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PRODUTIVIDADE E EFICIÊNCIA DO USO DO NITROGÊNIO NA CULTURA DO MILHO INOCULADO COM A*zospirillum brasilense* E ADUBADO COM MOLIBDÊNIO

SÃO LUÍS
2021

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Engenheiro Agrônomo

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Tese apresentada ao Programa de Pós-Graduação em Agroecologia na Universidade Estadual do Maranhão, para obtenção do título de Doutor em Agroecologia.

Orientador: Prof. Dr. Heder Braun

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RESUMO

O nitrogênio (N) é um dos nutrientes mais importantes e exigidos pela cultura do milho e o que mais onera a produção agrícola, pois na maioria dos solos agricultáveis este elemento não está disponível em níveis necessários para atingir altas produtividades. Uma alternativa é a utilização de produtos biológicos (bactérias do gênero Azospirillum) e o micronutriente molibdênio (Mo), que também é importante, pois esse micronutriente interfere diretamente no metabolismo do N. O objetivo desse trabalho foi investigar o efeito da inoculação com *Azospirillum brasilense* associada a aplicação de Mo e N no desenvolvimento produtivo e na eficiência do uso do N e eficiência fotossintética do N em plantas de milho cultivada em solo da região trópico úmido maranhense. Três experimentos de campo foram conduzidos no município de São Luís (2° 30' S, 44° 18' O, 24 metros acima do nível do mar), Estado do Maranhão, Brasil. O delineamento experimental foi em blocos casualizados com quatro repetições e oito tratamentos. Quando o efeito do tratamento foi significativo, sete contrastes ortogonais foram analisados. Os tratamentos foram uma dose de N 140 kg ha^{-1}, uma dose de Mo 90 g ha^{-1} e métodos de inoculação de *A. brasilense* (semente e foliar). As variáveis dependentes analisadas foram: índice de clorofila foliar, altura da planta, diâmetro do caule, altura de inserção da primeira espiga, peso de 100 sementes, produtividade de grãos, índice de colheita, matéria seca da parte aérea e eficiência de uso de nitrogênio. Avaliamos também características fisiológicas no início do estágio de florescimento (aproximadamente 20 dias após a adubação de cobertura) e do grão leitoso (aproximadamente 40 dias após a adubação de cobertura) as características foram: assimilação fotossintética de CO_2, condutância estomática, concentração intercelular de CO_2, índice de clorofila foliar e a eficiência do uso de N fotossintético. A inoculação com *A. brasilense*, nitrogênio e Mo mostrou-se potencialmente utilizável pois os resultados mostraram que a combinação (*A. brasilense*, Mo e N) aumentou 5,1% da eficiência do uso do nitrogênio e 12,68% da eficiência do uso de N fotossintético. No entanto, não houve aumento na produtividade de grãos. Por outro lado, Mo e N combinados aumentaram 17,8% a produtividade de grãos quando comparadas as plantas de milho inoculadas apenas com *A. brasilense*. Diante desses resultados é necessário propor novas pesquisas com o papel de elucidar melhor o papel do Mo na ligação com bactérias promotoras de crescimento e seus efeitos na rizosfera e na produtividade de grãos.

Palavras-chave: Tropico úmido, bactérias diazotróficas, nutrição de plantas.

ABSTRACT

Nitrogen (N) is one of the most important nutrients required by the maize crop and the most burdensome on agricultural production since in most arable soils this element is not available at the levels necessary to achieve high yields. An alternative is the use of biological products (bacteria of the genus Azospirillum) and the micronutrient molybdenum (Mo), which is also important, as this micronutrient directly interferes with N metabolism. The objective of this work was to investigate the effect of inoculation with *A. brasilense* associated with the application of Mo and N on the productive development and on the N use efficiency and N photosynthetic efficiency in maize plants grown in soil from the humid tropic region of Maranhão. Three field experiments were carried out in the municipality of São Luís (2° 30' S, 44° 18' W, 24 meters above sea level), State of Maranhão, Brazil. The experimental design was in randomized blocks with four replications and eight treatments. When the treatment effect was significant, seven orthogonal contrasts were analyzed. The treatments were a dose of N 140 kg ha^{-1}, a dose of Mo 90 g ha^{-1} and methods of inoculation of *A. brasilense* (seed and leaf). The dependent variables analysed were: leaf chlorophyll index, plant height, stem diameter, height of insertion of the first ear, weight of 100 seeds, grain yield, harvest index, shoot dry matter and nitrogen use efficiency. We also evaluated physiological characteristics at the beginning of the flowering stage (approximately 20 days after top dressing) and milky grain (approximately 40 days after top dressing). The characteristics were: photosynthetic CO2 assimilation, stomatal conductance and intercellular concentration of CO_2. leaf chlorophyll index and photosynthetic N use efficiency. Inoculation with *A. brasilense*, nitrogen and Mo proved to be potentially usable as the results showed that the combination (*A. brasilense*, Mo and N) increased 5.1% of the nitrogen use efficiency and 12.68% of the photosynthetic nitrogen use efficiency. However, there was no increase in grain yield. On the other hand, Mo and N combined increased grain yield by 17.8% when compared to corn plants inoculated only with *A. brasilense*. Given these results, it is necessary to propose further research with the role of better elucidating the role of Mo in the link with growth-promoting bacteria and its effects on the rhizosphere and on grain yield.

Keywords: Humid tropic, diazotrophic bacteria, plant nutrition.

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CAPÍTULO I

1. INTRODUÇÃO GERAL

A agricultura é uma atividade indispensável para o mundo porque desempenha um papel importante no fornecimento de alimentos para a população mundial. Por outro lado, a pesquisa agrícola tem um problema real e urgente a ser resolvido: produzir mais alimentos em um espaço menor, ou seja, aumentar a produtividade das lavouras. O foco é usar um método chamado "land sparing – efeito poupa-terra": para produzir mais alimentos em um espaço menor. Isso visa não expandir a área cultivável, mas intensificar a produção e fortalecer a proteção ambiental alocando mais espaço para fitorremediação (técnica de descontaminação de solo e água, através da utilização de plantas). Atualmente a corrida pela não expansão de novas áreas agrícolas, tem levado pesquisadores a se esforçarem para aumentar a produtividade das lavouras, com foco na quantidade e a qualidade dos produtos, a viabilização dos custos de investimento e a concretização final de planos ecologicamente corretos e socialmente justos. Nesse campo, podemos focar na cultura do milho (*Zea mays* L.), por ser de grande importância econômica e alimentar.

O milho é uma cultura de importância global em virtude da sua diversidade de utilização, extensão da área cultivada e elevada capacidade produtiva. Os maiores produtores mundiais são os Estados Unidos, Canadá e o Brasil. Na safra de 2019/2020 o Brasil obteve produtividade média de 5,531 Mg ha^{-1} (CONAB, 2020).

É sabido que as lavouras de milho são altamente dependentes de um bom manejo de fertilizantes de nitrogênio. Embora o manejo da fertilização das lavouras de milho tenha melhorado, o nitrogênio (N) ainda é um dos principais fatores que levam à baixa produtividade das lavouras de milho (USDA, 2017). O N é o nutriente mais necessário no ciclo produtivo da lavoura de milho, e sua deficiência pode causar perdas significativas de produtividade. Os fertilizantes nitrogenados respondem por grande parte do custo de fertilização das lavouras de milho, que pode variar dependendo da produtividade necessária (DARTORA *et al*., 2013). A maioria dos solos nos trópicos úmidos é deficiente em nitrogênio, apenas 30% a 40% do nitrogênio é usado pelas lavouras e 60% a 70% do nitrogênio é perdido por lixiviação, volatilização e desnitrificação (HIREL *et al*., 2007; KONG *et al*., 2016).

A fixação biológica de nitrogênio (FBN) é benéfica para o suprimento de parte do N necessário à cultura do milho durante seu ciclo produtivo. Nas monocotiledôneas, a FBN ocorre por meio de bactérias diazotróficas, ao qual iremos destaca o gênero Azospirillum Esses microrganismos são capazes de reduzir o N atmosférico (N_2) a amônia (NH_4^+) pela quebra da ligação tríplice do N através da enzima nitrogenase, com alto consumo de energia na forma de adenosina trifosfato (ATP) (SANGOI *et al.*, 2015). As bactérias diazotróficas também podem atuar no crescimento vegetal, produção de hormônios (auxinas, citocininas, giberelinas, etileno) e atuam como agentes de controle biológico de patógenos (CORREA *et al.*, 2008). Em geral, as bactérias diazotróficas geram benefícios ao desenvolvimento das plantas pela combinação de todos esses mecanismos listados acima (DOBBELAERE *et al.*, 2003). No entanto, a inconsistência dos resultados de pesquisas inviabiliza inferências sobre as possíveis interações das bactérias diazotróficas e a disponibilidade de N mineral no que diz respeito à resposta da planta (BREDA *et al.* 2020), pois o nitrato (NO_3^-) também é um modulador da arquitetura da raiz e pode atuar em um papel sinérgico na melhoria da exploração do solo pelas plantas (FORDE 2014). Nesse sentido, a genética das plantas e o ambiente afetam o metabolismo das bactérias diazotróficas e, consequentemente, a resposta de promoção do crescimento das plantas. Os estudos genéticos são fundamentais para descrever e revelar as etapas e funcionalidades das bactérias diazotróficas e seus efeitos nas monocotiledôneas.

Diante desse cenário, uma alternativa para redução da dependência de fertilizantes nitrogenados nas lavouras de milho, é a utilização consorciada de bactérias diazotróficas do gênero *Azospirillum* e o micronutriente molibdênio (Mo). Essa associação tem como objetivo, aumentar o rendimento produtivo da lavoura de milho e manter o balanço nutricional adequado.

O Mo é um micronutriente exigido pelas plantas em pequenas quantidades. No entanto, sua deficiência é tão prejudicial quanto a falta de um macronutriente (N, K e P), principalmente pois afeta diretamente o metabolismo do nitrogênio (TAIZ; ZEIGER, 2017). A utilização de N pelas plantas de milho pode ser potencializada com o uso de Mo, uma vez que, este micronutriente é constituinte de enzimas que permitem a assimilação de N via fixação biológica por microrganismos diazotróficos (nitrogenase) e/ou N sintético (nitrato redutase) (PICAZEVICZ *et al.*, 2017).

As plantas absorvem Mo na forma de molibdato (MoO_4^{-2}) (VALENTINI *et al.*, 2005). Embora a quantidade necessária seja pequena, o teor nos tecidos da planta é geralmente inferior a 1,0 mg kg^{-1} de matéria seca. (MENGEL; KIRKBY, 1987). Estudos constataram que o uso de Mo com *Azospirillum brasilense* (GANAPATHY; SAVALGI, 2006) e fertilizantes nitrogenados sintéticos (VALENTINI *et al.*, 2005) pode aumentar o crescimento e a produtividade das plantas de milho. No entanto, o efeito da pulverização de Mo na produtividade do milho é diferente, dependendo da localização e do tipo de solo.

Diante dos relatos apresentados, a comunidade cientifica possui um déficit de informações sobre o uso de bactérias diazotróficas (*Azospirillum brasilense*) associadas a adubação com o micronutriente Mo e seus efeitos em plantas de milho em solo da região do tropico úmido maranhense, o que pode contribuir para a redução parcial de uso do N sintético aplicado em cobertura e potencializar os benefícios dessa técnica com o consequente aumento da produtividade.

2. REVISÃO BIBLIOGRÁFICA

2.1. Cultura do milho

O milho (*Zea mays* L.), é uma espécie, pertencente à família das Poáceas (antiga família das gramíneas), possivelmente tem origem americana, foi encontrado em pequenas ilhas próximas ao litoral mexicano. Para alguns membros da comunidade cientifica, o milho é originário do Teosinte (*Zea mexicana* L.), uma gramínea anual originária do México e da Guatemala, por meio de seleção feita pelo homem, outros membros defendem a hipótese de que o milho e o Teosinte diferenciam-se a mais tempo de um ancestral comum (SOUZA, 2017).

Após o descobrimento das Américas, o milho foi levado para o continente europeu, onde plantas foram cultivadas em jardins até seu valor alimentício tornar-se conhecido (OKUMURA *et al*., 2011). Na contemporaneidade, devido à sua alta capacidade de adaptação a diversas condições de cultivo, e em virtude da grande variedade de genótipos existentes, a cultura do milho está presente em todos os continentes e sua produção mundial só perde para a cultura do trigo e a do arroz (SILVA *et al*., 2014).

A cultura do milho, possui papel incontestável na economia mundial e brasileira devido a sua posição entre as espécies agrícolas com maior área de cultivo (MÔRO; FRITSCHE, 2015). No Brasil, os maiores produtores de milho na safra de 2019/2020 foram os estados de Mato Grosso, Paraná, Goiás, Mato Grosso do Sul e Minas Gerais. O estado do Maranhão participou com uma produção de aproximadamente 977,3 mil toneladas (CONAB, 2020).

Nas condições de exploração do milho no Brasil, grande parte das cultivares possui ciclo de produção entre 105 e 180 dias, período compreendido entre a semeadura e a colheita (MOREIRA, 2015). Segundo Magalhães et al. (2006) a cultura do milho, para expressar seu potencial produtivo, necessita em média de 600 mm de precipitação e temperaturas entre 25 e 30 ºC.

A importância econômica do milho grão é caracterizada pelas diversas formas de sua utilização, que vai desde a alimentação animal, como forragem conservada para o período da seca e na fabricação de farelos até a indústria química, como matéria prima de mais de 500 produtos e a indústria alimentícia, como amido, farinhas e óleo (OKUMURA *et al*., 2011).

Há vários fatores que limitam a produtividade do milho, como por exemplo, doenças, pragas, déficit hídrico, disponibilidade e assimilação de nutrientes. Em relação à fertilidade, a cultura do milho é muito exigente em fertilizantes, principalmente nitrogenados (FERNANDES *et al*., 2008). Neste sentido, estudos devem ser intensificados com o intuito de desenvolver técnicas alternativas e promissoras para a eficácia da incorporação do nitrogênio nas plantas de milho, o que refletirá em estratégia econômica e de menor impacto ambiental.

2.2. Nitrogênio

O nitrogênio (N) é um nutriente essencial absorvido pelas raízes, convertido em aminoácidos para compor diversas moléculas nas raízes e na parte aérea das plantas durante todo o período de crescimento (WANG; XING, 2017). É importante, sobretudo nos estádios iniciais do desenvolvimento vegetal, quando sua disponibilidade se relaciona diretamente com as maiores eficiências de utilização pelas plantas. Nas plantas de milho é importante na fase de aproximadamente quatro folhas, quando se define o potencial produtivo (RITCHIE *et al.*, 2003).

