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MOBILIZAÇÃO DE COMPOSTOS CARBÔNICOS E NITROGENADOS EM PLANTAS DE MILHO SOB DESFOLHA E ATUAÇÃO DO ÓXIDO NÍTRICO NA MITIGAÇÃO DO ESTRESSE

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Tecnologias Ambientais Aplicadas.

Orientador: Prof. Dr. Plinio Rodrigues dos Santos Filho
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A Banca examinadora abaixo-assinada aprova a Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Ciências Ambientais.

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RESUMO

A agricultura enfrenta desafios decorrentes das mudanças climáticas e da exaustão de recursos naturais, resultando em desequilíbrios que afetam o crescimento das plantas, incluindo o milho. Este cultivo é afetado por fatores ambientais e biológicos, como a desfolha, que diminui a área de fotossíntese e prejudica a produção. As plantas têm mecanismos de detecção e resposta ao estresse para recuperar sua homeostase, mas os processos metabólicos subjacentes à recuperação após a desfolha são pouco compreendidos. No entanto, esses processos têm o potencial de melhorar as colheitas, pois o metabolismo vegetal é altamente adaptável às mudanças ambientais. Nesse contexto, os bioestimulantes, como o óxido nítrico (NO), têm sido estudados. Onde, sua aplicação exógena mostrou regular o equilíbrio redox e a fotossíntese, atenuando os efeitos negativos de estresses ambientais no milho. Este estudo visa preencher lacunas no conhecimento ao investigar a bioquímica do milho pós-desfolha e examinar o potencial dos NO para mitigar os efeitos prejudiciais da desfolha e promover o crescimento do milho. O estudo se concentra na análise das trocas gasosas, morfologia das raízes e teores de aminoácidos, açúcares e amido na parte aérea e nas raízes durante a recuperação das plantas após a desfolha com a aplicação de GSNO. Os resultados demonstram que a desfolha afeta significativamente os metabólitos primários, alterando as concentrações de aminoácidos, açúcares e amido. No entanto, a aplicação de GSNO como bioestimulante pode mitigar esses efeitos negativos, promovendo respostas que beneficiam a recuperação das plantas após a desfolha. Sugerindo um potencial do GSNO como uma ferramenta para melhorar a resistência das plantas de milho ao estresse e promover um crescimento mais saudável.

Palavras-chave: *Zea mays* L*;* GSNO; Estresse mecânico.

ABSTRACT

Agriculture faces challenges stemming from climate change and the depletion of natural resources, resulting in imbalances that impact plant growth, including corn. This crop is affected by environmental and biological factors such as defoliation, which reduces the photosynthetic area and hampers production. Plants possess stress detection and response mechanisms to restore their homeostasis, but the underlying metabolic processes following defoliation remain poorly understood. Nevertheless, these processes hold the potential to enhance crop yields as plant metabolism is highly adaptable to environmental changes. In this context, biostimulants like nitric oxide (NO) have been under investigation. Exogenous application of NO has shown to regulate redox balance and photosynthesis, mitigating adverse environmental stress effects on corn. This study aims to bridge knowledge gaps by investigating post-defoliation corn biochemistry and examining the potential of NO to alleviate detrimental defoliation effects and promote corn growth. The study focuses on gas exchange analysis, root morphology, and the levels of amino acids, sugars, and starch in both the aerial and root portions during plant recovery after defoliation with GSNO application. Results demonstrate that defoliation significantly affects primary metabolites by altering concentrations of amino acids, sugars, and starch. However, GSNO application as a biostimulant can mitigate these negative effects, promoting responses that benefit plant recovery post-defoliation. This suggests the potential of GSNO as a tool to enhance corn plant resilience to stress and foster healthier growth.

Keywords: *Zea mays* L*;* GSNO; Mechanical stress.

SUMÁRIO

1 INTRODUÇÃO GERAL

A agricultura como um todo, enfrenta muitos desafios, os quais incluem, principalmente, efeitos deletérios ocasionados pela crescente escala do aquecimento global e das mudanças climáticas, bem como o esgotamento dos recursos naturais (Zhao *et al.*, 2017). O que proporciona um quadro acentuado de interações desequilibradas entres as plantas e o ambiente, promovendo condições de estresse, que dependendo do nível e tempo, podem comprometer o estabelecimento e progressão das culturas (Taiz *et al.*, 2017).

As plantas de milho enfrentam muitos desafios ao longo do seu crescimento e desenvolvimento. Os quais incluem os fatores abióticos como chuvas de granizo, seca, nutrientes, temperaturas, ventos intensos (FAO, 2021) e os fatores bióticos como ataques de pragas e doenças. Dentre os fatores abióticos, o estresse mecânico gera um dano nas folhas e parte aérea, caracterizando uma desfolha, que reduz a área fotossinteticamente ativa das plantas. Este estresse pode provocar em efeitos negativos no crescimento das plantas, capacidade de sobrevivência, sucesso reprodutivo e até levar a perdas produção. Sendo dependente da fase de desenvolvimento e da intensidade da desfolha (Battaglia *et al.*, 2019).

As plantas como organismos sésseis desenvolveram diversos mecanismos de detecção e respostas a este estresse, que buscam reestabelecer a homeostase novamente através de complexas cascatas de sinalização, a fim de remodelar o sistema bioquímico, fisiológico e morfológico (Kouhen *et al.*, 2023). Contudo, os processos metabólicos que regulam a recuperação das plantas de milho submetidas a desfolha ainda foram pouco explorados, sendo de suma importância compreendê-los. Visto que, podem oferecer uma gama de oportunidades para melhoramento dos cultivos, já que, o metabolismo é diretamente responsivo em relação a mudanças ambientais, sendo moldado através da realocação de recursos disponíveis a fim de levar a adaptações benéficas para o desenvolvimento e manutenção da vitalidade (Arnold; Sajitz-Hermstein; Nikoloski, 2015; Geiger; Servaites, 1994).

Além disso, há uma crescente demanda de insumos agrícolas de baixo custo e baixo impacto ambiental. Nesse sentido, pesquisas acima dos efeitos dos bioestimulantes na mitigação de efeitos de estresses bem como melhorias nas culturas em geral, vem ganhando destaque. Os bioestimulantes são bioprodutos derivados de inúmeras fontes e desempenham diversificadas funções nos sistemas agrícolas (Halpen *et al*., 2015). Podem ser capazes de melhorar a adaptabilidade das plantas a diversos estresses abióticos (Masondo *et al*., 2018) ajudando no estabelecimento da cultura. São caracterizados como uma abordagem inovadora para a regulação de processos fisiológicos, possibilitando o decréscimo de impactos

ambientais, permitindo que seja mantido uma sustentabilidade econômica e ambiental nos sistemas agrícolas (Halpern *et al.*, 2015).

