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Inoculação com *Herbaspirillum seropedicae* associada à fertilização nitrogenada (^{15}N) no capim-marandu

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Dissertação apresentada para obtenção do título de Mestre em Ciências. Área de concentração: Solos e Nutrição de Plantas

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RESUMO

Inoculação com *Herbaspirillum seropedicae* associada à fertilização nitrogenada (^{15}N) no capim-marandu

A compatibilidade entre quatro níveis de fertilização nitrogenada (N-ureia) foi avaliada em plantas de capim-marandu (*Urochloa brizantha* cv. Marandu) inoculadas com bactéria diazotrófica endofítica (BDE) emergente (*Herbaspirillum seropedicae* HRC54), via foliar. O experimento foi desenvolvido em condição de casa-de-vegetação, em delineamento em blocos casualizados, obedecendo a um esquema fatorial 4x2. Essa questão foi baseada no recorrente apelo ambiental e econômico em torno da baixa eficiência de uso do N-ureia em culturas não-leguminosas. Procedimentos mais usais no nosso campo de pesquisa foram aliados a técnicas precisas para tratar especialmente da contribuição de N-fixado e da recuperação do N-fertilizante aplicado nessa interação planta-bactéria. Por meio da combinação de técnicas isotópicas ^{15}N (abundância natural $\delta^{15}N$ ‰ e diluição isotópica) foi constatado que a inoculação contribuiu significativamente com N-fixado, porém observou-se redução substancial na fixação biológica do nitrogênio com o incremento dos níveis de N (de 21,5% para 8,6%). Em alto nível de N-fertilização (100 mg kg^{-1} de solo) pouco ou nenhum efeito da inoculação foi constatado, fornecendo base suficiente para classificá-lo como inibitória à atuação do microrganismo. Plantas de capim-marandu inoculadas e providas de condições mais modestas de N-fertilização (nível ausente, baixo e médio ou 0, 25 e 50 mg kg^{-1} de solo, respectivamente) produziram mais biomassa de raízes (até +37,7%) e da parte aérea (até +28,5%).Também exploravam mais os recursos do solo (N, P, K, Mg e Fe foram mais acumulados na parte aérea), bem como o N-fertilizante aplicado (recuperando até 2 vezes mais), o que foi atribuído especialmente ao maior desenvolvimento de raízes de menores diâmetros (especialmente <1mm). Aqui, comunicamos ainda em pequena escala, como tirar melhor proveito da inoculação com BDE no capim-marandu, e, portanto, contribuir para o uso mais racional e eficiente de N-fertilizantes. Finalmente, abrimos questões para novas investigações baseadas em técnicas isotópicas ^{15}N com grande potencial de aplicabilidade.

Palavras-chave: 1. Bactérias promotoras de crescimento de plantas 2. *Urochloa brizantha* cv. Marandu 3. Ureia ^{15}N 4. Bactérias endofíticas 5. Recuperação de N

ABSTRACT

Inoculation with *Herbaspirillum seropedicae* associated with nitrogen (^{15}N) fertilization on marandu grass

The compatibility between four levels of nitrogen fertilization (N-urea) was evaluated in marandu grass (Urochloa brizantha cv. Marandu) inoculated by leaf application with emerging endophytic diazotrophic bacteria (EDB) (*Herbaspirillum seropedicae* HRC54). The experiment was developed under a randomized block design under greenhouse conditions, following a 4x2 factorial scheme. This approach was based on the recurrent environmental and economic appeal around the low use efficiency of N-urea in non-legume crops. More usual procedures in our field of research were combined with precise techniques to address the contribution of N-fixed and the recovery of applied N-fertilizer in this plant-bacteria interaction primarily. A combination of ^{15}N isotopic techniques (natural abundance $\delta^{15}N$ ‰ and isotopic dilution) found that inoculation contributed significantly to N-fixed, but a substantial reduction in biological nitrogen fixation was observed with increasing N levels (from 21.5% to 8.6%). At a high N-fertilization level (100 mg kg^{-1} of soil), little or no effect of inoculation was found, providing the sufficient basis to classify it as inhibitory to microorganism action. Inoculated marandu grass plants provided with more modest N-fertilization conditions (default, low and medium level or 0, 25, and 50 mg kg^{-1} soil, respectively) produced more root (up to +37.7%) and shoots (up to +28.5%) dry matter. They also exploited more soil resources (N, P, K, Mg, and Fe were accumulated more in shoots) as well as applied N-fertilizer (recovering up to 2 times more), which was attributed mainly to the more significant development of smaller diameter roots (especially <1mm). Here, we communicate, on a small scale, how to take better advantage of EDB inoculation on marandu grass, thus contributing to more rational and efficient N-fertilizer use. Finally, we open questions for further investigations based on ^{15}N isotopic techniques with great potential for applicability.