O N possui papel fundamental no metabolismo vegetal por participar diretamente na biossíntese de proteínas e clorofilas (ANDRADE *et al.*, 2003). É constituinte de proteínas, enzimas, coenzimas, ácidos nucléicos, fitocromos e integra a molécula da clorofila. Além disso, afeta as taxas de iniciação e expansão foliar, o tamanho final e a intensidade de senescência das folhas, o desenvolvimento da área foliar e a taxa de fotossíntese, o crescimento radicular, o rendimento biológico, o tamanho e número de espigas, a massa de grãos e índice de espiga, a altura de planta, o comprimento da espiga, o diâmetro de colmo, a inserção de espiga, o número de plantas acamadas e quebradas e a qualidade de grãos (SILVA, 2014). A maioria dos fertilizantes nitrogenados empregados na cultura milho são hidrossolúveis, e rapidamente liberam para o solo NO_3^- e NH_4^+, sendo desta forma prontamente assimiláveis pela planta. Assim, as plantas de milho, por remover grandes quantidades de nitrogênio, requer o uso de adubação nitrogenada em cobertura para complementar a quantidade suprida pelo solo. O N é predominantemente derivado de fertilizantes, fixação biológica de N_2, mineralização do N orgânico de esterco animal, resíduos de culturas e matéria orgânica do solo (SOUZA, 2017), no entanto, por sua alta mobilidade, o N está susceptível a perdas e pode ocasionar riscos ambientais (MENDES, 2016).

Em grande parte dos solos brasileiros, a quantidade de N é insuficiente, fazendo-se necessário o fornecimento externo do nutriente em concentração adequada para garantir o crescimento, desenvolvimento e a produtividade das plantas de milho (BELARMINO *et al.*, 2003), e isso, deve ser feito respeitando-se os limites do solo, para evitar a degradação. Nesse contexto, o manejo da adubação nitrogenada é realizado com o intuito de garantir boa produtividade, em função da dinâmica do N, grandes quantidades desse nutriente são adicionadas ao solo, o que pode levar a perdas e degradação do ambiente. E tendo em vista a crescente demanda por fertilizantes nitrogenados e a preocupação com as possíveis perdas e contaminação do ambiente (FERNANDES; LIBARDI, 2007), faz-se necessária a aplicação de N na forma parcelada em cobertura (YAMADA; ABDALA, 2000), como também, a investigação de alternativas para suprimento de N via fixação biológica ou fertilizantes alternativos. Isso reduzirá as perdas do nutriente e aumentará sua eficiência de uso (BOTREL *et al.*, 1999).

Com esse conhecimento, surge a necessidade de buscar alternativas para diminuir as perdas através do parcelamento da adubação de cobertura, assim como buscar alternativas para suplementar com N os plantios. Uma opção é favorecer a fixação biológica de N, essa técnica pode incrementar o rendimento da cultura do milho sem prejuízos aos recursos naturais (BASI, 2013).

2.3. Eficiência do Uso do Nitrogênio (EUN)

Nas últimas décadas, os esforços têm sido direcionados no sentido de otimizar a eficiência de utilização de nutrientes pelas plantas, visando reduzir os custos de produção, evitar a degradação dos recursos ambientais e aumentar o rendimento das culturas (KOLCHINSKI; SCHUCH, 2003). Nesse sentido, existem diversos caminhos possíveis para aumentar a eficiência do uso do nitrogênio (EUN). Um dos caminhos mais simples é a redução nas doses de adubos nitrogenados para níveis que sejam produtivos e seguros ambientalmente (FERNÁNDEZ *et al.* 1998).

O uso de fertilizantes em culturas de grãos e fibras também é importante na manutenção das reservas de N do solo. Alta produtividade com doses baixas de N, normalmente significa que a quantidade de N exportada com a colheita é maior do que a adicionada, o que contribui para o empobrecimento do solo (ALVES *et al.*, 2006). Fernandes et al. (2005) estudando doses de N em seis cultivares de milho e a eficiência de uso desse nutriente pela cultura, em região de cerrado, verificaram que a eficiência do uso de nitrogênio de todos os híbridos testados diminuiu com o aumento da dose de N aplicada. Além disso, observaram ainda que as doses de N influenciaram principalmente a massa de 100 grãos e a produtividade de grãos. Dessa forma, nos estudos sobre a dinâmica do N no sistema solo-planta, muitas vezes, é difícil quantificar a origem deste nutriente (SCIVITTARO *et al.*, 2000).

A eficiência nutricional, pode ser definida como a quantidade de matéria seca ou grãos produzidos por unidade de nutriente aplicado (FAGERIA, 1998). A eficiência da utilização do nitrogênio adicionado ao solo, por sua vez, se refere ao grau de recuperação desse elemento pelas plantas, considerando as perdas que geralmente ocorrem (BREDEMEIER; MUNDSTOCK, 2000). A eficiência nutricional depende de vários processos fisiológicos, tais como absorção, assimilação e retranslocação do nitrogênio pela planta (MOLL *et al.*, 1982; CARVALHO, 2011) e pode ser aumentada com a adoção de práticas de manejo apropriadas.

Especificamente em milho, Moll et al. (1982) definiram a eficiência de uso do N (EUN) como a massa de grãos dividida pela massa de N aplicado no solo (Gw/Ns), ambas expressas na mesma unidade, como por exemplo, gramas por planta. Entretanto, a produtividade de grãos também se destaca como bom parâmetro da eficiência de utilização do N (CARVALHO, 2011). Para Fageria (1998) ao avaliar experimentos de campo, a produção de grãos foi o melhor parâmetro para avaliação da eficiência nutricional em culturas anuais.

Os principais componentes de avaliação da EUN são a eficiência de absorção do N (EAN), a eficiência de utilização do N (EUtN) e a eficiência de remobilização do N (ERN) (LE GOUIS *et al.*, 2000). A EAN representa a capacidade das plantas em absorver o N disponível do solo. A EUtN é definida como a relação entre o rendimento da cultura e o N total absorvido pela planta (N nos grãos + N na biomassa), ou seja, essa medida indica o rendimento de grãos obtido para cada unidade de N absorvido pela planta. Em adição, a eficiência de remobilização do N (ERN) representa a capacidade das plantas em translocar o N após a antese da parte vegetativa para os grãos. Cultivares com uma maior ERN tendem a acelerar a senescência e aumentar os níveis de N nos grãos (GAJU *et al.*, 2014).

Segundo Fidelis et al. (2007), a identificação de genótipos capazes de absorver e utilizar o nitrogênio de forma eficiente é um dos caminhos para aumentar a EUN na cultura do milho, incrementar a produção, minimizar as perdas e reduzir a contaminação do meio ambiente. Entretanto, a melhor eficiência nutricional é aquela obtida sob nível de nutriente adequado em que a produtividade máxima é obtida, visto que a eficiência nutricional diminui com níveis crescentes de um nutriente, devido ao suprimento desse nutriente exceder as necessidades da cultura (FAGERIA, 1998). Fernandes et al. (2005) e Farinelli; Lemos (2010), relataram que a eficiência do uso de nitrogênio em todos os híbridos testados nas pesquisas, diminuiu com o aumento da dose de N aplicada.

Nesse contexto, o conhecimento da relação entre os caracteres envolvidos na eficiência nutricional e o uso racional da adubação nitrogenada é fundamental, não apenas para aumentar a eficiência de recuperação do nitrogênio, mas também para aumentar a produtividade da cultura e diminuir o custo de produção (FAGERIA *et al.*, 2007).

2.4. *Fixação Biológica do Nitrogênio (FBN)*

O nitrogênio ainda que seja o gás mais abundante na atmosfera, não é prontamente assimilado pelas plantas. A forma que as plantas assimilam o N difere entre as espécies vegetais, as quais absorvem principalmente as formas inorgânicas deste nutriente, o nitrato ou o amônio (WILLIANS; MILLER, 2001; FAGERIA *et al.* 2003; SOUZA; FERNANDES, 2006).

O nitrogênio gasoso (N_2) compõe 78% da atmosfera terrestre. A fixação de nitrogênio requer a quebra da ligação tripla covalente de excepcional estabilidade do N_2. Contudo, os gases atmosféricos também se difundem para o espaço poroso do solo e o N_2 pode ser aproveitado por alguns microrganismos, principalmente bactérias que ali habitam, graças à ação da enzima dinitrogenase, que tem a capacidade de romper a tripla ligação do N_2 e reduzi-lo a NH_3, a mesma forma obtida no processo industrial (HUNGRIA *et al.*, 2011; TAIZ; ZEIGER,2017).

A fixação biológica de nitrogênio (FBN) é um processo de transformação do N_2 na forma inorgânica combinada NH_3. A FBN envolve uma sucessão de processos que começam com a adaptação da bactéria à planta e culminam na fixação do N_2 atmosférico (FAGAN, *et al.*, 2007)

Todo o sistema de fixação biológica de nitrogênio é coordenado pelo complexo enzima nitrogenase, que é formado por duas unidades proteicas, a ferro-proteína (Fe-proteína) e a molibdênio-ferro-proteína (MoFe-proteína) que são responsáveis pela fixação de nitrogênio (BURRIS, 1999; MYLONA *et al.*, 1995; TAÍZ; ZIEGER, 2017). Para que ocorra a FBN é necessário que a nitrogenase esteja em condições anaeróbicas. Na reação de redução do N_2 a nitrogenase é auxiliada por uma enzima transportadora de elétrons, a ferredoxina, originária do fotossistema I da fase fotoquímica da fotossíntese (BURRIS, 1999; TAIZ; ZIEGER, 2017).

A capacidade de reduzir o nitrogênio atmosférico a amônia, está restrita a um pequeno grupo de microrganismos denominados diazotróficos, ou fixadores de N_2 (NOVAKOWISKI *et al.*, 2011). Pesquisas sobre as bactérias diazotróficas tiveram início no Brasil há mais de 40 anos. Essas pesquisas foram realizadas pela renomada pesquisadora Johanna Döbereiner e seus colaboradores (MOREIRA *et al.* 2010).

Esses microrganismos estão distribuídos em diversos grupos filogenéticos e habitam vários ecossistemas em vida livre, em simbiose com leguminosas como o feijoeiro e o feijão caupi, ou endofiticamente em raízes ou parte aérea de poaceae como milho, bem como de espécies forrageiras como *Brachiaria* ssp*., Paspallum notatum* F. entre outras (WEBER *et al*., 2000; MOREIRA *et al*., 2010).

Dentre as diazotróficas, bactérias do gênero Azospirillum associam-se à rizosfera da planta de milho e podem contribuir com a nutrição nitrogenada da cultura (FIGUEIREDO *et al*., 2009). Pesquisa realizada por Novakowiski et al. (2011), demonstrou que as bactérias do gênero Azospirillum podem promover o crescimento vegetal através da produção de fitoreguladores e sideróforos ou por aumentar a disponibilidade de fósforo.

No processo de FBN em gramíneas, somente uma parte do nitrogênio fixado diretamente para a planta associada é secretado para suprir parcialmente suas necessidades. Já em leguminosas, a inoculação das culturas com microrganismos, ainda que fixem nitrogênio, não conseguem suprir totalmente as necessidades das plantas em relação ao N (HUNGRIA, 2011).

O grande interesse na fixação biológica em gramíneas é devido à maior facilidade de aproveitamento de água das mesmas em relação às leguminosas, pela maior efetividade fotossintética. As gramíneas apresentam um sistema radicular fasciculado, tendo vantagens sobre o sistema pivotante das leguminosas para extrair água e nutrientes do solo; e por serem as gramíneas largamente utilizadas como alimento pelo homem. Por isso, a FBN é um processo importante para a economia em adubos nitrogenados e equilíbrio ambiental (DÖBEREINER, 1992).

2.5. Azospirillum brasilense

As bactérias diazotróficas não simbióticas (BDNS) atuam no desenvolvimento das plantas por meio da FBN e também pela produção e liberação de substâncias reguladoras do crescimento vegetal (SILVA; MELLONI, 2011). No grupo das bactérias diazotróficas não simbióticas (BDNS), destaca-se o gênero Azospirillum. Pesquisas consideram as bactérias do gênero Azospirillum como diazotróficas facultativas, capazes de colonizar raízes de plantas não leguminosas interna e externamente (BALDANI *et al*., 1997). Esse gênero é caracterizado por organismos de metabolismo bastante versátil, o que confere características adaptativas, que permitem a sobrevivência em meio nutritivo rico e protetor existente na rizosfera das plantas (STEENHOUDT; VANDERLEYDEN, 2000). As BDNS podem desempenhar importante papel na sustentabilidade dos ecossistemas, uma vez que incorporam N_2 por meio da fixação biológica (DOBBELAERE *et al*., 2003).

A fixação de nitrogênio pelas bactérias diazotróficas não simbióticas (BDNS) ocorre em ambiente natural, temperatura ambiente e seu crescimento ideal varia numa faixa de temperatura entre 28 e 41° C (ECKERT *et al.*, 2001), ocorrem em níveis bem menores de energia, consumindo os açúcares da planta, mas que são compensados pelo aporte de N fornecido ao sistema (ALVES, 2007).

As bactérias do gênero Azospirillum são de vida livre, rizobactérias capazes de promover o crescimento das plantas e aumentar a produtividade em muitas culturas de importância econômica. Essas bactérias podem atuar no crescimento da planta através das sínteses de hormônios, principalmente auxinas (ácido 3-indolacético), giberelinas e citocininas (FIGUEIREDO *et al.*, 2009). Ocorre também a síntese de etileno, podendo agir como solubizador de fosfato ou acelerador do processo de mineralização (PERSELLHO-CARTINEAUX *et al.*, 2003; SÁ JÚNIOR, 2012). Todo esse processo de síntese tem como consequência uma maior absorção de água e nutrientes (CORREA *et al.*, 2008) resultando em uma planta mais vigorosa e produtiva (BASHAN *et al.*, 2004; HUNGRIA, 2011).

As bactérias diazotróficas foram descobertas no início da década de 1970 pela pesquisadora da Embrapa Drª. Johanna Döbereiner. Essas bactérias auxiliam por diversos mecanismos na nutrição nitrogenada das culturas. Dentre esses mecanismos, destacam-se a produção de hormônios, que interferem no crescimento das plantas e podem alterar a morfologia das raízes, possibilitando a exploração de maior volume de solo (BASHAN; HOLGUIN, 1997; ZAIED *et al.*, 2003), o aumento do processo da redução assimilatória de nitrato disponível no solo (BODDEY *et al.*, 1986) e a fixação biológica do N_2 (INIGUEZ *et a*l., 2004). Entre esses mecanismos, o aumento do sistema radicular, estimulado pela presença de bactérias, através da produção de substâncias promotoras do crescimento radicular, pode resultar em maior absorção de minerais e de água (OKON; LABANDERA-GONZALEZ, 1994).

As características benéficas destas bactérias podem ser resumidas em: capacidade de penetrar na raiz das plantas, antagonismo a agentes patogênicos, associação com várias gramíneas e com não gramíneas (morango, tabaco, café e outras), produção de hormônios promotores de crescimento e desenvolvimento, baixa sensibilidade às variações de temperatura e ocorrência em todos os tipos de solo e clima (ARAÚJO, 2008).