Dentre a diversidade de bioestimulantes, o óxido nítrico (NO) vem ganhando grande espaço dentro das pesquisas. Isso se deve ao fato que o mesmo pode exercer efeitos como modulador do equilíbrio redox e sistema fotossintético, quando aplicado de forma exógena, atuando como um grande mitigador das implicações de estresses abióticos em plantas. O NO é um gás solúvel, altamente difundível através de membranas sem necessidade de transportadores, com capacidade de reagir com certas quantidades de macromoléculas, como proteínas, lipídios, ácidos nucléicos, entre outras (Corpas *et al*., 2011; Domingos *et al*., 2015) o que justifica seus efeitos positivos quando aplicado nas plantas.

É importante destacar que que devido há uma escassez de estudos que abordem a bioquímica do milho após o processo de desfolha, e também de pesquisas que relacionem substâncias bioestimulantes na mitigação dos efeitos deletérios da desfolha, bem como auxiliem no crescimento e rentabilidade da cultura do milho, justificando assim a relevância desta investigação. Portanto, a hipótese central deste estudo postula que a remoção das folhas desencadeia uma alteração no equilíbrio de carbono e nitrogênio nas raízes, favorecendo as folhas, enquanto a aplicação exógena de GSNO induz a reestruturação do metabolismo de nitrogênio e carbono nas plantas de milho que sofreram desfolha. O objetivo principal desta pesquisa foi avaliar as trocas gasosas, examinar a morfologia das raízes e quantificar os teores de aminoácidos, açúcares solúveis totais, açúcares redutores e amido na parte aérea e nas raízes durante o processo de recuperação das plantas após o estresse de desfolha e com a aplicação de GSNO.

2 REVISÃO DE LITERATURA

2.1 A CULTURA DO MILHO: ASPECTOS GERAIS

O milho (*Zea mays*) pertence à família Poaceae. É originário da América Central, com domesticação ocasionada via seleção artificial cruzando subespécies de indivíduos selvagens há cerca de nove mil anos (García-Lara; Serna-Saldivar, 2019). É uma planta veranica, com alta adaptabilidade ambiental, permitindo que seja cultivado nas mais diversas zonas (Martinez; Fernandez, 2019). Se encontra como um dos cereais de destaque, categorizado como uma *commodity* (FAO, 2021) apresentando grande importância econômica mundial e notoriedade agrícola (FAO, 2021; Muimba-Kankolongo, 2018). Isso se deve a sua alta eficiência em termos de rendimento por unidade de terra. Além disso, é um dos grãos de maior relevância para a humanidade devido a sua variedade de usos, sendo fonte para alimentação humana e animal, além de ser matéria para a indústria, incluindo a produção de combustível (Martinez; Fernandez, 2019).

No Brasil tem um cultivo geograficamente difundido (Bergamaschi; Matzenauer, 2014), com produção estimada em 131.865, 9 mil toneladas para a safra 2022/2023, em uma área plantada de 22.267,4 mil hectares (CONAB, 2023). O milho pode ser semeado em diversas épocas ao longo do ano e pode compor diversos sistemas de cultivo, como na sucessão após colheita da soja, cultivo consorciado, sistemas integrados de produção lavoura-pecuária ou ainda, em esquema de rotação de cultura no sistema de plantio direto (Contini *et al.*, 2019; Karam *et al.*, 2020).

No país, a semeadura é subdividida em três épocas, em razão do regime de chuvas: primeira safra, semeada de agosto a dezembro; segunda safra ou safrinha, de janeiro a março e terceira safra, de abril a julho, sendo esta última, restrita a poucas áreas, e as duas primeiras safras de maior relevância (CONAB, 2023; Karam *et al.*, 2020). Na primeira safra, o plantio é feito no período chuvoso do ano, época considerada a ideal para o desenvolvimento das culturas pois possui as melhores condições climáticas (temperatura, luminosidade e umidade) para o cultivo. Já o plantio da safrinha ocorre em condições ambientais consideradas sub-ótimas para seu cultivo devido aos riscos veranicos (CONAB, 2023; Contini *et al.*, 2019).

O safrinha tem um limitante temporal, onde, quanto mais tarde for semeado, mais o ciclo da cultura se estenderá para o inverno, havendo grandes possibilidades de perda de produtividade. Isso se deve ao fato de que o final do verão e as posteriores estações frias, são caracterizadas por baixos níveis de precipitação e baixas temperaturas, limitando a

disponibilidade de água no solo, podendo acometer a cultura nas fases de maior sensibilidade, como nas de florescimento e enchimento de grãos (Karam *et al.*, 2020; Magalhães *et al.*, 2020). Contudo, sua proporção de cultivo é crescente, sendo a principal safra atualmente, com uma área plantada no período de 2022/2023 de 77,15% da área total de cultivo de milho, apresentando uma produtividade no mesmo período de cerca de 75,48% em relação ao total produzido (CONAB, 2023).

2.2 SISTEMA ANTECIPE

A Antecipe é um sistema de cultivo antecipado desenvolvido pela Empresa Brasileira de Pesquisa Agropecuária (Embrapa) e parceiros e tem como objetivo aumentar a produtividade do sistema soja-milho safrinha no Brasil. Consiste de um método de semeadura intercalar do milho nas leiras da soja, pouco tempo antes da colheita da mesma, permitindo que haja o estabelecimento precoce do milho, reduzindo riscos de perda de produtividade do safrinha em função das condições climáticas adversas do final da estação chuvosa e início das estações secas. Em sua formulação, o Sistema Antecipe, contou com estudos em relação aos danos mecânicos ocasionados nas plantas de milho no momento da colheita da soja, a fim de encontrar a melhor época de semeadura antes da colheita (Karam, *et al*., 2020).

Durante tais estudos sobre danos, foi demonstrado que se houver poda (desfolha) das plantas durante os estágios V4 e V5 de desenvolvimento, as plantas conseguem se recuperar do dano, pois seu meristema apical ainda se encontra abaixo do solo, permitindo que brote novamente (K*ARAM, et al*., 2020). Fato importante, pois define o tempo certo de colheita da soja e semeadura do milho, para que ambos não sofram perdas neste período, pois o período em que são realizados a colheita da soja e o cultivo de safrinha, não proporcionam condições ótimas para a máxima eficiência da produção deste segundo, e quanto mais tarde ocorrer sua semeadura, mais propicio ficará a condições ambientais adversas em estágios fenológicos sensíveis. E ter este conhecimento é de suma importância, visto que, cerca de 35% da área utilizada no país para o cultivo de safrinha são de áreas de cultivo de soja (CONAB, 2020).

Dessa maneira o Sistema Antecipe promove uma maior segurança para aqueles períodos que ocorrem atrasos na semeadura ou mesmo na colheita da soja, permitindo que a produção do milho não sofra drásticas perdas devido ao adiantamento de seu cultivo. Além disso, o Sistema Antecipe realiza uma ligação entre a produção agrícola com a sustentabilidade, pois permite incrementos de produção, sem a necessidade de expansão das áreas agrícolas (efeito poupa-terra) (Magalhães *et al*., 2020).