Keywords: 1. Plant growth-promoting bacteria 2. *Urochloa brizantha* cv. Marandu 3. Urea ^{15}N. 4. Endophytic Bacteria 5. N recovery

INTRODUÇÃO

A oferta de nitrogênio via fertilização mineral é essencial para que se atenda a demanda nutricional de culturas não leguminosas e, consequentemente, se atinja produtividades satisfatórias, cuja demanda acompanha o crescente aumento da população global (DIMPKA et al., 2020; LI et al., 2022). Entretanto, um aumento acelerado e desmedido no uso de fertilizantes nitrogenados (N-fertilizantes) estão associados a altas taxas de perdas do mesmo (são mais de 100 tg N ano^{-1}, com aproveitamento médio de 50%) (JENSEN et al., 2020). Práticas ineficazes, onerosas, e que embutem riscos poluidores (como contaminação do solo e da água por nitrato e emissão de óxido nitroso) distanciam a agricultura da sustentabilidade, e, contrastam com tópicos acordados entre vários países e a Organização das Nações Unidas (ONU, 2018).

Com uma ocupação estimada em cerca de 180 milhões de hectares (Mha), espécies do gênero *Urochloa* são as mais utilizadas na América do Sul. Dentre suas cultivares, o capim-marandu (*Urochloa brizantha* cv. Marandu) é a mais cultivada no Brasil (86 Mha), com destacada representatividade na pecuária, bem adaptada as condições edafoclimáticas tropicais e bem responsiva a adubação (LEITE et al., 2019; HUNGRIA et al., 2021). Relatos anteriores demonstraram que a N-fertilização aumenta a produtividade e melhora a qualidade da forragem (SALES et al., 2020; LEITE et al., 2021). Contudo, em taxas demasiadas, o N-fertilizante é subutilizado pela planta (apenas 40-60% é aproveitado), o que geralmente é traduzido em baixa eficiência de uso e/ou menor recuperação do mesmo (DELEVATTI et al., 2019). O N-ureia (fertilizante com melhor custo benefício e maior concentração de N), que é majoritariamente usado nesses casos, sofre intensamente com perdas em solos tropicais (principalmente por volatilização da amônia) (TORRALBO et al., 2022).

A inoculação de bactérias promotoras de crescimento de plantas tem se mostrado uma alternativa viável do ponto de vista ambiental e econômico na redução da dependência ou mesmo no aumento na recuperação do N-fertilizante aplicado (GALINDO et al., 2020; WANG et al., 2020). Dentre os benefícios proporcionados por essas interações, o aprimoramento do sistema radicular merece destaque, mas na maioria das vezes os resultados são comunicados meramente em aumento da biomassa da raiz. As raízes possuem funções intimamente relacionadas à nutrição (exploração dos recursos do solo e fertilizante) (GU et al., 2018), além de mecanismos subjacentes a flutuações no suprimento de nitrogênio que alteram a arquitetura das raízes (JIAN e VON WIREN, 2020). Ainda, é um órgão de intensa atividade e recrutamento de microrganismos, especialmente em regiões limítrofes com a rizosfera (endorizosfera) (SALAS-GONZÁLEZ et al., 2020).