É importante salientar que o processo de fixação biológica por essas bactérias em associação com gramíneas supre apenas parcialmente as necessidades das plantas em nitrogênio (HUNGRIA *et al.*, 2011). Não é recomendado a substituição total da adubação nitrogenada por bactérias diazotróficas não simbióticas.

2.6. Molibdênio

O molibdênio (Mo) tem sua principal função associada ao metabolismo do nitrogênio (N), e relaciona-se às enzimas redutase do nitrato e nitrogenase, de modo que os sintomas de deficiência se confundem com aqueles do nitrogênio (MARSCHNER, 1995). É capaz de mediar diversas reações de oxirredução nos sistemas biológicos (SRIVASTAVA, 1997). Participa como co-fator de enzimas redutase do nitrato, a oxidase da xantina, a oxidase de aldeído e a oxidase de sulfeto. A deficiência de Mo provoca redução na concentração de clorofilas nas folhas, acarretando decréscimo de fotossíntese e prejuízo no metabolismo do N, tendo como consequência o acúmulo de nitrato no tecido das plantas (BORKET, 1989). Portanto, qualquer deficiência desse elemento pode comprometer o metabolismo do N, diminuindo o rendimento das culturas.

O N absorvido pelas plantas na forma de nitrato (NO_3^-) é reduzido a amônia (NH_3), possibilitando assim sua assimilação. A primeira reação do processo redutivo é catalisada pela redutase do nitrato, sendo o Mo um cofator dessa enzima que reduz o NO_3^- a nitrito (NO_2^-). O (NO_2^-) é reduzido a amônia e assimilado na forma orgânica por meio do sistema glutamina sintetase glutamina oxoglutarato unida transferase (GS-GOGAT) com síntese de aminoácidos e, posteriormente, de proteínas, clorofila e outros compostos (CRAWFORD *et al*., 1989). Assim, a produção de metabólitos nitrogenados (aminoácidos e proteínas) é afetada pela deficiência de Mo o uma vez que ocorre decréscimo na atividade da redutase do nitrato na ausência do cofator. Com isso, ocorre diminuição na síntese de aminoácidos e, consequentemente, de proteínas. Dessa forma, o Mo exerce papel direto no crescimento e desenvolvimento das plantas (VALETINI *et al*., 2005).

As respostas à adubação molibídica estão relacionadas ao requerimento de Mo por vários tipos de molibdoenzimas (Mo-enzimas) presentes nas plantas. Essas Mo-enzimas podem estar envolvidas na redução e assimilação do N (nitrato redutase, NR), fixação do N (nitrogenase), catabolismo de purinas (xanthine dehydrogenase/oxidase), síntese de ácido abscísico, ABA, e ácido indol-3 acético (aldeído oxidase, AO) e metabolismo do enxofre (sulfite oxidase, SO) (KAISER *et al*., 2005). Destas as de maior relevância para as plantas estão a nitrato redutase e a nitrogesnase (HAMLIN, 2007).

O Mo utilizado pelas plantas pode ser originado do próprio solo ou resultante da aplicação de produtos químicos e/ou orgânicos que o contenham em sua composição (PEREIRA *et al*., 2012). Geralmente, as fontes de micronutrientes de produtos químicos variam de modo considerável na sua forma física, reatividade química, custo, teor do nutriente e eficiência agronômica. O fornecimento do fertilizante molíbdico às plantas tem sido feito de três formas principais: aplicação direta no solo, aplicação foliar e aplicação direta na semente (PEREIRA, 2010).

O Mo interfere diretamente no crescimento e desenvolvimento do milho e consequentemente, na produção de grãos, por meio do metabolismo do N (PEREIRA *et al.*, 1999). A faixa crítica de concentração de Mo no milho é de 0,1 a 0,2 mg kg^{-1} (DIOS; BROYER, 1965). No milho, a deficiência de Mo encurta os internós, reduz a área foliar e causa o desenvolvimento de clorose nas folhas (AGARWALA et al., 1978). O manejo adequado da adubação com molibdênio é imprescindível para o melhor aproveitamento da adubação nitrogenada (FERREIRA *et al.*, 2001).

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The effects of nitrogen, *Azospirillum brasilense* and molybdenum on the grain yield and nitrogen use efficiency on maize plants in the sub-humid tropics of Brazil.

CAPÍTULO II

The effects of nitrogen, *Azospirillum brasilense* and molybdenum on the grain yield and nitrogen use efficiency on maize plants in the sub-humid tropics of Brazil.

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Highlights

- The combination (*A. brasilense*, Mo and N) increased 5.1% nitrogen use efficiency;
- Application methods *A. brasilense* on seed increased 13.4% nitrogen use efficiency;
- Molybdenum and nitrogen increased 17.8% grain yield;

ABSTRACT

The objective this is study was to evaluated the effectiveness of inoculation methods of *A. brasilense*, N rate and molybdenum on yield and N use efficiency in maize plants cultivated in the sub-humid tropical regions of Brazil. Three field trials were conducted in a randomized complete block design with four replicates and eight treatments. The treatments were 140 kg ha^{-1} of N, 90 g ha^{-1} of Mo and inoculation methods of *A. brasilense* (seed and leaf). In experiment 2, all treatments did affect grain yield, 100 - seed weight, harvest index, leaf chlorophyll index (LCI), stem diameter, insertion height of the first ear (IHFE) and shoot dry matter. In experiment 3, all treatments did affect grain yield, nitrogen use efficiency (NUE), harvest index, leaf chlorophyll index (LCI), stem diameter, insertion height of the first ear (IHFE), plant height and shoot dry matter. The inoculation with *A. brasilense*, N and Mo showed the potential to be used, and the results showed that Mo is the limiting factor for grain yield and NUE.

Keyswords: nitrogen fertilization, biological nitrogen fixation, plant growth-promoting rhizobacteria, *Zea mays.*

1. INTRODUCTION

Nitrogen (N) is the most significant agricultural input for crops to achieve high yield, is consumed by the crop roots throughout the growing season (Wang and Xing, 2017; Picazevicz, Krudra and Moreno, 2017; Galindo et al. 2017; Bloch et al. 2020), but excessive N inputs make it a difficult problem for optimal use of N (Norr, 2017). Excess or low supply of the nutrients will result in reduced nitrogen use efficiency (NUE) and cause significant losses in grain yield and grain quality (Haroon et al, 2019).

Nitrogen is considered a major limiting factor for maize (*Zea mays* L.) grain yield because it is an essential component of all proteins and enzymes, nucleic acids that make up DNA, and chlorophyll that enables the process of photosynthesis in plants (Leghari et al. 2016). Low yields are generally attributed to low fertility soils that require high fertilizer inputs for optimal productivity (Martins et al, 2018). In this context cultivation of maize in tropical soil from the Amazonian periphery do not adequately supply the plant's demand for nitrogen.

Agronomic management for improved nitrogen use efficiency (NUE), the use of agroecological practices is the key to increase maize production while reducing environmental pollution. One possibility such as inoculation by plant growth-promoting bacteria (PGPB) can represent a sustainable alternative for increase nutrient use efficiency in tropical agriculture (Perreira et al. 2020). Inoculation technology with PGPB has been presented worldwide as an important tool for reaching sustainability in agriculture due to its low environmental and production costs compared with industrial inputs (Oliveira et al. 2017).

PGPB colonize rhizosphere or plant root and improve plant health and growth. Some of the most important plant growth promoting bacteria activity include biological nitrogen fixation (BNF)(Pankievicz et al., 2019), production of indolic compounds and siderophores, increase on 1−aminocyclopropane−1−carboxylate deaminase activit (Ambrosini and Passaglia, 2017), solubilization of mineral phosphates (Ludueña et al., 2018; Qi et al., 2018) and production of phytohormones, such as salicylic acid, gibberellins, cytokinins and indole-3-acetic acid (IAA) (Cassán and Diaz-Zorita, 2016; Fukami et al., 2017; Dahal et al., 2017; Gouda et al., 2018). The diazotroph *Azospirillum brasilense* (free-living diazotrophic bacteria) is considered a model PGPB, and a great amount of information regarding the physiology of its growth and development has been published (Fendrik et al., 1995; Cassán et al., 2015, Cassán et al, 2020). Several positive results in the development and productivity of corn have been reported with inoculation with *Azospirillum brasilense* (strains Ab-V5 and Ab-V6) in tropical conditions (Martins et al., 2018; Oliveira et al., 2018; Galindo et al., 2019).

Molybdenum is the micronutrient required in the least amount by plants. However, there is a close relationship between the supply of Mo, the activity of nitrate reductase, and the growth of plants. (Kirkby and Römheld, 2004). Therefore, the supply of Mo is closely associated with the utilization and metabolism of N. Nitrogen utilization by maize can be potentiated by molybdenum (Mo), since it is a constituent of enzymes that allow the assimilation of this macronutrient via biological fixation by diazotrophic microorganisms (nitrogenase) and/or N fertilization (nitrate reductase) (Picazevicz et al, 2017). The increase in maize growth and production has already been observed with the use of Mo combined with Azospirillum brasilense (Ganapathy and Savalgi, 2006) and N fertilizer (Valentini et al., 2005)

The combined use of chemical and biological inputs in the cultivation of non-legumes can contribute to reduce costs and optimize production (Picazevicz et al, 2017). Less is known about the effects of inoculation methods of *A. brasiliense*, N rates and molybdenum on physiological characteristics, yield, and N use efficiency on maize plants grown in the sub-humid tropical regions of Brazil. Thus, we evaluated the effectiveness of inoculation methods of *A. brasilense*, nitrogen and molybdenum on yield and N use efficiency in maize plants cultivated in the sub-humid tropical regions of Brazil.

2. MATERIALS E METHODS

2.1. Field site description

Three field trials were conducted in the municipality of São Luís (2°30'S, 44°18'W, 24 m above sea level), State of Maranhão, Brazil. The first trial was stablished from February 2018 to May 2018, the second trial from October 2018 to January 2019 and third trial from March 2019 to July 2019. The region has a hot, semi-humid, equatorial climate, with mean annual rainfall of 2,200 mm and two well-defined seasons: a rainy season from January to June, and a dry season with pronounced water deficits from July to December. The climatic conditions during the trials are shown in Figure 1.

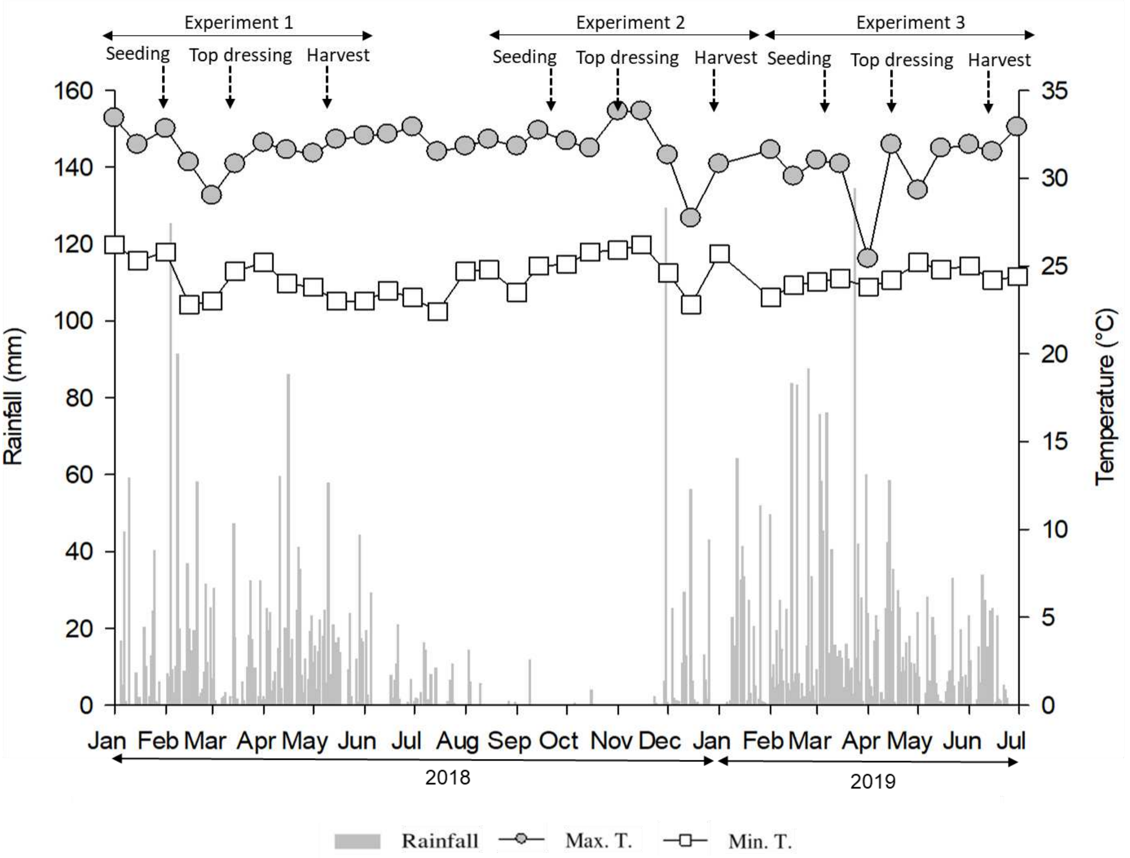

Figure 1. Rainfall (mm) and maximum and minimum temperatures obtained from the data base National Institute of Meteorology of Brazil (INMET) during the corn cultivation (all seasons) in the period from January 2018 to July 2019.

The trials were conducted under a no-tillage system. The field had not been cultivated with any agricultural crop for at least 10 years (2003-2013). Maize and cowpea (*Vigna unguiculata* L. Walp) had been cultivated in the field after 2013. The remaining straws from previous maize and cowpea crops were left on the soil surface. The soil in this area is a Typic Hapludult (Soil Survey Staff, 1999), with pH in CaCl2 (soil:solution ratio of 1:2.5) = 5.3; organic matter = 5 g dm^{-3}; P (resin) = 31 mg dm^{-3}; K^+ = 1.7 mmolc dm^{-3}; Ca^{2+} = 24 mmolc dm^{-3}; Mg^{2+} = 15 mmolc dm^{-3}; $H^+ + Al^{3+}$ = 19 mmolc dm^{-3}; sum of bases = 40.7 mmolc dm^{-3}; cation exchange capacity at pH 7 = 59.7 mmolc dm^{-3}; base saturation = 68%; and coarse sand = 260 g kg^{-1}, fine sand = 640 g kg^{-1}, silt = 20 g kg^{-1}, and clay = 120 g kg^{-1} and texture sandy loam.

No mechanical soil preparation was carried out before the maize sowing. At 30 days before sowing in 2017 was applied 1.0 Mg ha-1 of dolomitic limestone (32% CaO, 15% MgO, and total neutralizing power of 91%) without incorporation.