2.3 DESFOLHA

O estresse por desfolha pode ser ocasionado nas plantas por diversas condições abióticas e bióticas, como tempestades, granizo, doenças foliares, ataques herbívoros (Battaglia *et al.*, 2019) e implementações agrícolas (Karam *et al.*, 2020). Tais tipos de estresse produzem diferentes graus de danos as culturas, sendo dependente da intensidade/grau da desfolha e do estádio de desenvolvimento em que as plantas se encontram (Battaglia *et al.*, 2019).

Nas plantas sob desfolha há uma clara priorização do reestabelecimento do aparato fotossintético por meio da retomada de crescimento. Dessa forma, é necessário uma remobilização de C e N entre fonte-dreno (Meuriot *et al.*, 2018). Onde, a demanda energética das novas folhas deve ser suprida pela degradação de proteínas, oxidação de aminoácidos e degradação de compostos de armazenamento como o amido, até o completo reestabelecimento da capacidade fotossintética (Galili *et al.*, 2014; Hildebrandt *et al.*, 2015).

Em plantas de milho, sabe-se que, se a desfolha ocorrer até os estádios de desenvolvimento V4-V5 (de quatro a cinco folhas completamente expandidas), as plantas conseguem retomar seu crescimento e ainda pode-se evitar a perda de produtividade (Karam *et al.*, 2020; Magalhães *et al.*, 2020). Isso pode ser explicado pelo fato de que o meristema apical foliar ainda se encontra abaixo do solo e as folhas perdidas no processo, futuramente passariam por senescência, não interferindo na produção de grãos (Magalhães; Durães, 2008). Contudo, é necessária uma maior exploração de como ocorrem os processos de remobilização e as vias bioquímicas envolvidas.

2.4 BIOESTIMULANTES

Os bioestimulantes são produtos que podem ser obtidos de diversificados materiais orgânicos ou sintéticos, e são tidos como substâncias de baixo impacto ambiental. Podem ser utilizados em diferentes concentrações, proporções e composições (Du Jardin, 2015). São divididos em quatro grupos de acordo com sua composição: os biorreguladores, as substâncias húmicas, os microrganismos e os aminoácidos (Barbosa da Silva *et al.*, 2023; Halpern *et al.*, 2015). Podem auxiliar na melhoria e eficácia nutricional, tolerância a estresses e incrementar a produção. Em outras palavras, podem desempenhar funções relacionadas a capacidade de estimular e acelerar o processo de germinação e crescimento vegetativo e proporcionar um ambiente homeostático, favorecendo o desenvolvimento das plantas,

promover a antecipação do processo de maturação para a colheita (Halpern *et al.*, 2015; MAPA, 2021).

Os bioestimulantes também podem ser capazes de reduzir a competição das culturas com plantas invasoras e melhorar a adaptabilidade das plantas (Masondo *et al.*, 2018) ajudando o estabelecimento da cultura, entre outros. Dessa maneira, são caracterizados por serem uma abordagem inovadora para a regulação de processos fisiológicos nas plantas., proporcionar um menor uso de agroquímicos, possibilitando o decréscimo de impactos ambientais, permitindo que seja mantido uma sustentabilidade econômica e ambiental nos sistemas agrícolas (Barbosa da Silva *et al.*, 2023; Halpern *et al.*, 2015).

2.5 ÓXIDO NÍTRICO: CONCEITOS E INTERAÇÕES

O óxido nítrico (NO) é um gás solúvel, altamente lipofílico, incolor e inorgânico. Pertence as moléculas do tipo radical livre por ter um elétron não emparelhado em um dos seus orbitais (Corpas *et al.*, 2011). Devido a sua natureza gasosa, consegue atravessar membranas e se difundir rapidamente, sem necessidade de um transportador. Além disso, detém a capacidade de reagir com certas quantidades de macromoléculas, como proteínas, lipídios, ácidos nucléicos, entre outras (Corpas *et al.*, 2011; Domingos *et al.*, 2015). Assim, é tido como um dos mais importantes sinalizadores de processos intra e extracelular.

O óxido nítrico endógeno pode ser gerado por mecanismos enzimáticos e não enzimáticos. Os mecanismos enzimáticos incluem as óxido nítrico sintase (NOS) e a nitrato redutase (NR) (Neill; Desikan; Hancock, 2003). Essas moléculas podem catalisar a formação de NO a partir da L-arginina, NO^{2-} ou NADH (Yamasaki; Sakihama; Takahashi, 1999), dependendo da molécula ativa e do substrato disponível. Já os mecanismos não enzimáticos são dependentes dos ciclos de nitrificação e de desnitrificação, os quais fornecem o NO como um subproduto da oxidação do N_2O para a atmosfera. Além disso, tem-se que a redução não enzimática do nitrito também pode levar à formação de NO (Corpas *et al*., 2007), ou ainda, alguns autores como Cooney et al. (1994), sugerem que possa ocorrer a formação de NO através da redução mediada por luz de N_2O por carotenóides.

O NO atua como sinalizador nas plantas e, estudos acerca de suas funções nos processos fisiológicos, indicam que está envolvido na regulação do crescimento e desenvolvimento e nas respostas ao estresse biótico e abiótico nas plantas (Kaiser *et al.*, 2016; Salgado *et al.*, 2013; Sanz *et al.*, 2015). Mais especificamente, o óxido nítrico está envolvido direto ou indiretamente, nas modificações pós-traducionais, incluindo ligação a centros

metálicos, a S-nitrosilação de grupos tiol e a nitração da tirosina, a qual pode estar associada na sinalização celular sob as condições fisiológicas e de estresse (Corpas *et al.*, 2011). É responsável pelo ajuste fino da homeostase do nitrogênio nas plantas. Função dada devido a capacidade do NO de regular por feedback as vias de assimilação do nitrogênio, onde, dependendo de sua concentração podem aumentar ou reduzir esse processo (Frungillo *et al.*, 2014).

Aplicações exógenas de óxido nítrico são, geralmente, realizadas através de um doador, ou seja, uma molécula que irá gerar NO, ao passar para as células vegetais. Essa abordagem favorece a utilização de variadas concentrações, proporcionando a investigação de resposta das plantas de acordo com as doses. Os doadores de NO mais empregados são o nitroprussiato de sódio (SNP) e o S-nitrosoglutationa (GSNO) (Neill; Desikan; Hancock, 2003).

O GSNO é uma molécula formada a partir da interação entre o NO e a glutationa reduzida (GSH), em um processo denominado S-nitrosilação. Essa reação envolve a formação de N_2O_3 ou a adição de NO a um radical glutationil produzido durante esse processo (Broniowska; Diers; Hogg, 2013; Corpas; Alché; Barroso, 2013; Khajuria *et al.*, 2019). É uma molécula que desempenha um papel significativo nas plantas, sendo associada à formação e degradação de S-nitrosotióis dentro das células, reservatório de NO durante processos de sinalização do mesmo, além de exercer papel importante na mitigação de estresse nitrosativo (Khajuria *et al.*, 2019).