Herbaspirillum seropedicae, uma bactéria diazotrófica endofítica (BDE), possui grande potencial em fixar N atmosférico e outros efeitos bioestimulantes (por exemplo, solubilização de nutrientes, produção de reguladores de crescimento, mecanismos contra estresses bióticos e abióticos), consequência de uma colonização intercelular, protegida de altos níveis de oxigênio, fatores edafoclimáticos externos à planta, ou mesmo competição com outros microrganismos indígenas do solo (SILVA et al., 2017; MATTEOLI et al., 2020). Há resultados promissores acerca da capacidade desse endófito em colonizar e promover o crescimento de gramíneas tropicais (OLIVARES et al., 2017), porém, não existem relatos específicos sobre como essa interação pode beneficiar o capim-marandu, especialmente quando cultivado em níveis de N-fertilização.

O uso de isótopos estáveis como o ^{15}N pode contribuir com o entendimento dos mecanismos da inoculação de *H. seropedicae* no capim-marandu, como os níveis de N podem impactar essa associação, além de auxiliar no desenvolvimento de melhores práticas de manejo do nutriente. Desde que comparadas com plantas referência, a técnica de abundância natural ($\delta^{15}N$ ‰) permite entender e quantificar a quantidade do N na planta oriundo da fixação atmosférica, enquanto que a diluição isotópica possibilita quantificar o N proveniente do fertilizante enriquecido em átomos ^{15}N (CHALK et al., 2016). Ainda, essas técnicas podem ser combinadas, avaliando as fontes do N para a cultura de interesse, com precisão e alta confiabilidade, através da espectrometria de massas (GALINDO et al., 2021).

A hipótese deste trabalho é que a inoculação com *H. seropedicae* promove o crescimento do capim-marandu, fornecendo N-fixado e maior recuperação do do N-fertilizante aplicado. No entanto, também espera-se que níveis mais altos de de N-fertilização promovam impactos negativos ao organismo inoculado, com menor contribuição de N-fixado na nutrição da planta. Para validar essas hipóteses, objetivou-se examinar a atuação de *H. seropedicae* associado a níveis de N-fertilização na contribuição de N-fixado e recuperação do N-fertilizante especialmente por meio de técnicas isotópicas ^{15}N (diluição isotópica e abundância natural - $\delta^{15}N$ ‰), levando-se também em consideração à avaliação de raízes (comprimento e área superficial), avaliações nutricionais (acúmulo de macro e micronutrientes) e produtivas (biomassa áerea, de raízes e densidade de perfilhos).

CONCLUSÃO

A promoção de crescimento de forrageiras por BDE é dinâmica e superestimada por métodos indiretos. Análises isotópicas ^{15}N (abundância natural ^{15}N em conjunto a diluição do isotópo ^{15}N) podem ser utilizadas como ferramentas sensíveis e precisas para compreensão dessa interação em diferentes regimes de N-fertilização. Essa janela pode ser expandida para outras não-leguminosas que ocupam áreas significantes de cultivo e ajudar na compreensão das mais variadas intempéries na eficiência da FBN, ou propriamente no progresso para um melhor manejo do N-fertilizante.

Nesse caso particular, avaliamos como níveis de N-fertilização podem regular a relação cruzada entre a estirpe HRC54 de *H. seropedicae* e o capim-marandu. No geral, quanto mais N-fertilizante foi aplicado, menor foi a promoção de crescimento conferida. De forma mais específica: i) a inoculação foi mais eficaz em nível ausente e baixo de N. Nesses tratamentos, *H. seropedicae* HRC54 contribuiu com quantidades satisfatórias de N-fixado (>20%) e aumentou muito o crescimento de raízes de menores diâmetros. Os parâmetros avaliados foram eficientes em demonstrar um efeito cascata positivo entre aprimoramento do sistema radicular com maior exploração dos recursos do solo e fertilizante aplicado, e finalmente com incrementos na produção de forragem; ii) o nível alto de N-fertilização foi classificado como inibitório para atuação do organismo, com drástica redução de N-fixado e impacto negativo sobre o crescimento da raiz; e iii) indícios de que a eficácia da inoculação não pôde se sustentar em nível médio de N-fertilização foram levantados especialmente pelas técnicas isotópicas ^{15}N.

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