2.1. Experimental design, treatment, and field management

The experimental design was a randomized complete block design with four replicates and eight treatment (Table 1). The treatments were composed from one N rate of 140 kg ha^{-1}, one Mo rate of 90 g ha^{-1} and inoculation methods of *A. brasilense* (seed and leaf). Each plot consisted of four 4 m-long rows, spaced 0.8 m apart. The outer rows of each plot and 0.5 m from each end of the rows were used as borders.

Table 1. Treatment arrangement of all seasons 2018/2019 with *Azospirillum brasilense* (seeds and leaf), nitrogen and molybdenum rates.

Treatments	Nitrogen *(kg/ha^{-1})*		Molybdenum *(g/ha^{-1})*	Azospirillum seeds *(ml/ha^{-1})*	Azospirillum leaf *(ml/ha^{-1})*
	Sowing	Topdressing	Topdressing	Sowing	Topdressing
1	-	-	-	-	-
2	40	100	-	-	-
3	40	100	-	100	-
4	40	100	-	-	200
5	40	100	90	-	-
6	40	100	90	100	-
7	40	100	90	-	200
8	40	100	90	100	200

At sowing furrow, 40 kg ha^{-1} of N (granulated urea), 80 kg ha^{-1} of P_2O_5 (simple superphosphate), and 100 kg ha^{-1} of K_2O (potassium chloride) were applied in all trials. Seeds of the maize simple hybrid PIONNER 30F35® was sown at a density of four seeds per meter (5 plants m^{-2}). Seedling emergence occurred between four and six days after sowing for all trials. All plots were irrigated after the sowing with 15 mm of water, using a drip tape system and 2-day intervals, for a good and uniform seedling emergence. Approximately every 3 days without rainfall after seedling emergence, the plots were irrigated with 15 mm of water (2-hour irrigation). The plants were irrigated when there was no or low rainfall in the previous week. When there was a need to irrigate, we use the drip tape with flat emitters inside, 16mm diameter, thickness from 0.6mm, spacing from 20cm.

Topdressed N (100 kg ha^{-1} of N) (granulated urea, with 46% of N) was manually applied when the maize plants had six leaves completely expanded (V6) and was evenly applied along the furrows at 10 cm away from the plants. All plots were immediately irrigated after N fertilizer with approximately 15 mm of water to minimize ammonia volatilization. Topdressed Mo (90 g ha^{-1}) (ammonium molybdate) applied when the maize plants had six leaves completely expanded (V6) by spraying the solution, using a sprayer equipped with one cone nozzle (XR 11002; Teejet®, Wheaton, USA), with a flow rate of 200 L ha^{-1} of water. A plastic sheet was used to protect adjacent plots from unwanted spray drift.

The seeds and leaves of maize plants were inoculated with *Azospirillum brasilense*, using the commercial strains Ab-V5 and Ab-V6 (Nitro 1000 Gramíneas; Nitro 1000, Cascavel, Brazil) at rates of 100 mL and 200 mL of the liquid inoculant per hectare (2×108 CFU [colony forming unity] mL^{-1}), respectively. The seeds were microbiolized one hour before sowing the crop, and the leaves were inoculated at V6 stage maize by spraying the solution, using a sprayer equipped with one cone nozzle (XR 11002; Teejet®, Wheaton, USA), with a flow rate of 200 L ha^{-1} of water. A plastic sheet was used to protect adjacent plots from unwanted spray drift.

Deltamethrin (5g ha^{-1} active ingredient) was applied to control fall armyworm (*Spodoptera frugiperda*), when needed. Weeds were controlled by manual hoeing until the N topdressing application.

2.3. Measurements collected

The following nutritional evaluations were performed: leaf chlorophyll index (LCI), measured indirectly using a portable non-destructive chlorophyll meter SPAD-502 (Minolta Co., Japan). The readings were performed in 5 plants per plot, and total N concentration in leaves, collecting 2 leaves in the flowering stage were analyzed using the Kjeldahl method (Tedesco et al., 1995).

The following productive component measurements were performed at 100 days after emergence for all experiments, plant height, defined as the distance (cm) from the ground level to the apex of the spike, at harvest time; stem diameter, insertion height of the first ear (IHFE). Five plants from each plot were collected from two central rows to determine plant dry weight and plant N concentration. These plants were separated into grain and shoot plant, then oven-dried at 70 °C to constant weight and weighed. Grain dry weight plus shoot dry weight represented the total plant dry weight. Total N concentration of each fraction (grain and shoot plant) was analysed using the Kjeldahl method (Tedesco et al., 1995) and the N content was calculated as the product of N concentration by its dry weight. Grain N content plus shoot N content represents the plant N content (aboveground). All ears from the central two rows of each plot were hand-harvested. Grain yield was calculated and adjusted to 13% moisture content and 100-seed weight. This moisture content is the minimum considered for the marketing of maize in Brazil.

Based on the measurements of plant dry weight and N content, we calculated harvest index dividing the grain dry weight by the plant dry weight and N harvest index dividing the grain N content by the plant N content. The N use efficiency and its two components were calculated according to Moll et al. (1982): i) N utilization efficiency (kg kg^{-1}, kg of grain per kg of N extracted) = grain yield/plant N content, ii) N uptake efficiency (kg kg^{-1}, kg of N in plant per kg of N applied) = plant N content/N rates, iii) N use efficiency (kg kg^{-1}, kg of grain per kg of N applied) = NUpE × NUtE = grain yield/N rates.

2.4. Statistical analyses

All data were initially tested for homogeneity of variance with O'Neill and Mathews test and for normality using the Shapiro Wilk test. When the effect of treatment was significant ($p < 0.05$) its sum of squares were partitioned into seven orthogonal contrasts (below). Values were reported as means ± standard deviation (n=4).

The following orthogonal contrasts are:

a) T1 vs. T2 to T8 (this contrast check the effect of N deficiency in maize plants)

b) T2 vs. T3 to T8 (this contrast check the effect of new production technology)

c) T3 and T4 vs T5 to T8 (this contrast check the effect of Azospirilum versus the combination Mo and Azospirilum)

d) T3 vs. T4 (this contrast check the effect of Azospirilum on seed versus Azospirilum on leaf, without Mo application)

e) T5 vs T6 to T8 (this contrast check the effect of Mo versus Azospirillum, regardless of the form of application)

f) T6 vs T7 and T8 (this contrast check the effect of Azospirilum on seed versus Azospirilum, regardless of the form of application)

g) T7 vs T8 (this contrast check the effect of Azospirilum on leaf versus combination Azospirilum on seed with Mo application).

Statistical analyses were performed using the statistical Software R version 4.0.2 (R Core Team, 2021) and the ExpDes.pt package (Ferreira; Calvacanti and Nogueira, 2014).

3. RESULTS

In experiment 1, treatments affected grain yield, 100-seed weight, nitrogen use efficiency (NUE), leaf chlorophyll index (LCI), stem diameter, insertion height of the first ear (IHFE) and shoot dry matter (Table 2). Treatments did not affect harvest index ($p = 0.50$, mean = 63.56 ± 2.93 %) and plant height ($p = 0.24$, mean = 1.54 ± 0.11 m).

In experiment 2, treatments affected grain yield, 100 - seed weight, harvest index, leaf chlorophyll index (LCI), stem diameter, insertion height of the first ear (IHFE) and shoot dry matter, however, treatments did not affect NUE ($p = 0.06$, mean = 36.50 ± 3.67 kg kg^{-1}) and plant height ($p = 0.10$, mean = 1.64 ± 0.08 m).

In experiment 3, all treatments did affect grain yield, nitrogen use efficiency (NUE), harvest index, leaf chlorophyll index (LCI), stem diameter, insertion height of the first ear (IHFE), plant height and shoot dry matter, however, treatments did not affect 100 - seed weight ($p = 0.19$, mean = 22.49 ± 1.78 g).

Maize plants N deficiency was either 31% for grain yield, 4.2% LCI, 5.3 % stem diameter, 5.9% IHFE, 4.4% shoot dry matter (Exp. 1), 23.6% grain yield, 14.7% LCI, 35.2% stem diameter, 13.8% IHFE, 4.8% shoot dry matter (Exp. 2), 39.3% grain yield, 17.5% harvest index, 28.9% LCI, 23.7% plant height, 10.5% stem diameter, 10.7% IHFE (Exp. 3) greater compared when maize plants fertilized of nitrogen. However, 3.24% shoot dry matter was increased with N deficiency in experiment 3 (1 vs T2 to T8, Table 3 and 4).

Maize plants fertilized with 140 kg ha^{-1} of nitrogen, 90 g ha^{-1} of Mo and inoculated *A. brasilense* (seed and leaf) was either 3.9% stem diameter and 5.5% shoot dry matter

(Exp.1), 8.3% for 100 seed weight, 15.3% stem diameter (Exp.2) and 5.1% NUE and 2.9% IHFE (Exp. 3) greater compared when maize plants only fertilized with 140 kg ha^{-1} of nitrogen. However, there was a reduction 6.3% for 100 - seed weight, 4.2% IHFE (Exp. 1), 10.6% IHFE, 9.3% shoot dry matter (Exp. 2), 20.5% LCI, 4.8% stem diameter and 8.5% shoot dry matter (Exp. 3) (2 vs T3 to T8, Table 3 and 4).

Maize plants only inoculated of *A. brasilense* was either 1.95% IHFE (Exp.1), 8.55% 100 – seed weight, 3.4% harvest index and 17% stem diameter (Exp.2) increased compared when maize plants combination inoculated of *A. brasilense* and fertilized with 90 g ha^{-1} of Mo. However, there was a reduction 10% for NUE, 3.8% LCI and 8.9% stem diameter (Exp.1), 5.8% harvest index and 16% LCI (Exp 3) (T3 an T4 vs T5toT8, Table 3 and 4).

The effect of *A. brasilense* on seed increased 13.4% NUE, 6.1% shoot dry matter (Exp. 1), 13.3% 100 – seed weight, 2.1% LCI (Exp. 2), 4.8% stem diameter, 8.8% shoot dry matter (Exp. 3), when compared the effect *A. brasilense* on leaf without Mo. However, there was a reduction 8.4% harvest index, 2.8% shoot dry matter (Exp. 2), 11.9% harvest index (Exp. 3) (T3 vs T4, Table 3 and 4).

The effect of Mo increased 4.4% stem diameter (Exp. 1), 17.8% grain yield, 10.4% 100 – seed weight, 11% harvest index, 8.1% stem diameter (Exp. 2), 10.3% NUE, 6.6% LCI and 2.4% shoot dry matter (Exp. 3) when compared maize plants inoculated *A. brasilense*, regardless of the form of application. However, there was a reduction 5.9% for IHFE and 7.7% shoot dry matter (Exp. 1), 21.5% grain yield, 7.2% harvest index, 3.88% stem diameter (Exp.3) (T5 vs T6 to T8, Table 3 and 4).

The effect of *A. brasilense* on seed increased 8.1% for 100 – seed weigh, 12.4% NUE, 4.7% LCI, 6% IHFE (Exp. 1), 13.4% grain yield, 8.8% shoot dry matter (Exp.2), 8.6% harvest index, 7.9% NUE, 3.7% stem diameter (Exp. 3) when compared *A.*

brasilense regardless of the form of application. However, there was a reduction 3% for shoot dry matter (Exp. 1), 5.6% LCI, 6.4% IHFE (Exp. 2) and 5.8% shoot dry matter (Exp. 3) (T6 vs T7 and T8, Table 3 and 4).

The effect of *A. brasilense* on leaf increased 5.7% for LCI (Exp. 1), 22.5% grain yield, 7.9% harvest index, 20.1% stem diameter, 21.4% IHFE, 6.7% shoot dry matter (Exp. 2) when compared *A. brasilense* on seed with Mo. However, there was a reduction 10.5% for NUE, 10.2% for shoot dry matter (Exp. 1), 12.4% harvest index and 5.1% IHFE (Exp. 3) (T7 vs T8, Table 3 and 4).

4. DISCUSSION

We wanted to understand how the combined use of chemical (N and Mo) and biological (*A. brasilense*) inputs affects the yield and N use efficiency in maize plants cultivated in the sub-humid tropical regions of Brazil. Although it is known that microorganisms favor the growth of crops, less is known about the interactions between diazotrophic (*A. brasiliense*), N rates and molybdenum in maize plants and how this influences plant growth promoting properties, yield and N use efficiency.

Notably, N is the nutrient that is most demanded by maize plants and directly affects crop development and yield (Galindo et al. 2020). Nitrogen fixation is a key service in the ecology of plants, but it is an energy-expensive process. Therefore, diazotrophs are critical in the nitrogen fixation process, but generally represent a minority among bacterial communities in plants (Somers et al. 2005; Rajendran et al. 2008; Cassán et al. 2009; Hungria et al. 2010; Cassán et al. 2014), and nitrogen fixation is a tightly regulated process that is deactivated when the fixed nitrogen is available.

In the present study, exact mechanisms of the effect of Azospirillum inoculation, nitrogen and molybdenum on the development of maize plants were not evaluated, but it is very likely that the improvement in grain yield, LCI and NUE (except experiment 2), reflected in the improvement of shoot dry matter, plant height, length of ear height. The due effect of new production technology is associated inoculated Azospirillum its known ability to promote plant growth (Hungria et al. 2010; Galindo et al. 2016; Fukami et al. 2017; Martins et al. 2018; Salvo et al. 2018). To Molybdenum, for being a constituent of enzymes (nitrate reductase - NR and glutamine synthetase - GS) and to nitrogen, which enables plant development.

The growth-promoting mechanisms due to *A. brasilense* may have improved the plants' ability to exploit the soil more efficiently, as indicated in studies using *A. brasilense* (Martins et al. 2018; Fukami et al. 2018a; Fukami et al. 2018b, Leite et al. 2019, Zeffa et al. 2019). Furthermore, the application of Mo reduces these adverse effects (N health, increased tissue nitrate concentration), plant growth and development, and thus increases crop yield (Kaiser et al. 2005; Kovács et al. 2015). Finally, the important use of nitrogen maize can be enhanced by molybdenum (Mo), as it is a constituent of enzymes that allow the assimilation of this macronutrient via biological fixation by diazotrophs. microorganisms and/or fertilization with N (Imran et al. 2019).