Estudos como de Rigui et al. (2019) e Silveira et al. (2016) demonstram que aplicações exógenas dessa molécula estão fortemente relacionadas a recuperação de plantas estressadas. E, apesar do conhecimento do papel na tolerância das plantas a estresses, o NO e seus doadores, como o GSNO, ainda possuem muitas questões a serem compreendidas, como seu papel na regulação da homeostase e vias associadas a respostas a estresses (Khajuria *et al.*, 2019).

3 ARTIGO

Mobilization of carbon and nitrogen compounds in maize plants under defoliation and action of nitric oxide in stress mitigation

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Contributing authors: ACCS, DMM and TFSRM: responsible for implementing the experiment, carrying out, collecting and analyzing data and writing the text. EB, DK and PCM: responsible for guiding the research. PRSF, TCS and DMM: responsible for reviewing the writing of the article and conceptualizing the project.

Abstract

Main Conclusion Maize defoliation causes sugars, starch, proteins and amino acids mobilization from roots to shoots. Nitric oxide changes the amino acids and root morphology, and enhances carbon compounds, aiding plant recovery

Abstract

The study aimed to assess the mobilization of carbon and nitrogen compounds (sugars, starch, amino acids, proteins) in maize plants undergoing early defoliation, along with examining alterations in photosynthesis and root morphology. The plants were grown in 20-L pots, distributed in four treatments (control; defoliation; defoliation with 50 µM S-Nitroglutathione (GSNO) and defoliation with 100 µM GSNO). Defoliation was carried out when the plants reached four fully expanded leaves, with a section in the leaves keeping the shoot 5 cm above the soil. The sixth fully expanded leaf, as well as the roots, were collected on the 14th, 21st and 28th days after defoliation for analyses of sugars, proteins and amino acids, leaf gas exchange and root morphology. The result was a marked decrease in the content of starch, sugars and total amino acids in plants that underwent

defoliation, mainly in the roots. Amino acids associated with nitrogen transport, such as asparagine, increased in the leaves of plants that underwent defoliation in relation to the roots, indicating N mobilization and a source-sink relationship. Nitric oxide caused an increase in amino acids important in stress response pathways such as phenylalanine and tyrosine. Nitric oxide favors root modifications so that the roots can better explore the soil. Therefore, the results demonstrate that plants that underwent defoliation do not differ in their development after 28 days of stress. Nitrogen mobilization through amino acids is essential for the initial recovery of maize plants and nitric oxide can favor this process.

Keywords: *Zea mays* L. S-Nitroglutathione. Amino acids. Sugars. Mechanical stress. WinRhizo

Introduction

Maize (*Zea mays* L.) is a cereal with great global economic importance and agricultural notoriety, being categorized as a commodity (FAO 2021; Muimba-Kankolongo 2018). This crop has high environmental adaptability, allowing it to be cultivated in the most varied regions of the world (Santos et al. 2013). In recent decades, this cereal has reached the level of the largest agricultural crop in the world, being the only one to have surpassed the 1 billion ton yield mark (Contini et al. 2019).

Maize plants face many biotic and abiotic factors that can become stress factors that reduce their growth and development. Among the abiotic factors, mechanical stress damages the leaves and shoot, characterizing defoliation, reducing the photosynthetically active plant leaf area. Mechanical stress as a function of defoliation is still very little studied and can occur due to mechanization, herbivory, pests, hail (Lopes 2015).

Depending on the stage of development and intensity of defoliation, it can result in detrimental effects on plant growth, integrity, reproductive success and survivability. There are studies that show that if defoliation occurs at the beginning of corn development (early defoliation) it does not change the productivity of corn plants (Karam et al. 2020; Magalhães et al. 2020). However, little is known about the mechanisms involved in the response pathways to this type of stress. It is only known that plants have detection mechanisms and responses to this stress, which seek to reestablish homeostasis again through complex signaling cascades, in order to remodel the biochemical, physiological and morphological system (Kouhen et al. 2023).

These signaling cascades can modify plant metabolism under stress conditions, using alternative respiratory substrates, such as proteins. Consequently, there is an increase in protein degradation (Hildebrandt et al. 2015) and transcription of amino acid catabolic genes, in order to generate energy (ATP) (Araújo et al. 2011; Galili et al. 2014). However, which amino acids are used and how the translocation and remodeling of these amino acids occur in the root and shoot are still not very clear in the case of maize crops under defoliation. Understanding carbon and nitrogen metabolism for the reestablishment of maize plants after early defoliation also needs elucidation, as this stress modifies the source-sink relationship. These responses can offer a range of opportunities for improving crops and understanding the effects of stress, since metabolism is directly responsive to environmental changes (Arnold; Sajitz-Hermstein; Nikoloski 2015; Geiger and Servaites 1994).

The use of biostimulants has gained great prominence in mitigating environmental stress. Exogenous nitric oxide (NO) is already known as a biostimulant for its effects as a modulator of redox balance in the photosynthetic system and as a regulator of plant growth and development under stress (Corpas et al. 2011; Domingos et al. 2015; Wimalasekera et al. 2011). Among NO donors is S-Nitrosoglutathione (GSNO), a molecule formed non-enzymatically by NO in the presence of glutathione (GSH) (Broniowska et al. 2013). GSNO is a molecule that can function as a store of NO bioactivity. Exogenous applications of GSNO have been

associated with promising results in the recovery of plants under stress (Rigui et al. 2019; Silveira et al. 2016).

In this context, the study of the combination of a substance with biostimulant properties, such as GSNO in plants under defoliation, can provide results that help in maize growth and profitability, in addition to favoring its application in crops that face production problems due to mechanical damage as a function of defoliation, as well as extreme environmental conditions. It is also worth highlighting that few studies have delved into the morphophysiological (gas exchange and root morphology) and biochemical (sugars and amino acids) issues of plants undergoing defoliation. Therefore, the hypothesis of this research is that defoliation causes a change in the carbon and nitrogen balance in the roots, favoring the leaves, and the exogenous application of GSNO leads to the remodeling of nitrogen and carbon metabolism in maize plants under defoliation. The objective was to analyze gas exchange, root morphology and quantify the levels of amino acids, total soluble sugars, reducing sugars and starch content in shoots and roots in the recovery of plants subjected to defoliation with the application of GSNO, given that there are few studies on maize biochemistry under defoliation.

Materials and methods

Plant material and growing conditions

The experiment was conducted in a greenhouse at the Santa Clara Educational Unit of the Federal University of Alfenas (UNIFAL-MG), located in the city of Alfenas, Minas Gerais, Brazil, at 818 m altitude, 21° 25’ 20”S and 45° 59’00” W, average temperature of 21ºC, maximum of 34ºC and minimum of 6ºC.

The hybrid 4080 SHS (Santa Helena Sementes^{TM}) was used, with an early cycle and excellent mulching, being indicated for whole plant forage and recommended by the company for summer and off-season crops in the region. For cultivation, 20-L pots previously filled with Dystrophic Red Oxisol were used, keeping two plants per pot. Fertilization was carried out in accordance with the recommendation of soil chemical analysis and all phytosanitary treatments necessary for the crop were applied (Ribeiro 1999). Daily irrigation was carried out to keep the soil close to 70% of its maximum water retention capacity throughout the experimental period.

A randomized block design (RBD) was used, consisting of four treatments with sixteen replications each and two plants per pot. The treatments were: control (without mechanical damage due to defoliation), defoliation (with mechanical damage due to defoliation at stage V4), and two defoliation treatments with the application of two concentrations of S-nitrosoglutathione (GSNO), 50 µM and 100 µM. Therefore, the treatments were arranged as follows: Control; Defoliation; 50 µM GSNO and 100 µM GSNO.

When the plants reached four fully expanded leaves (V4) (31 days after sowing), the treatments were applied. Defoliation was carried out with the aid of scissors to eliminate the entire plant shoot in treatments that received defoliation, maintaining a cutting height 5 cm above the soil surface. In the control treatment, the plants were grown without mechanical damage.

The treatments that received GSNO application had two spraying stages. In the first, the plants were sprayed with GSNO 12 hours before defoliation; approximately 5 mL of solution per plant were applied with the aid of a manual pump. In the second stage, after 5 days of defoliation, the plants received the application of the NO donor following the previous protocol, but applying around 2 mL of solution per plant. In both stages, the control and defoliation treatments were sprayed with distilled water in the same proportions.

For all evaluated parameters, three material and data collection times were standardized: 14th, 21st and 28th day after mechanical damage. Leaf analyses were standardized at V6, which corresponds to the sixth fully

expanded leaf, in the control treatment. In addition, in the defoliation treatment, the first leaf that expanded after mechanical damage due to defoliation was standardized, also corresponding to V6.

Analysis of the content of reducing sugars, soluble sugars and starch

Leaf and root samples (200 mg) were homogenized in an ice bath with 2 mL of methanol/chloroform/water solution (12:5:3, v/v), and incubated at room temperature for 24 hours. They were then centrifuged at 1500 g for 30 minutes and, subsequently, the supernatant was collected and added with a chloroform and water solution (4:1:1.5, v/v). For the analysis of reducing sugars (RS) and total soluble sugars (TSS), the aqueous phase of this mixture was used and, for starch, the precipitate obtained from centrifugation.

The methodology described by Miller (1959) was used to quantify RS, where an aliquot of the aqueous phase and distilled water (totaling 750 µL) was added to 500 µL of a solution consisting of 10 mL of 2N NaOH, 0.5 g of dinitro-3,5-salicylic acid (DNS), and 15 g of sodium potassium tartrate. The samples were vortexed and then heated in a water bath at 100 °C for 5 minutes. Subsequently, 3750 µL of distilled water was added at room temperature, and absorbance was read at 540 nm.

TSS concentration in plant tissues was analyzed by adding an aliquot of the aqueous phase and distilled water (totaling 1 mL), with 2 mL of anthrone reagent (prepared with 20 mg of anthrone, 500 µL of water and 10 mL of concentrated sulfuric acid). The samples were vortexed and then heated in a water bath at 100 °C for 3 minutes. Subsequently, absorbance was read at 620 nm, at room temperature (Yemm and Willis 1954).

Starch contents were determined according to McCready et al. (1950), by adding 1 mL of 30% (v/v) perchloric acid to the precipitate resulting from the extraction. The samples were then vortexed for approximately 5 minutes. The liquid phase of the obtained solution was used to react with anthrone in the same way as mentioned above.

Quantification of proteins and amino acids

Protein levels were quantified from leaf and root extracts obtained by macerating 200 mg of the material with 2 mL of 50 mM potassium phosphate buffer, pH 7.5 (containing EDTA, 5% PMSF and PVP). After extraction, the material was subjected to the Bradford method (1976), in ELISA, using BSA as a standard.

Amino acids (AA) were separated and analyzed by reversed-phase High Performance Liquid Chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan), through derivatization with o-phthaldialdehyde, using a C-18 column (5 µm, 250 µM x 4.60 mm). The solution for the mobile phase consisted of 65% methanol (solvent A) and phosphate buffer pH 7.25 (50 µM sodium acetate, 50 µM dibasic sodium phosphate, 1.5 mL acetic acid, 20 mL tetrahydrofuran, 20 mL methanol in 1 L water, solvent B), at a flow rate of 0.8 mL min^{-1}. The solvent A gradient increased in proportions of: 20 to 60% between 0 and 25 min, 60 to 75% between 25 and 31 min and 75 to 100% between 31 and 50 min. Later, he returned to initial conditions to rebalance the spine.

The column eluent was monitored with a fluorescence detector operating at an excitation wavelength of 250 nm and an emission wavelength of 480 nm (Puiatti and Sodek 1999). A pool containing the following amino acids was used as a standard: Aspartate (Asp), Glutamate (Glu), Asparagine (Asn), Serine (Ser), Glutamine (Gln), Histidine (His), Glycine (Gly), Threonine (Thr), Citrulline, Arginine (Arg), Alanine (Ala), Tyrosine (Tyr), Methionine (Met), Valine (Val), Phenylalanine (Phe), Isoleucine (Ile), Leucine (Leu) and Lysine (Lys). Among

the AA mentioned, only those that presented higher concentrations in the control treatment leaves and roots were represented and discussed individually, serving as a form of standardization. Furthermore, it was not possible to identify citrulline in the samples, nor was it possible to analyze glycine and threonine separately, as they were presented together in the study. Total amino acids were quantified by summing the concentration of all amino acids identified in each treatment at each of the evaluation times.

Gas exchange and root morphology

Gas exchange analyses, as well as the collection of material for the analysis of root morphology, were carried out on three different days: 14, 21 and 28 days after defoliation. For gas exchange, an infrared gas analyzer (LI-6400XT - Li-COR Biosciences, Lincoln, Nebraska, USA) was used. Net photosynthetic rate (A) and stomatal conductance (gs) were evaluated. The analyses were in the middle section of the leaves of plants in the control treatment and in the available area of plants that underwent defoliation, corresponding to the completely expanded sixth leaf. Measurements were taken in the morning, from 8 to 10 am. A photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1} was used, with a blue-red LED light source, and the chamber temperature was 28°C.

To analyze the morphology of the root system, the WinRhizo Pro 2007ª image analysis system (Regent Instruments, Sainte-Foy, QC, Canada), coupled to a professional scanner (Epson, Expression 10000 XL, Epson America, Inc., USA) was used, equipped with an additional light unit (TPU). The procedures for obtaining the images were carried out in accordance with Marques et al. (2023). The following characteristics were determined: length (cm), surface area (cm^2), average diameter (mm) and root volume (cm^3). The roots were then stored in paper bags and transported to a forced air circulation oven at 65 ºC until constant mass was obtained. Other attributes involving morphological and dry mass data were performed: specific root length (SRL cm g^{-1}) and root fineness (RF cm cm^3) (Marques et al. 2023).

Data analysis

For all analyzed parameters, the means and standard deviation of the replications were calculated. For statistical analysis, analysis of variance (ANOVA) and the Tukey test were used at 0.05% significance ($p \leq 0.05$), in the Sisvar software version 5.6 (Federal University of Lavras, Lavras, Brazil).