We also observed that the effect of *A. brasilense* on seed increased NUE and shoot dry matter (experiment 1 and 2), when compared the effect *A. brasilense* on leaf without Mo. The stimulation of plant root growth by *A. brasilense* induces an increase in the water absorption and nutrient acquisition rates (including N), which clearly improves the assimilation of N in the biomass and, more generally, plant growth. This capacity is mediated by the bacterial colonization of the roots and their ability to produce different phytohormones, mostly during early stages of plant development and absolve root exudates, it is known maize exudates contain carbohydrates, amino acids and organic acids that serve as carbon sources for bacteria in the rhizosphere (Carvalhais et al. 2011; Van Deynze et al. 2018). On the hand, foliar application of *A. brasilense* creates an unknown interaction between the leaf surface of the plant and the microorganism, which needs to be further investigated (Preininger et al. 2018). Fukami et al. (2016) reported that spray inoculation with *A. brasilense* either on leaves or soil can increase plant growth and can replace 25% of N fertilization on maize. In addition, abiotic factors such as humidity and light have a great impact, furthermore biotic factors including herbivores and the competition between colonizing microbes.

Nitrogen use efficiency is controlled by a complex set of interactions among genotype, growing environment, and agronomic management (Hirel et al. 2011; Cañas et al. 2010). In the present study, the new production technology increased NUE. According to Cormier et al. (2013), two strategies can be designed to improve the NUE: increasing the yield on a constant N supply and/or maintaining a high yield by reducing the N supply, a role that can be performed by Mo and as a consequence, helping to achieve a higher grain yield (Munareto, 2016; Galindo et al., 2019a). On the other hand, Calonego et al. (2010) discovered that the absence of Mo foliar supply made for the accumulation of nitrate in common bean leaves: this as a result of the increased nitrogen availability in the soil, which indicated the inefficiency of nitrogen assimilation of plants in the absence of Mo. Srivastava (1997) came to a similar conclusion, stating that in molybdenum-deficient plants, nitrate-reductase activity is often reduced, which results in the buildup of a high concentration of NO_3^-. Mo deficiency likely resulted in an unbalanced nitrogen metabolism, so nitrogen metabolism is seen as a cycle that can be affected by a plant's Mo status (Hu et al., 2002, Yu et al., 2006). Nitrate accumulation in crop plants due to molybdenum deficiency might have serious consequences for human health. Excess nitrate consumption can increase the risk of cancer in adults and causes serious health damage especially in children. (Sanchez-Echaniz et al., 2001)

Due to the several mechanisms reported to promote plant growth, Bashan and de-Bashan (2010) proposed the “theory of multiple mechanisms” in which the bacterium leads to a cumulative or sequential pattern of effects, resulting from mechanisms occurring simultaneously or consecutively. According to these authors, there is no single mechanism involved in the promotion of plant growth by specie *A. brasilense* but a combination of a few or many mechanisms determined by inoculant-plant-environment interactions. (Galindo et al. 2020).

5. CONCLUSIONS

The inoculation with *A. brasilense*, N and Mo, showed potential to be used, because in our study the combination increased 5.1% NUE. However, there was no increase in grain yield. On the other hand, Mo and N increased 17.8% grain yield when compared maize plants inoculated *A. brasilense*.

Therefore, studies conducted under conditions that challenge the maize plants in terms of stress, biotic or/and abiotic, are necessary to better understand the role of Mo, applied alone or in combination with growth-promoting bacteria in the sub-humid tropical regions of Brazil

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Credit authorship contribution statement:

Heder Braun: Conceived and designed the experiment, and wrote and reviewed the original draft of the manuscript;

Marcelo Marinho Viana: Performed the experiments in field, collected all samples, collected data in field and performed analysis in the laboratory and wrote and reviewed the original draft of the manuscript.

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Table 2. Mean value (± SD) of grain yield, 100-seed weight, harvest index, nitrogen use efficiency (NUE) and leaf chlorophyll index (LCI), experiments 1,2 and 3 in the maize under field conditions in research farm, Maranhão State University, Maranhão State, Brazil.

Treatments	Experiment 1				Experiment 2				Experiment 3			
	Grain Yield	100-seed weight	NUE	LCI	Grain Yield	100-seed weight	Harvest Index	LCI	Grain Yield	Harvest Index	NUE	LCI
	t/ha^{-1}	g	kg kg $^{-1}$	-	t/ha^{-1}	g	%	-	t/ha^{-1}	%	kg kg $^{-1}$	-
1	3,6±0,2	30,0±2,1	-	51,5±1,3	4,3±0,4	27,3±1,7	68,4±1,9	54,0±3,7	1,8±0,2	40,9±1,6	-	73,8±3,6
2	4,8±0,7	32,8±1,2	36,0±2,2	52,9±2,6	6,0±0,4	25,7±1,5	66,5±2,5	58,5±2,6	3,04±0,7	50,2±2,9	23,6±2,1	111,2±2,8
3	5,2±0,1	30,9±1,4	37,1±1,2	52,5±2,6	5,8±0,6	31,3±1,6	66,6±2,5	58,6±2,4	3,2±0,8	44,6±3,5	20,7±2,2	88,9±2,5
4	4,8±0,7	32,2±1,3	32,7±2,1	52,2±1,5	5,7±0,2	27,6±2,3	72,1±2,7	62,0±2,6	2,9±0,6	50,6±2,4	22,5±2,7	88,9±2,7
5	5,3±0,5	29,8±1,2	37,6±3,7	54,6±1,2	6,3±0,6	29,8±1,6	73,7±2,4	60,7±2,1	2,6±0,7	48,0±1,5	17,0±2,2	95,4±2,9
6	5,8±0,9	32,6±1,1	43,8±2,3	56,6±1,2	5,6±0,4	25,6±1,5	66,7±1,6	59,8±1,9	3,6±0,5	54,6±2,3	24,2±1	103,1±3,7
7	5,3±0,7	29,2±1,4	36,4±3,2	55,5±2,2	5,5±0,4	27,2±2,2	67,7±1,6	63,9±2,4	3,2±0,2	47,0±2,6	22,6±1,7	105,6±2,4
8	5,4±0,8	30,8±1,4	40,3±2,6	52,3±1,5	4,3±0,1	27,2±1,3	62,3±1,8	62,4±2,2	2,9±0,3	52,8±1,6	20,8±2,3	102,5±2,8
Contrast						**p value**						
1 vs 2 to 8	0.001	0,128	-	0,026	<0,001	0,670	0,720	<0,001	<0,001	<0,001	-	<0,001
2 vs 3 to 8	0,165	0,027	0,195	0,326	0,104	0,014	0,175	0,068	0,910	0,660	0,051	<0,001
3,4 vs 5 to 8	0,140	0,131	<0,001	0,004	0,152	0,013	0,048	0,224	0,993	0,009	0,627	<0,001
3 vs 4	0,419	0,245	0,030	0,810	0,733	0,005	<0,001	0,077	0,583	0,002	0,218	0,974
5 vs 6 to 8	0,529	0,212	0,104	0,908	<0,001	0,004	<0,001	0,378	0,074	0,023	<0,001	<0,001
6 vs 7,8	0,240	0,006	0,003	0,022	0,015	0,143	0,237	0,043	0,116	0,005	0,055	0,519
7 vs 8	0,897	0,136	0,055	0,018	0,001	0,957	0,003	0,400	0,517	0,003	0,223	0,085

Table 3. Mean value (± SD) of plant height, stem diameter, insertion height of the first ear (IHFE) and shoot dry matter, experiments 1,2 and 3.

Treatments	Experiment 1			Experiment 2			Experiment 3			
	Stem diameter	IHFE	Shoot Dry Matter	Stem diameter	IHFE	Shoot Dry Matter	Plant height	Stem diameter	IHFE	Shoot Dry Matter
	………… cm ………….		kg ha^{-1}	………… cm ………….		kg ha^{-1}	……..………… cm ………..…….			kg ha^{-1}
1	11,09±0,3	89,18±3,6	3575,2±44,5	11,06±3,9	46,70±2,1	3867,08±78,8	1,11±0,18	15,1±0,3	50,9±4,1	4670,8±25,8
2	11,23±0,4	97,60±1,1	3536,7±55,1	14,09±2,8	58,15±1,9	4361,7±89,5	1,46±0,20	17,3±0,6	54,9±3,1	4875,6±20,9
3	10,96±0,4	95,09±3,7	3865,11±59,8	19,32±2,3	51,32±3,5	3985,1±49,9	1,50±0,10	17,1±0,2	58,4±2,5	4658,4±53,3
4	10,92±0,5	97,16±1,2	3644,1±81,5	18,84±2,1	53,50±2,8	4043,7±72,4	1,41±0,11	16,3±0,6	57,5±3,2	4279,5±37,7
5	12,63±0,7	89,47±2,6	3574,2±65,9	15,84±3,6	57,68±1,2	4100,6±47,3	1,48±0,10	16,2±0,2	58,1±2,9	4546,6±44,7
6	12,16±0,6	98,78±3,6	3776,3±70,0	17,68±2,6	50,81±2,2	4229,9±72,5	1,45±0,06	17,3±0,7	55,6±2,2	4270,6±59,4
7	12,35±0,5	91,47±2,3	3700,1±65,5	18,68±1,7	60,56±3,6	3991,5±77,7	1,47±0,11	16,4±0,6	56,1±2,1	4546,1±79,6
8	11,72±0,7	94,12±2,4	4079,9±87,7	14,93±2,2	47,62±3,3	3723,8±85,9	1,43±0,15	16,9±0,1	58,9±1,7	4492,4±72,7
Contrast					**p value**					
1 vs 2 to 8	0,025	<0,001	0,003	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
2 vs 3 to 8	0,044	0,041	<0,001	0,008	0,003	<0,001	0,901	0,057	0,031	<0,001
3,4 vs 5 to 8	<0,001	0,037	0,377	0,023	0,126	0,181	0,924	0,960	0,427	0,814
3 vs 4	0,913	0,302	<0,001	0,822	0,241	0,043	0,213	0,042	0,544	<0,001
5 vs 6 to 8	0,057	0,003	<0,001	0,338	0,004	0,172	0,577	0,045	0,329	<0,001
6 vs 7,8	0,666	<0,001	0,017	0,525	0,048	<0,001	0,969	0,053	0,154	<0,001
7 vs 8	0,079	0,189	<0,001	0,026	<0,001	<0,001	0,594	0,179	0,066	0,134

Application methods of *Azospirillum brasilense* associated with nitrogen and molybdenum on ecophysiological parameters on maize plants cultivated in the sub-humid tropical regions of Brazil

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CAPÍTULO III

Application methods of *Azospirillum brasilense* associated with nitrogen and molybdenum on ecophysiological parameters on maize plants cultivated in the sub-humid tropical regions of Brazil

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Highlights

- The combination of *A. brasilense*, Mo and N increased 12,68% photosynthetic nitrogen use efficiency;
- Application methods *A. brasilense* on seed average increased 18.5% photosynthetic nitrogen use efficiency;
- Molybdenum and nitrogen increased at 6.73% photosynthetic nitrogen use efficiency

ABSTRACT

Nitrogen (N) plays a vital role in plant growth, as it is a constituent of nucleotides and proteins. However, the effects induced by combination molybdenum (Mo), N and *Azospirillum brasilense* on photosynthetic efficacy have not been investigated. The objective this is study, evaluated the of inoculation methods *A. brasilense*, nitrogen and molybdenum on ecophysiological parameters on maize plants cultivated in the sub-humid tropical regions of Brazil. Three field trials were conducted in a randomized complete block design with four replicates and eight treatment. The treatments were 140 kg ha^{-1} of N, 90 g ha^{-1} of Mo and inoculation methods of *A. brasilense* (seed and leaf). In experiment 1, treatments 20 days after top dressing did affect stomatal conductance (g_s), intercellular CO_2 concentration (Ci), leaf chlorophyll index (LCI) and photosynthetic nitrogen use efficiency (PNUE). In experiment 2, treatments did affect Ci, LCI and PNUE 20 and 40 days after top dressing. In experiment 3, treatments did affect A, g_s, 20 days after top dressing, Ci, LCI, PNUE. The average of the experimental results showed a positive effect of the application N, Mo and *A. brasilense* compared to only N or without N. This combination increased PNUE 12,68%. Therefore, it is evident from this study that the application of *A. brasilense* in combination with N and Mo improves N retention, N uptake, photosynthetic apparatus and PNUE of corn grown in sandy soils with poor physical and chemical properties.

Keyswords: *Zea mays*; photosynthesis; ecophysiological; biological nitrogen fixation, nitrogen fertilization.

1. INTRODUCTION

Maize (*Zea mays* L.) is a cereal widely cultivated throughout the world due to the important role it plays in the different world agribusiness supply chain. The culture stands out as a source of products for human and animal nutrition (Santos et al, 2017). Nitrogen (N) is typically a limiting nutrient for the production of most of the cereals, and most importantly for maize (Yue et al. 2021), because it is an essential component of all proteins and enzymes, nucleic acids that make up DNA, and chlorophyll that enables the process of photosynthesis in plants (Leghari et al. 2016), moreover nitrogen (N) plays a vital role in plant growth and productivity.

N supply has a significant impact on photosynthesis by affecting leaf structure and nitrogen distribution in the leaf (Mu and Chen, 2021). Photosynthesis, or the conversion of light energy into chemical energy, control a variety of physiological, biochemical and molecular processes that substantially contribute to the plant growth and development. (Sener et al. 2016, Imran et al. 2019).

Physiologically, the relationship between leaf N and net photosynthetic rate is well documented (Chen et al, 2016). However poor N management has contributed to increased N losses by ammonia (NH_3) volatilization, nitrate (NO_3) leaching and nitrous oxide (N_2O) emissions, which have both economic and environmental consequences (Linquist et al. 2013; Abalos et al. 2014; Martins et al. 2015, 2017; Galindo et al. 2020). These problems indicate the need for a new strategy or alternative, to increased production on maize, nitrogen use efficiency and photosynthetic capacity per unit leaf N.

Many studies have shown molybdenum (Mo) plays an essential role in many biochemical processes in plants and is a constituent of nitrogenase (Fageria et al., 2011; Marschner, 2012; Kovács et al, 2015; Barbosa et al, 2021), the enzyme that catalyzes the reduction of atmospheric nitrogen into ammonia, and a cofactor in nitrate reductase (Silva et a., 2017). Mo is less available to plants in acidic soils (typical tropical soils). The limitations of molybdenum in acidic soils indicate the need for strategy Mo foliar supply for plants (Calonego et al., 2010, Kovács et al., 2015).

The use of biological techniques such as plant growth-promoting bacteria (PGPB) can represent a sustainable alternative for cereal growth in sub-humid tropical regions of Brazil. (Martins et al., 2018; Galindo et al., 2019). In this sense, inoculation and application of PGPB especially *A. brasilense* is an important strategy in maize cultivation. Several benefits have been attributed to the inoculation with *A. brasilense*, including the supply of N by the biological nitrogen fixation (BNF) process (Hungria et al., 2010; Fukami et al., 2017), stimulation of root growth (Lin et al., 2012; Santi et al., 2013), phosphate solubilization (Rodriguez et al., 2004), and increased tolerance to abiotic (Bulgarelli et al., 2013, Fukami et al., 2018) and biotic (Correa et al., 2008) stresses. In the case of the Brazilian commercial strains of *A. brasilense* Ab-V5 and Ab-V6, the main effects have been attributed to the production of phytohormones (Hungria et al., 2010; Fukami et al., 2017).