Results

Reducing sugars, total soluble sugars and starch

On the 14th day after defoliation, it is possible to observe a reduction in the content of starch, soluble sugars (TSS) and reducing sugars (RS) in maize leaves and roots in the defoliation treatment in relation to control plants (Fig. 1). The defoliated plants that received the application of GSNO at two concentrations (50 and 100 µM) showed the same concentration of RS in the leaves as the control plants (Fig. 1 a). Starch content in roots was higher with the spraying of 100 µM GSNO (Fig. 1 f), and in leaves at both concentrations, when compared to plants under defoliation (Fig. 1 e).

On the second day of evaluation (21st), there was also a reduction in total soluble sugars and reducing sugars in leaves and roots of plants under defoliation in relation to the control (Fig. 1). In the same treatment, starch content also decreased in the leaves, however, in the roots, it increased (Fig.1 a-b). The application of

GSNO demonstrated an effect at a concentration of 50 µM, increasing the content of reducing sugars in the leaves, and, at a concentration of 100 µM, reducing the starch content in the roots, both statistically resembling the content found in plants in the control treatment. (Fig.1).

On the 28th day after defoliation, there was a reduction in the RS content in the roots, and in the TSS content in the leaves and roots, in addition to starch content of the leaves for the plants under defoliation (Fig. 1). Meanwhile, there was an increase in the content of reducing sugars in the leaves and starch in the roots. The application of GSNO caused a positive effect at both concentrations, reducing the content of reducing sugars in the leaves and starch in the roots that had been high in plants that had undergone defoliation (Fig. 1). Moreover, the content of soluble sugars in the leaves increased to a level similar to that of control plants (Fig. 1 c). Starch content in the leaves, which had been reduced by defoliation, increased at 100 µM (Fig. 1 e).

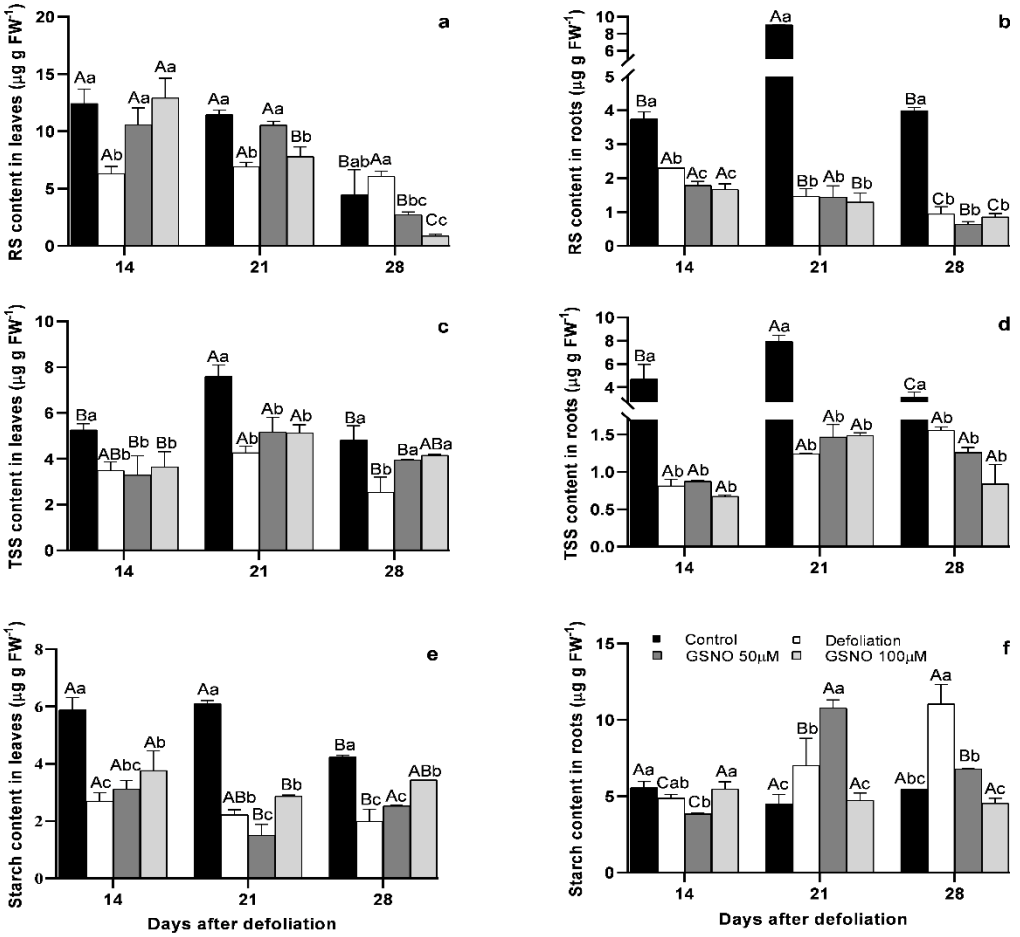

Fig.1: Content of reducing sugars (a and b), total soluble sugars (c and d) and starch (e and f) in maize leaves and roots subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

Protein and amino acid content

Protein content showed a difference between treatments and evaluation days after defoliation (Fig. 2.a-b). In

the leaves, it is possible to observe that plants in the control treatment showed a higher concentration of proteins on all days of evaluation, followed by plants under defoliation (Fig. 2 a). In general, plants that received the application of GSNO at both concentrations showed lower protein concentrations (Fig. 2.a). In the roots, protein concentration was higher in control plants compared to other treatments on all days evaluated (Fig. 2 b). On the 28th day, plants sprayed with 100 µM GSNO showed higher protein concentrations when compared to plants under defoliation (Fig. 2.b). Furthermore, in general, protein content in the leaves was higher than the protein content in the roots on both days of evaluation (Fig. 2.a-b).

The total concentration of amino acids was higher on the 14th day after defoliation, mainly for leaves, with a decreasing tendency over time (Fig. 2.c-d). In the leaves of plants corresponding to the control treatment, there was a higher concentration of amino acids, with a reduction on the other days of evaluation (Fig. 2.c). The application of 50 and 100 µM GSNO provided an increase in the concentration of amino acids, especially on the 21st day (Fig. 2.c). In the roots, the concentration of amino acids was higher in control plants on the 14th and 21st days in relation to the other treatments (Fig. 2.d). The application of GSNO to maize plants increased amino acid content in the roots when compared to plants under defoliation on the 14th day, at both concentrations (Fig. 2.d).