The most frequent technique of inoculation is via seeds, however, another strategy of application of the PGPB is the foliar, where the PGPB are sprayed over the leaves of the cultivation. The foliar application creates an unknown interaction between the plant leaf surface and the microorganism, which needs to be further investigated (Preininger et al., 2018; Efthimiadou et al., 2020). It should also be noted that the efficiency of inoculation via seeds can be affected by the use of chemicals during treatment, such as fungicides and insecticides, which reinforces the importance of researches and analysis to use supplementary forms of inoculation (Boleta et al., 2020).

The need for agriculture that maintains ecosystems and biodiversity, the search for solutions that combine increased production and increasingly sustainable agricultural practices is necessary. Some studies analyze crop production, however, the interaction of the ecophysiological system is still not very explored. Plants can look healthy and no productive. Therefore, the objectives of this study were to evaluate the effects of the combined use of chemical (N and Mo) and biological (*A. brasiliense*) factors on physiological parameters on maize plants grown in the sub-humid tropical regions of Brazil. It was hypothesized that co-application N, Mo, and *A. brasilense* would enhance the photosynthetic efficiency of maize plants.

2. MATERIALS E METHODS

2.1. Field site description

Three field trials were conducted in the municipality of São Luís (2°30'S, 44°18'W, 24 m above sea level), State of Maranhão, Brazil. The firts trial was stablished from February 2018 to May 2018 (Experiment 1), second from October 2018 to January 2019 (Experiment 2) and third from March 2019 to July 2019 (Experiment 3). The region has a hot, semi-humid, equatorial climate, with mean annual rainfall of 2,200 mm and two well-defined seasons: a rainy season from January to June, and a dry season with pronounced water deficits from July to December. The climatic conditions during the trials are shown in Figure 1.

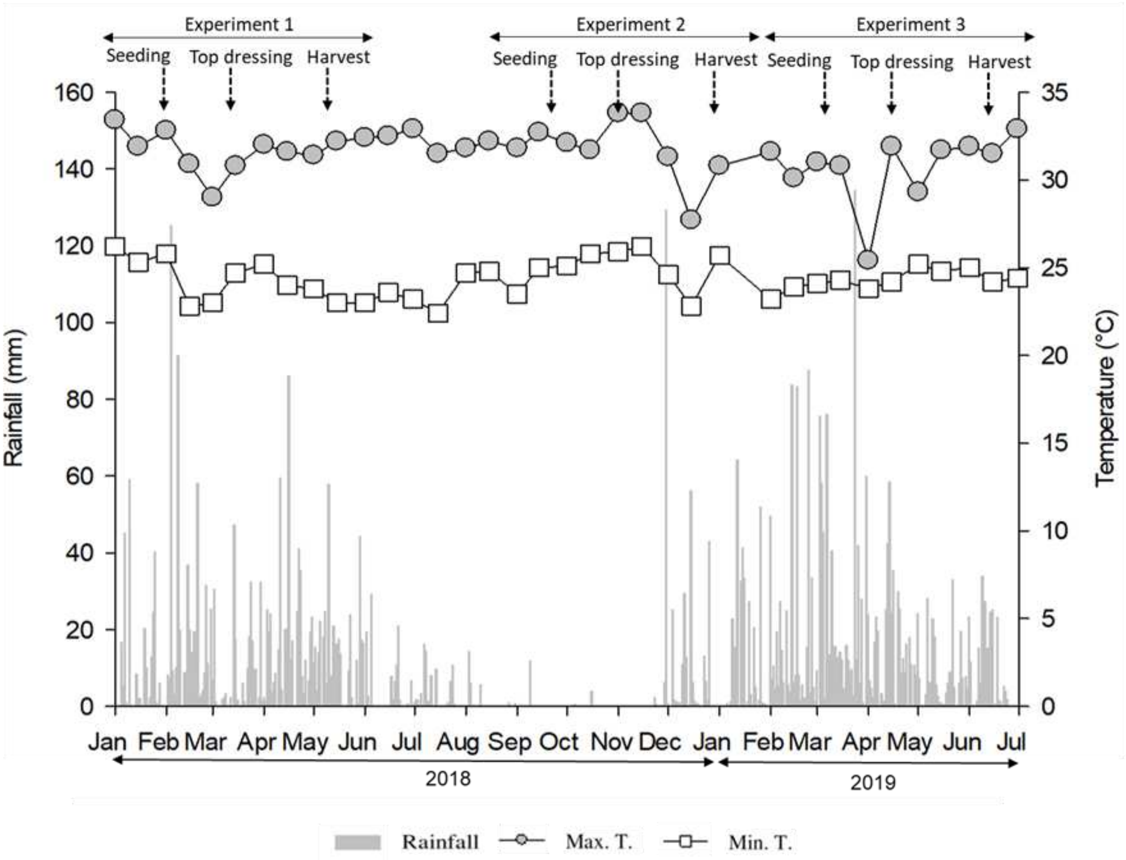

Figure 1. Rainfall (mm) and maximum and minimum temperatures obtained from the data base National Institute of Meteorology of Brazil (INMET) during the corn cultivation (all seasons) in the period from January 2018 to July 2019.

The trials were conducted under a no-tillage system. The field had not been cultivated with any agricultural crop for at least 10 years (2003-2013). Maize and cowpea (*Vigna unguiculata* L. Walp) had been cultivated in the field after 2013. The remaining straws from previous maize and cowpea crops were left on the soil surface. The soil in this area is a Typic Hapludult according to USDA soil taxonomy (Soil Survey Staff, 1999), with pH in CaCl2 (soil:solution ratio of 1:2.5) = 5.3; organic matter = 5 g dm^{-3}; P (resin) = 31 mg dm^{-3}; K^{+} = 1.7 mmolc dm^{-3}; Ca^{2+} = 24 mmolc dm^{-3}; Mg^{2+} = 15 mmolc dm^{-3}; H^{+} + Al^{3+} = 19 mmolc dm^{-3}; sum of bases = 40.7 mmolc dm^{-3}; cation exchange capacity at pH 7 = 59.7 mmolc dm^{-3}; base saturation = 68%; and coarse sand = 260 g kg^{-1}, fine sand = 640 g kg^{-1}, silt = 20 g kg^{-1}, and clay = 120 g kg^{-1} and texture sandy loam.

No mechanical soil preparation was carried out before the maize sowing. At 30 days before sowing in 2017 was applied 1.0 Mg ha-1 of dolomitic limestone (32% CaO, 15% MgO, and total neutralizing power of 91%) without incorporation.

2.2. *Experimental design, treatment, and field management*

The experimental design was a randomized complete block design with four replicates and eight treatment (Table 1). There were one N rate 140 kg ha^{-1}, one Mo rate 90 g ha^{-1} and inoculation methods of *A. brasilense* (seed and leaf). Each plot consisted of four 4 m-long rows spaced 0.8 m apart. The outer rows of each plot and 0.5 m from each end of the rows were used as borders. Treatment arrangement of all seasons 2018/2019 with *Azospirillum brasilense* (seeds and leaf), nitrogen and molybdenum rates.

Table 1. Treatment arrangement of all seasons 2018/2019 with *Azospirillum brasilense* (seeds and leaf), nitrogen and molybdenum rates.

Treatments	Nitrogen *(kg/ha^{-1})*		Molybdenum *(g/ha^{-1})*	Azospirillum seeds *(ml/ha^{-1})*	Azospirillum leaf *(ml/ha^{-1})*
	Sowing	Topdressing	Topdressing	Sowing	Topdressing
1	-	-	-	-	-
2	40	100	-	-	-
3	40	100	-	100	-
4	40	100	-	-	200
5	40	100	90	-	-
6	40	100	90	100	-
7	40	100	90	-	200
8	40	100	90	100	200

At sowing furrow, 40 kg ha^{-1} of N (granulated urea), 80 kg ha-1 of P_2O_5 (simple superphosphate), and 100 kg ha^{-1} of K_2O (potassium chloride) were applied in all trials. Seeds of the maize simple hybrid PIONNER 30F35® was sown at a density of four seeds per meter (5 plants m^{-2}). Seedling emergence occurred between four and six days after sowing for all trials. All plots were irrigated after the sowing with 15 mm of water, using a drip tape system and 2-day intervals, for a good and uniform seedling emergence. Approximately every 3 days without rainfall after seedling emergence, the plots were irrigated with 15 mm of water (2-hour irrigation). The plants were irrigated when there was no or low rainfall in the previous week. When there was a need to irrigate, we use the drip tape with flat emitters inside, 16mm diameter, thickness from 0.6mm, spacing from 20cm.

Topdressed N (100 kg ha^{-1} of N) (granulated urea) was manually applied when the maize plants had six leaves completely expanded (V6) and was evenly applied along the furrows at 10 cm away from the plants. All plots were immediately irrigated after N fertilizer with approximately 15 mm of water to minimize ammonia volatilization. Topdressed Mo (90 g ha^{-}1) (ammonium molybdate) applied when the maize plants had six leaves completely expanded (V6) by spraying the solution, using a sprayer equipped with one cone nozzle (XR 11002; Teejet®, Wheaton, USA), with a flow rate of 200 L ha-1 of water. A plastic sheet was used to protect adjacent plots from unwanted spray drift.

The seeds and leaves of maize plants were inoculated with *Azospirillum brasilense*, using the commercial strains Ab-V5 and Ab-V6 (Nitro 1000 Gramíneas; Nitro 1000, Cascavel, Brazil) at rates of 100 mL and 200 mL of the liquid inoculant per hectare (2×108 CFU [colony forming unity] mL-1), respectively. These strains have not been used in sub-humid tropical regions of the State of Maranhão, Brazil. The seeds were inoculated one hour before sowing the crop, and the leaves were inoculated at V6 stage by spraying the solution, using a sprayer equipped with one cone nozzle (XR 11002; Teejet®, Wheaton, USA), with a flow rate of 200 L ha-1 of water. A plastic sheet was used to protect adjacent plots from unwanted spray drift.

Deltamethrin (5 g ha-1 active ingredient) was applied to control fall armyworm (Spodoptera frugiperda), when needed. Weeds were controlled by manual hoeing until the N topdressing application.

2.3. *Measurements collected*

The features were evaluated at the beginning of tasseling stage, (approximately 20 days after topdressed) and milk stage (approximately 40 days after topdressed). The photosynthetic CO_2 assimilation (A), stomatal conductance (g_s) and intercellular CO2 concentration (Ci) was measured with a portable LI-COR gas exchange system LI-6400 (LI-COR, Lincoln, USA), operating as an open system. The measurements were conducted under a clear and sunny day, and with photosynthetically active radiation of 1,500 μmol m-2 s-1. The average leaf temperature inside the chamber was 33.6±0.2 °C, and a CO_2 concentration of 400±1 μmol CO_2 mol s^{-1}. At least two measurements were performed on third leaf above the ear leaf in two plants, and more were performed when the first two values presented high variation. The average of the four readings was taken as a replicate. The leaf chlorophyll index (LCI), was measured indirectly using a portable non-destructive chlorophyll meter SPAD-502 (Minolta Co., Japan).

The same leaves that had been used for the photosynthetic CO_2 assimilation measurements were harvested. Subsequently, 20 leaf disks of approximately 1.8 cm^2 were taken and the remaining leaf tissues were oven-dried at 70 °C to a constant weight to calculate the specific leaf area (leaf area per unit of dry weight). Total N concentration in leaves were analyzed using the Kjeldahl method (Tedesco et al., 1995). In addition, the photosynthetic N use efficiency (PNUE) was calculated by dividing photosynthetic CO_2 assimilation by specific leaf N (N concentration per unit leaf area) (Sinclair; Horie, 1989; ; Rodríguez-López et al., 2014).

2.4. *Statistical analyses*

All data were initially tested for homogeneity of variance with O'Neill and Mathews test and normality using the Shapiro and Wilk test. When the effect of treatment was significant

($p < 0.05$), a set of seven orthogonal contrasts (below) was analysed. The precise p values of these contrasts were reported. Values were reported as means ± SD.

Orthogonal contrasts:

a) T1 vs. T2 to T8 (this contrast check the effect of N deficiency in maize plants)

b) T2 vs. T3 to T8 (this contrast check the effect of new production technology)

c) T3 and T4 vs T5 to T8 (this contrast check the effect of Azospirilum versus the combination Mo and Azospirilum)

d) T3 vs. T4 (this contrast check the effect of Azospirilum on seed versus Azospirilum on leaf, without Mo application)

e) T5 vs T6 to T8 (this contrast check the effect of Mo versus Azospirillum, regardless of the form of application)

f) T6 vs T7 and T8 (this contrast check the effect of Azospirilum on seed versus Azospirilum, regardless of the form of application)

g) T7 vs T8 (this contrast check the effect of Azospirilum on leaf versus Azospirilum on seed with Mo application).

The data were analysed using the statistical software R v. 3.6.1 (R Core Team, 2019) with the RStudio Version 1.2.5019 interface (RStudio Team, 2019) and the ExpDes.pt package (Ferreira; Calvacanti and Nogueira, 2014).

3. RESULTS

In experiment 1, treatments were evaluated at 20 days after topdressing (20 DATD) did affect stomatal conductance (g_s), intercellular CO_2 concentration (Ci), leaf chlorophyll index (LCI) and photosynthetic nitrogen use efficiency (PNUE). Treatments were evaluated at 40 days after topdressing (40 DATD) did affect Ci, LCI, PNUE. Treatments did not affect CO_2 assimilation (A) ($p = 0.06$, mean = 43.17 ± 3.66), 20 DATD and CO_2 assimilation ($p = 0.93$, mean =44.67 ± 3.42) and stomatal conductance ($p = 0.62$, mean = 0.34 ± 0.05) 40 DATD).

In experiment 2, treatments were evaluated at 20 DATD did affect Ci, LCI and PNUE and treatments were evaluated at 40 DATD did affect PI_{abs} (40 DATD). Treatments did not affect CO_2 assimilation (, p = 0.124, mean = 51.1 ± 2.62) and stomatal conductance (, p = 0.39, mean = 0.55 ± 0.13) at 20 DATD. CO_2 assimilation (p = 0.09, mean = 26.22 ± 2.81) and stomatal conductance (p = 0.57, mean = 0.59 ± 0.14) at 40 DATD.

In experiment 3, treatments were evaluated at 20 DATD did affect A, g_s, Ci, LCI, PNUE and treatments were evaluated at 40 DATD did affect PNUE Treatments did not affect CO_2 assimilation (p = 0.06, mean = 23.3 ±2.52) and stomatal conductance (p =0.65, mean = 0.6 ± 0.68) at 40 DATD.