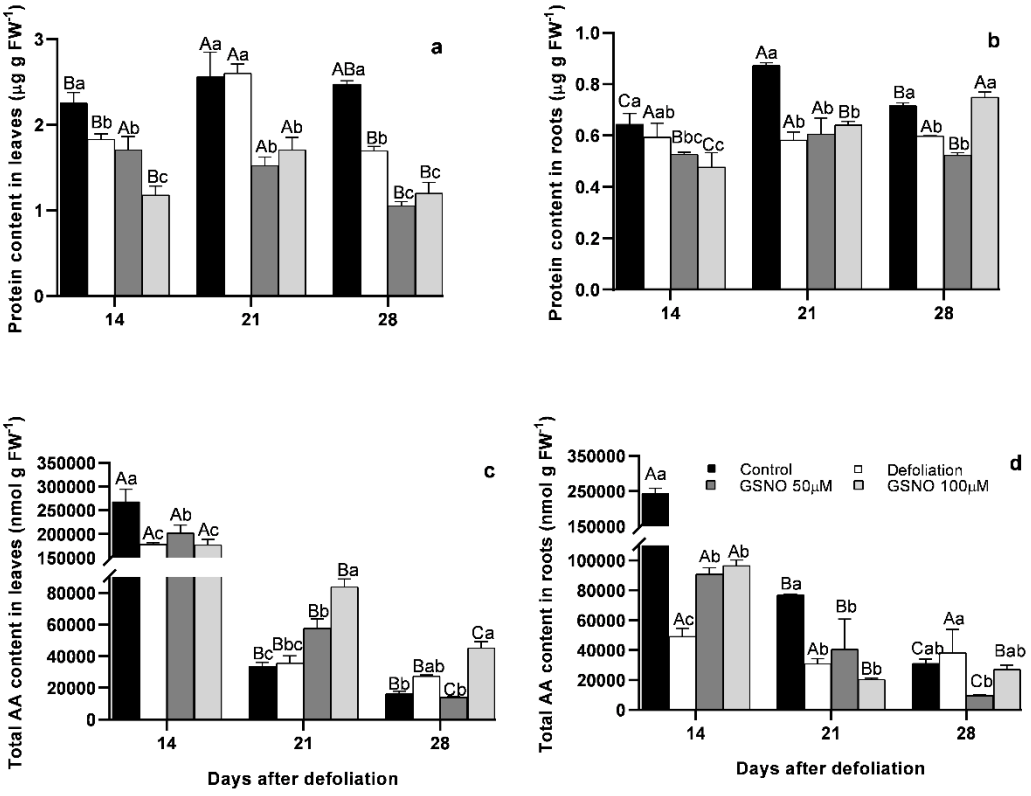

Fig. 2: Protein (a and b) and total amino acid (c and d) contents in maize leaves and roots subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

On the 14th day after defoliation, it was possible to observe that the concentration of aspartate in the leaves reduced with defoliation (Fig. 3.a), as well as the concentration of glutamate (Fig. 3.c), serine (Fig. 4 a), and arginine (Fig. 4 e). Asparagine (Fig. 3 e) had its content increased under defoliation. Regarding treatments with GSNO, at a concentration of 50 µM, asparagine (Fig. 3 e) and serine (Fig. 4 a) presented concentrations similar to the control treatment. In the treatment with GSNO 100 µM, the concentration of asparagine (Fig. 3 e) was also higher than that in defoliation and control.

On the 14th day after defoliation, the AA in the roots that suffered a reduction in their content, when comparing defoliation with the control treatment, were aspartate (Fig. 3 b), glutamate (Fig. 3 d), serine (Fig. 4 b), glutamine (Fig. 4 d) and arginine (Fig. 4 f). Asparagine (Fig. 3 f) had its content increased with defoliation. Glutamate (Fig. 3 d) showed higher concentration with the application of 50 µM GSNO compared to defoliation. The serine levels (Fig. 4 b) were higher in relation to the defoliation and control treatments at the same concentration (50 µM). In the 100 µM GSNO treatment, the concentrations of aspartate (Fig. 3 b), glutamine (Fig. 4 d) and arginine (Fig. 4 f) were higher than in the defoliation treatment, while asparagine (Fig. 3 f) presented a higher concentration than the control and defoliation. Serine was similar to the control treatment (Fig. 4 b).

On the 21st day after defoliation, there was an increase in the levels of glutamate (Fig. 3 c) and asparagine (Fig. 3 e) in the leaves in the defoliation treatment in relation to the control treatment. Furthermore, compared to the two treatments, there was a reduction in the concentration of arginine (Fig. 4 e), while plants that received 50 µM GSNO increased the concentration of aspartate (Fig. 3 a), glutamate (Fig. 3 c) and arginine (Fig. 4 e), with levels higher than the defoliation and control treatments. In comparison, plants receiving 100 µM GSNO had increased aspartate (Fig. 3 a), glutamate (Fig. 3 c), and arginine (Fig. 4 e) content above defoliation and control treatments.

Regarding the roots, on the 21st day after mechanical damage, there was a reduction in the content of glutamate (Fig. 3 d), serine (Fig. 4 b), glutamine (Fig. 4 d) and arginine (Fig. 4 f) in the roots. plants that suffered defoliation in relation to plants in the control treatment. Plants that received the application of 50 µM GSNO showed an increase in glutamate concentration (Fig. 3 d) in relation to defoliation. There was also an increase in glutamine content (Fig. 4 d) in relation to defoliation and control. Furthermore, the application of GSNO at this concentration increased the arginine content (Fig. 4 f), similar to the control treatment.