About the contrasts, maize plants N deficiency (T1) was either at 20 DATD in experiment 1 (16.4% LCI, 13.7 % PNUE), experiment 2 (16.5% Ci, 14.5% LCI), experiment 3 (2% Ci, 26.1% LCI) and at 40 DATD in experiment 1 (18.5% LCI), experiment 2 (7.2% Ci, 8.9% LCI, 9.9 PNUE) and experiment 3 (7.61% Ci, 21.6% LCI) greater compared when maize plants fertilized of nitrogen. However, at 20 DATD in experiment 1 (4.4% Ci), experiment 3 (8.6% A, 22.1% g_s) and at 40 DATD experiment 1 (8.3% Ci, 5.3% PNUE), experiment 3 (6.5% PNUE) was increased with N deficiency (T1 vs T2 to T8, Table 2, 3, 4 and 5).

Maize plants fertilized with 140 kg ha^{-1} of nitrogen, 90 g ha^{-1} of Mo and inoculated *A. brasilense* (seed and leaf) was either at 20 DATD in experiment 1 (18.2% gs,), experiment 2 (3.3% LCI) and at 40 DATD in experiment 1 (18.8% Ci, 5.8% LCI), experiment 2 (2% LCI, 11.7% PNUE) and experiment 3 (10.2% LCI, 9.6% PNUE) greater compared when maize plants only fertilized with 140 kg ha^{-1} of nitrogen. However, there was a reduction at 20 DATD in experiment 1 (2.6% Ci, 6.9% LCI), experiment 2 (13.3% PNUE), experiment 3 (10.6% Ci, 20.6% LCI) and at 40 DATD in experiment 1 (12.9% PNUE), experiment 2 (3% Ci), experiment 3 (7.3% Ci) (T2 vs T3 to T8, Table 2, 3, 4 and 4).

Maize plants only inoculated of *A. brasilense* was either at 20 DATD in experiment 1 (3.4% LCI) and at 40 DATD in experiment 1 (9.3 Ci) and experiment 3 (10.2% LCI) greater compared when maize plants combination inoculated of *A. brasilense* and fertilized with 90 g ha^{-1} of Mo. However, there was a reduction at 20 DATD in experiment 1 (6.6% Ci, 2.7% PNUE), experiment 2 (10.6% Ci), experiment 3 (3.9% Ci, 16.2% LCI) and at 40 DATD in experiment 1 (0.5% PNUE), experiment 2 (0.1% Ci, 8.8% PNUE) (T3 and T4 vs T5 to T8, Table 2, 3, 4 and 5).

The effect of *A. brasilense* on seed increased at 20 DATD in experiment 1 (7.9% Ci and 7.8% PNUE), experiment 2 (11.7% Ci and 17% PNUE), experiment 3 (9.7% Ci) and at 40 DATD in experiment 1 (30.7% PNUE) respectively, when compared the effect *A. brasilense* on leaf without Mo. However, there was a reduction at 20 DATD in experiment 1 (27.2% Gs), experiment 3 (16.9% PNUE) and 40 DATD in experiment 1 (10.8% LCI), experiment 2 (3.2% Ci, 12.6% PNUE) and experiment 3 (9% PNUE) (T3 vs T4, Table 2, 3, 4 and 5).

The effect of Mo increased at 20 DATD in experiment 1 (3.9 Ci, 17.2% PNUE), experiment 3 (7% A, 13% Gs, 6.7% LCI and 4% PNUE) and 40 DATD in experiment 1 (10% Ci, 10.5% PNUE), experiment 2 (3.1% Ci, 8.7% PNUE), experiment 3 (14.3% LCI) when compared maize plants inoculated *A. brasilense*, regardless of the form of application. However, there was a reduction at 20 DATD in experiment 3 (6.1% Ci) and at 40 DATD in experiment 1 (10.6% LCI), experiment 3 (5.1% PNUE) (T5 vs T6T8, Table 2, 3, 3 and 5).

The effect of *A. brasilense* on seed increased at 20 DATD in experiment 2 (5.4% PNUE), experiment 3 (3.2% Ci) and 40 DATD in experiment 1 (6.35% Ci and 7.1% PNUE), experiment 2 (2.3% Ci and 4% LCI) when compared *A. brasilense* regardless of the form of application. However, there was a reduction at 20 DATD in experiment 1 (27.2% gs, 46.7% PNUE) and 40 DATD in experiment 2, 3 (12.7% and 14% PNUE) respectively (T6 vs T7 and T8, Table 2, 3, 4 and 5).

The effect of *A. brasilense* on leaf increased at 20 DATD in experiment 1 (17.1% gs and 14.6% Ci), experiment 2 (11.3% Ci and 15.6% PNUE), experiment 3 (14.5% gs, 2.4% Ci) and 40 DATD in experiment 1 (19.6% PNUE), experiment 3 (4.3% Ci and 7.4% LCI) when compared *A. brasilense* on seed with Mo. However, there was a reduction at 40 DATD in experiment 1 (7.6% Ci), experiment 2 (3.4% LCI and 16.6% PNUE) (T7 vs T8, Table 2, 3, 4 and 5).

6. DISCUSSION

In the present study, we wanted to understand how the combined use of chemical and biological inputs affects the Photosynthesis, PNUE and efficiency of photochemistry in maize plants cultivated in the sub-humid tropical regions of Brazil. Nitrogen (N) is an essential but generally limiting nutrient for biological systems (Udvardi et al. 2021) Bacteria of the genus *Azospirillum,* native to the soil, and its association with plants has a series of beneficial responses such as increasing the synthesis of photosynthetic pigments (chlorophyll) (Boleta et al., 2020; Bulegon et al., 2017). Molybdenum (Mo) is an essential micronutrient for higher plants and plays an important role in the photosynthetic process due to its major involvement in the chlorophyll biosynthesis pathway (Yu et al., 2006) and in the chloroplast configuration and ultrastructure (Yu et al., 2005).

PNUE is a ratio determined simultaneously by numerator (A) and denominator (foliar nitrogen concentration) (Gou et al. 2016). Photosynthesis is an enzyme-mediated process that largely depends on Ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco enzyme). Rubisco enzyme, as the key enzyme that accounts for up to 30% of total leaf nitrogen, is often positively correlated to nitrogen availability and could directly influence photosynthetic capacity (Zhou, 2005; Pooter and Evans, 1998; Sims et al., 1998). In the present study the feature PNUE is affected in all experiments in 20 and 40 DAAT. This suggested that maize plants have a higher light energy convention and electron transport rate. Maize plants invested

N to Rubisco. The CO_2 concentration is higher in the vicinity of Rubisco in maize plants. Thus, a lower amount of Rubisco is sufficient to achieve high photosynthetic rate (Seemann et al., 1984; Sage et al., 1987; Mu and Chen, 2021).

Nitrogen availability in leaves is strongly positively correlated with photosynthesis, chloroplast structure, and chlorophyll content (Liu et al., 2018). Mo plays a key role in N absorption and assimilation pathways through enzymatic regulation (Campbell, 2001; Schwarz et al., 2009), indicating that Mo application may trigger many nitrogen-dependent physiological, biochemical and molecular processes through effective N acquisition, leading to plant N overall response to availability. Our results indicated that the effect of the new production technology on PNUE at 20 DAAT is not improved when compared with maize plants only fertilized nitrogen. Probably the Mo leaf spray had a low translocation, due to the acidic pH (<5.2) in soil, resulting in low Mo phloem mobility and xylem certainly affected Mo absorption (Valenciano et al., 2011). Campo and Hungria (2002) reported that Mo foliar spraying presents rapid translocation, after application of five days, they observed a higher concentration of molybdenum in the nodules of soybean plants. On the other hand, the effect of combined use of Mo and N leads increase of 10% in PNUE when compared maize plants inoculated *A. brasilense*.

Chlorophyll content is strongly correlated with light, PNUE and the final growth and development of vegetative organs (Liu et al., 2018). In addition, chlorophyll a and b play a key role in photosynthetic CO_2 assimilation, participating in the absorption, transfer and conversion of light energy (Sui et al., 2010). In the present study, the leaf chlorophyll index increased in almost all experiments, due to the positive relationship with N and Mo supply. Suggesting that combined application N and Mo induced significant increases in the chlorophyll a and b contents. Irman et al., 2019 indicates that Mo might have an essential role in the chlorophyll biosynthesis. Yu et al. (2006) report that in their essays the chlorophyll biosynthesis process, the transformation of δ-aminolaevulinic acid (ALA) into uroporphyrinogen III (Uro III), intermediates in the chlorophyll biosynthesis pathway, occurs in the chloroplast matrix instead of the membranes thylakoids and in the absence of Mo the transformation process is blocked and results in a decrease in the chlorophyll content in the leaf tissues.

Generally, Gs is often used to denote the extent of opening of stomata and Ci to indicate the assimilation ability of mesophyll cells for CO_2 in plants, which are important indices of the photosynthesis of plants and have close relationships with CO_2 assimilation (Farquhar and Sharkey 1982). In the present study, the disparities in results may have been a function of series environmental factors including water and nutrient status, light, CO_2 levels and temperature.

According to the set of results, probably the increase in the efficiency of the use of photosynthetic nitrogen (PNUE) induced by Mo and *A. brasilense* occurred with the improvement of the photosynthetic apparatus and also by the better absorption and assimilation of N.

7. CONCLUSIONS

The average of the experimental results showed a positive effect of the application N, Mo and *A. brasilense*. This combination increased PNUE 12,68%. Therefore, this study showed that the application of *A. brasilense* in combination with N and Mo improves N retention, N uptake, photosynthetic apparatus and PNUE of corn grown in sandy soils with poor physical and chemical properties.

However, further studies should be carried out to explore the molecular basis of Mo-induced changes in the photosynthetic apparatus.

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Credit authorship contribution statement:

Heder Braun: Conceived and designed the experiment, and wrote and reviewed the original draft of the manuscript;

Marcelo Marinho Viana: Performed the experiments in field, collected all samples, collected data in field and performed analysis in the laboratory and wrote and reviewed the original draft of the manuscript.

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Table 2. Effect of treatments evaluated at 20 days after topdressing, mean value (± SD) of CO_2 assimilation (A, µmol $CO_2.m^{-2}.s^{-1}$), stomatal conductance (g_s mol $m^{-2}.s^{-1}$), intercellular CO_2 concentration (Ci, µmol CO_2/ mol^{-1}) and leaf chlorophyll index (LCI). Experiments 1,2 and 3.

Treatments	Experiment 1			Experiment 2		Experiment 3			
	g_s	Ci	LCI	Ci	LCI	A	g_s	Ci	LCI
1	0,42±0,07	143,9±2,6	41,4±1,9	105,0±3,0	51,1±2,4	40,2±0,9	0,67±0,04	151,9±4,4	70,2±3,5
2	0,38±0,03	147,6±2,9	51,4±1,7	126,2±2,9	57,2±3,7	36,9±2,0	0,59±0,08	168,8±2,9	112,0±2,6
3	0,37±0,06	141,9±3,3	51,7±2,1	136,5±1,4	60,5±3,3	35,0±2,3	0,55±0,05	157,6±2,5	86,3±5,5
4	0,51±0,08	131,4±2,6	49,7±1,2	122,2±5,1	59,3±3,6	37,2±1,6	0,55±0,03	143,6±2,8	87,4±3,1
5	0,53±0,11	150,8±3,3	48,3±2,3	120,8±3,9	59,8±3,9	39,6±1,7	0,60±0,02	147,2±2,4	106,9±5,1
6	0,39±0,05	143,8±2,8	47,9±2,6	127,6±3,1	58,7±3,7	36,7±2,0	0,54±0,02	152,8±4,5	101,9±4,7
7	0,55±0,05	156,3±2,7	48,1±2,9	130,7±3,9	62,1±3,7	37,8±1,1	0,55±0,08	159,7±4,0	104,1±4,4
8	0,45±0,02	133,4±3,9	49,4±2,9	115,8±3,5	61,9±3,2	36,0±3,2	0,47±0,05	155,8±2,6	93,6±4,7
Contrast				**p value**					
1 vs 2 to 8	0,297	<0,001	<0,001	<0,001	<0,001	0,009	<0,001	0,017	<0,001
2 vs 3 to 8	0,013	0,007	0,057	0,748	0,098	0,914	0,136	<0,001	<0,001
3,4 vs 5 to 8	0,160	<0,001	0,017	<0,001	0,653	0,121	0,609	0,003	<0,001
3 vs 4	0,004	<0,001	0,193	<0,001	0,619	0,156	0,947	<0,001	0,683
5 vs 6 to 8	0,106	0,002	0,911	0,075	0,592	0,032	0,019	<0,001	0,002
6 vs 7,8	0,010	0,589	0,512	0,068	0,138	0,859	0,364	<0,001	0,165
7 vs 8	0,043	<0,001	0,354	<0,001	0,936	0,140	0,044	0,023	<0,001

Table 3. Effect of treatments evaluated at 20 days after topdressing, mean value (± SD) of photosynthetic nitrogen use efficiency (PNUE, µmol CO_2 g^{-1}. N. s^{-1}). Experiments 1,2 and 3.

Treatments	Experiment 1	Experiment 2	Experiment 3
	PNUE	PNUE	PNUE
1	86,6±2,3	154,4±3,9	107,3±4,6
2	100,4±3,5	171,1±2,2	108,2±3,4
3	102,1±5,1	163,9±3,8	97,5±2,6
4	94,7±3,4	140,2±4,1	117,4±1,1
5	116,3±3,8	151,6±3,7	110,9±3,5
6	73,4±1,8	143,5±4,5	108,5±2,8
7	109,6±4,1	164,8±4,4	105,2±5,2
8	106,1±3,9	138,8±2,9	105,9±3,6
Contrast		**p value**	
1 vs 2 to 8	<0,001	0,604	0,813
2 vs 3 to 8	0,957	<0,001	0,719
3,4 vs 5 to 8	0,090	0,132	0,912
3 vs 4	0,014	<0,001	<0,001
5 vs 6 to 8	<0,001	0,2071	0,022
6 vs 7,8	<0,001	0,0013	0,136
7 vs 8	0,209	<0,001	0,738

Table 4. Effect of treatments evaluated at 40 days after topdressing, mean value (± SD) of CO_2 assimilation (A, µmol $CO_2.m^{-2}.s^{-1}$), stomatal conductance (g_s mol $m^{-2}.s^{-1}$), intercellular CO_2 concentration (Ci, µmol CO_2/ mol^{-1}) and leaf chlorophyll index (LCI). Experiments 1,2 and 3.

Treatments	Experiment 1		Experiment 2		Experiment 3	
	Ci	LCI	Ci	LCI	Ci	LCI
1	98,51±3,1	41,4±2,8	230,4±3,8	97,1±2,3	229,7±2,5	64,1±4,5
2	75,82±3,7	47,0±2,3	252,7±1,9	103,6±2,9	262,1±3,5	72,3±3,0
3	86,12±4,5	48,5±3,0	247,8±1,9	109,7±3,3	248,4±2,2	88,1±2,3
4	87,32±3,9	54,3±2,9	255,9±2,8	107,4±3,7	248,6±2,0	86,9±5,5
5	89,60±2,9	47,5±2,6	251,5±1,7	107,9±2,2	244,2±2,2	90,9±5,7
6	95,00±3,2	51,9±3,9	239,8±3,7	108,8±3,2	243,5±3,3	74,8±2,9
7	105,15±3,6	53,7±2,6	244,6±2,7	102,4±3,6	252,5±4,6	82,5±4,8
8	97,71±3,6	52,1±3,1	246,4±3,1	106,5±1,7	241,6±4,9	76,4±3,1
Contrast			**p value**			
1 vs 2 to 8	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
2 vs 3 to 8	<0,001	0,006	<0,001	0,034	<0,001	<0,001
3,4 vs 5 to 8	<0,001	0,952	<0,001	0,102	0,056	0,001
3 vs 4	0,650	0,005	<0,001	0,278	0,951	0,676
5 vs 6 to 8	<0,001	0,003	<0,001	0,249	0,400	<0,001
6 vs 7,8	0,009	0,577	<0,001	0,023	0,111	0,076
7 vs 8	0,009	0,420	0,317	0,060	<0,001	0,047

Table 5. Effect of treatments evaluated at 40 days after topdressing, mean value (± SD) of photosynthetic nitrogen use efficiency (PNUE, µmol CO_2 g^{-1}. N. s^{-1}). Experiments 1,2 and 3.

Treatments	Experiment 1	Experiment 2	Experiment 3
	PNUE	PNUE	PNUE
1	164,0±2,8	70,4±3,3	94,2±4,6
2	174,2±4,4	69,1±4,2	81,6±2,7
3	175,7±4,6	68,1±3,5	84,5±2,7
4	134,4±4,3	78,0±3,9	92,8±4,6
5	164,4±3,0	88,7±3,6	86,8±2,8
6	154,4±4,6	74,6±4,9	99,4±5,0
7	159,0±4,9	77,6±2,7	87,8±4,7
8	127,8±5,2	90,6±1,8	86,6±3,4
Contrast		**p value**	
1 vs 2 to 8	<0,001	<0,001	0,015
2 vs 3 to 8	<0,001	<0,001	<0,001
3,4 vs 5 to 8	0,049	<0,001	0,393
3 vs 4	<0,001	0,001	0,008
5 vs 6 to 8	<0,001	0,001	0,073
6 vs 7,8	0,002	<0,001	<0,001
7 vs 8	<0,001	<0,001	0,674

CONSIDERAÇÕES FINAIS

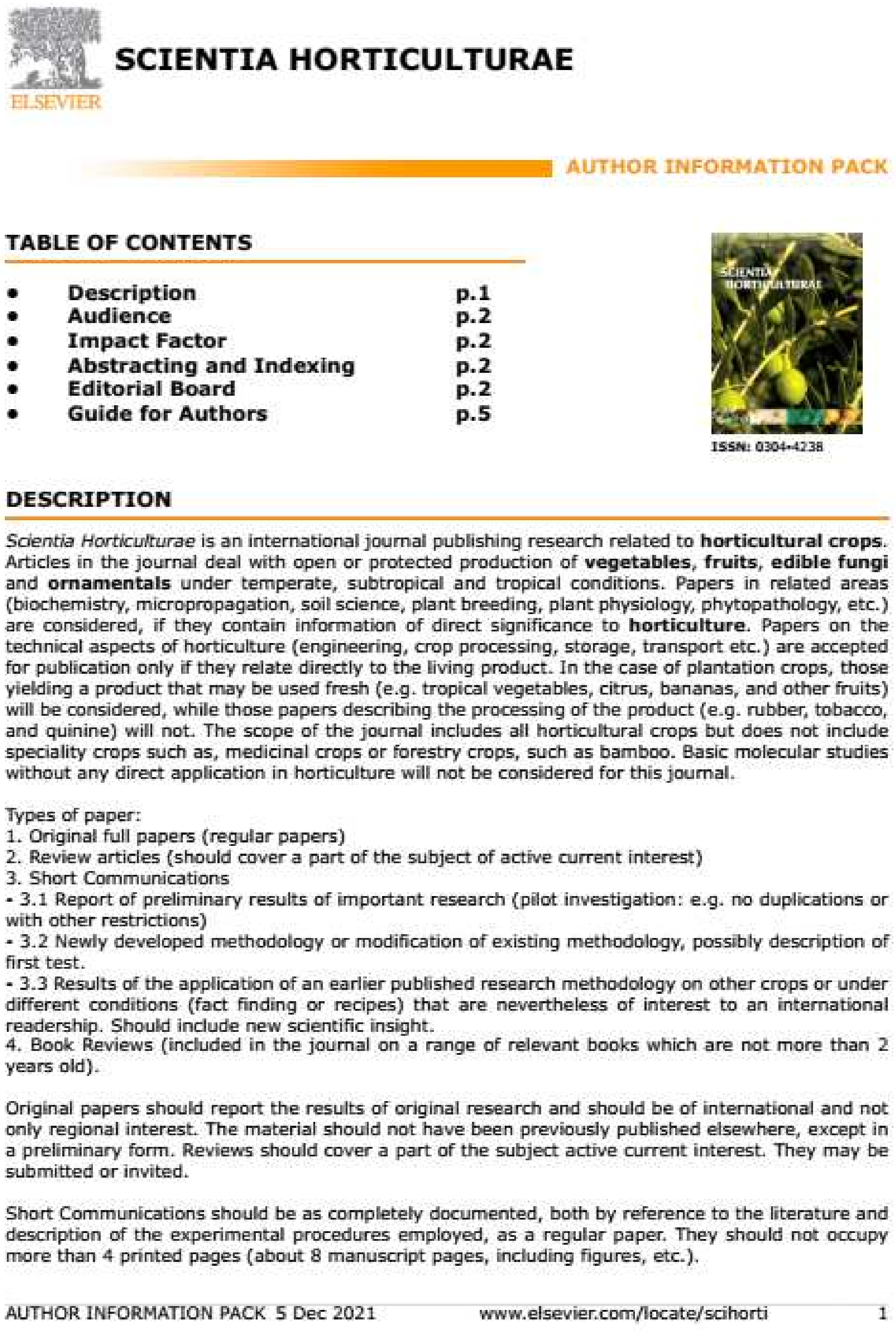
A dinâmica de aperfeiçoamento da produtividade dos cereais avança em direção a utilização combinada de produtos químicos e biológico, principalmente no viés da sustentabilidade com produtos de baixo custo.

Este trabalho abre a possibilidade de utilização de manejo nutricional da cultura do milho com a combinação de nitrogênio, molibdênio e bactérias diazotróficas (*A. brasilense*), gerando conhecimento cientifico sobre a interação de nutrientes e das bactérias diazotróficas em solo do tropico úmido brasileiro.

A tese abre novas linhas de pesquisas, sendo estas:

1. Teste em diferentes condições de solos do tropico úmido brasileiro;
2. Avaliação molecular da interação entre nitrogênio, molibdênio e bactérias diazotrófica na rizosfera;
3. Avaliação de produtividade, eficiência do uso do nitrogênio e eficiência fotossintética do uso do nitrogênio com a dose ótima de nutrientes e concentração ideal de bactérias diazotróficas para os solos do tropico úmido.

ANEXOS



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SCIENTIA HORTICULTURAE

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DESCRIPTION

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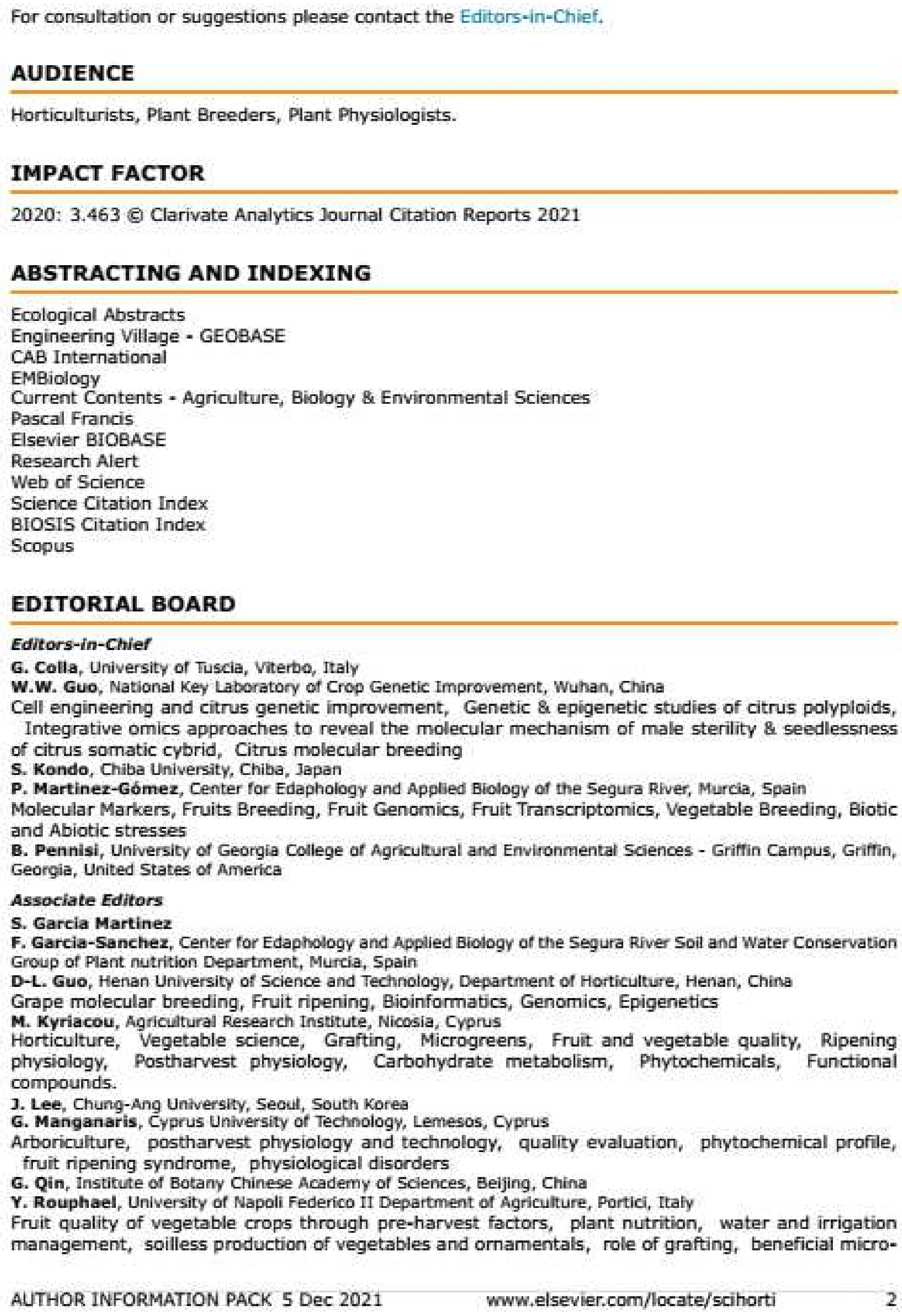

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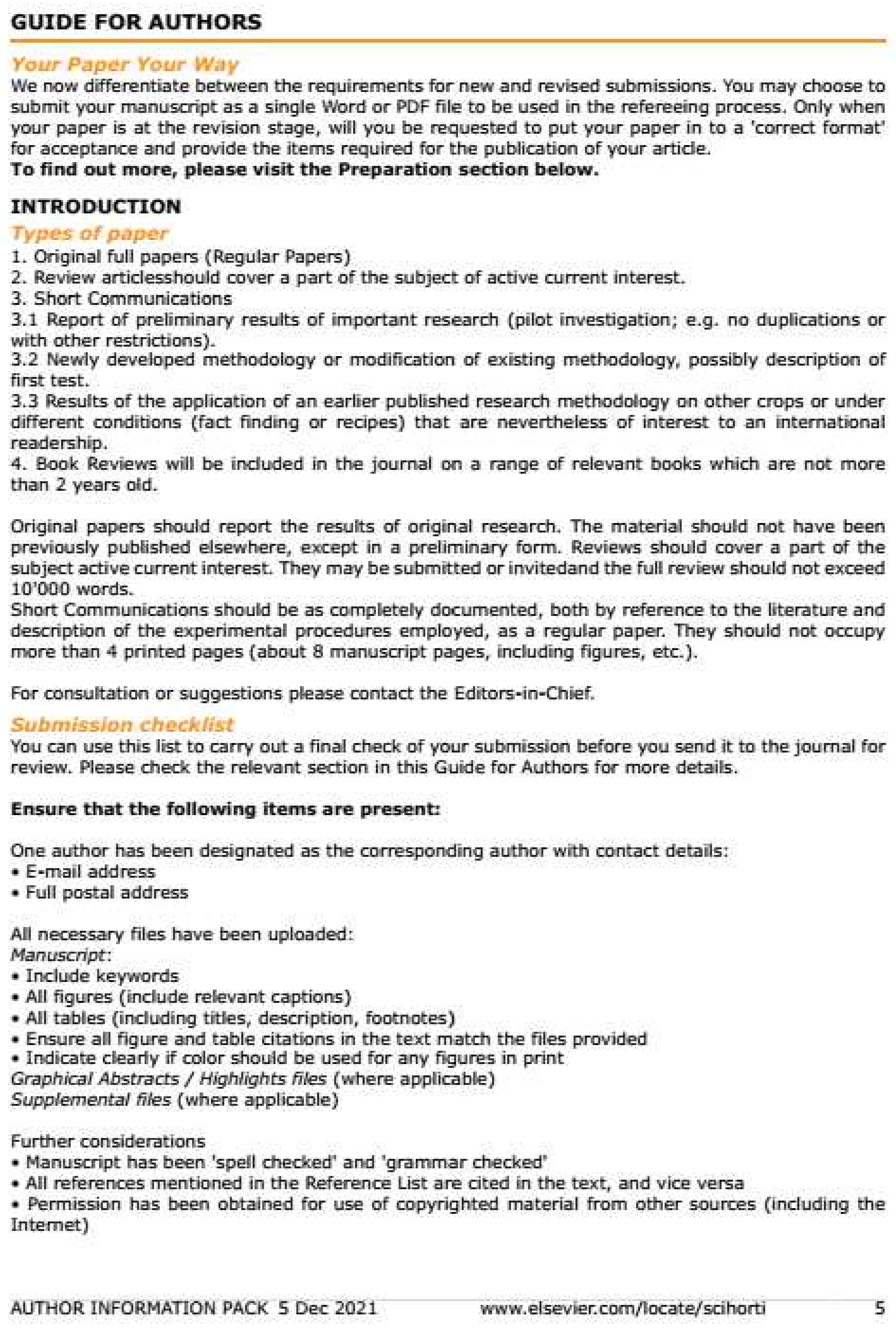

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