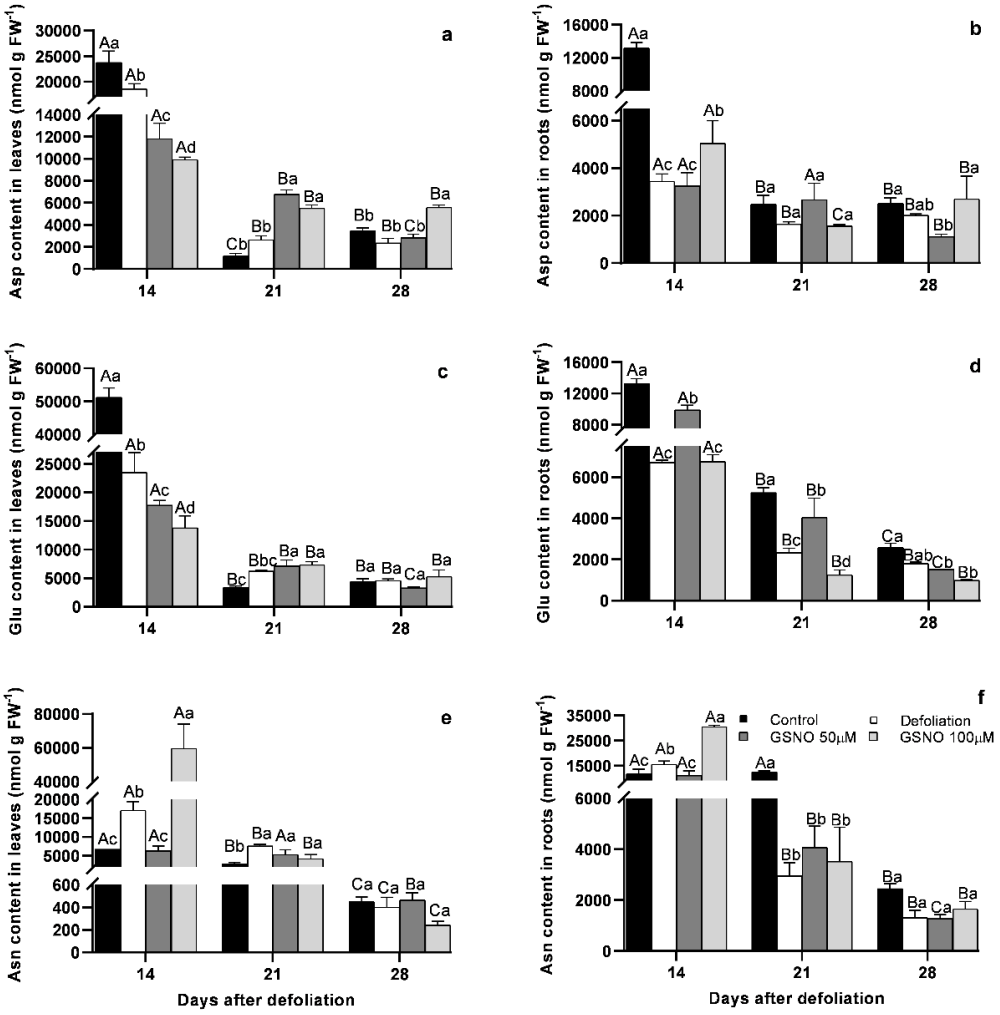

Fig. 3: Aspartate (a and b), glutamate (c and d) and asparagine (e and f) content in maize leaves and roots subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

On the 28th day after defoliation, the application of 100 µM of GSNO promoted an increase in aspartate (Fig. 3 a) in the leaves, higher than that of the defoliation and control treatments. On the 28th day, in the roots of plants that underwent defoliation there was a reduction in aspartate (Fig. 3 b), glutamate (Fig. 3 d) and arginine (Fig. 4 f). When subjected to treatments with 100 µM GSNO, there was an increase in aspartate, similar to the concentrations in the control treatment (Fig. 3 b). Furthermore, there was an increase in serine (Fig. 4 b) greater than the other treatments.

Finally, among the results obtained in the analysis of amino acids, it is possible to observe that glutamate (Fig. 3 c-d), arginine (Fig. 4 e-f), glutamine (Fig. 4 c-d), serine (Fig. 4 a-b) and asparagine (Fig. 3 e-f), present higher concentrations in the leaves in the defoliation treatment in relation to the same treatment in the roots at the beginning of growth resumption. In the same period, in the control treatment, arginine (Fig. 4 e) and glutamate (Fig. 3 c) are the amino acids with the highest concentration in the leaves, while glutamine (Fig. 4 d)

and arginine (Fig. 4 f) are the more abundant in the roots. In contrast, in the defoliation treatment, glutamine (Fig. 4 c) and arginine (Fig. 4 e) are more abundant in the leaves and serine (Fig. 4 b), together with asparagine (Fig. 4 f), in the roots.

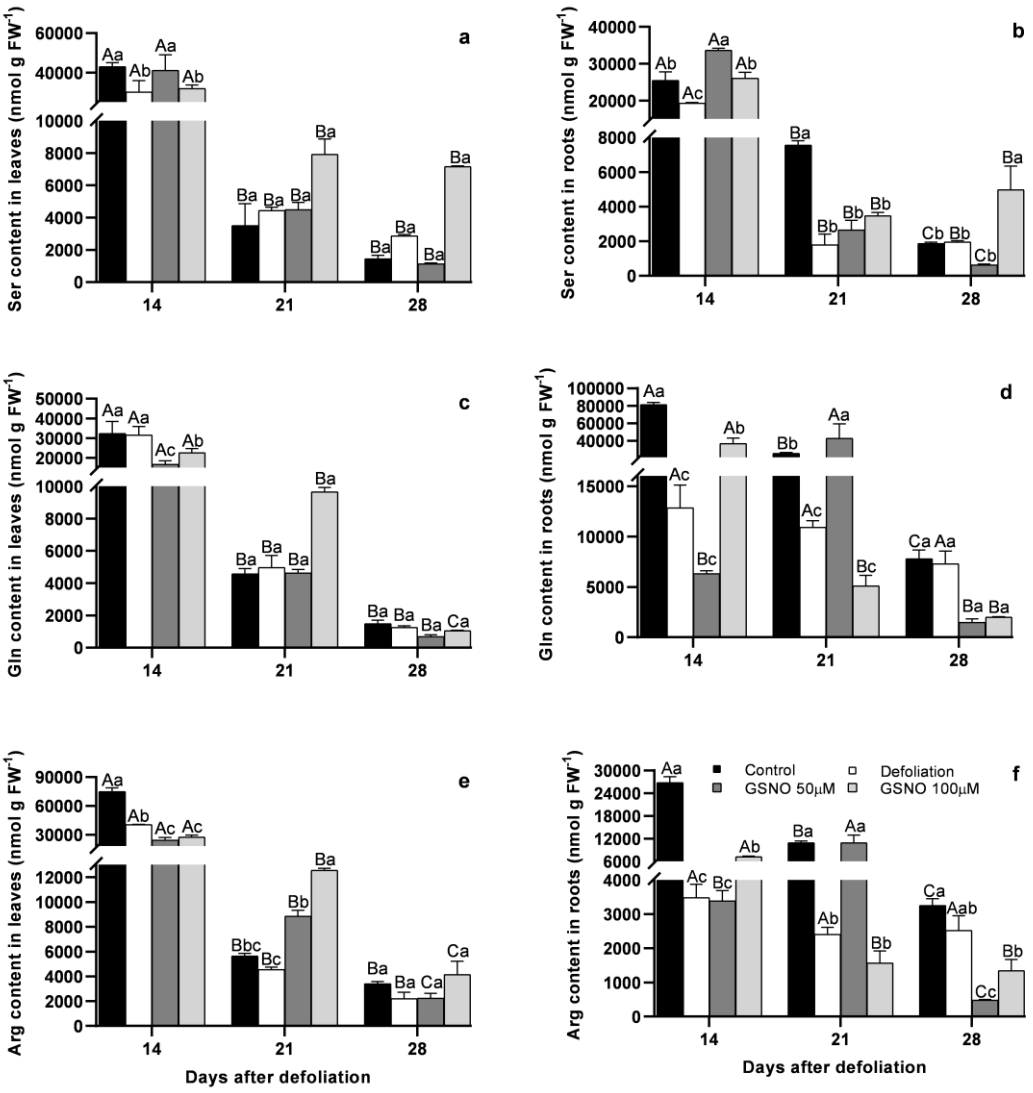

Fig. 4: Serine (a and b), glutamine (c and d) and arginine (e and f) content in maize leaves and roots subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

Gas exchange and root morphology

In gas exchange, photosynthesis (A) was greater in control plants on the 14th and 21st days (Fig. 5.a). However, spraying 50 µM GSNO increased A and stomatal conductance (gs) in relation to defoliation in the first evaluation (14th day) (Fig. 5.b). On the 21st day, A and gs showed the same behavior, with control plants

superior to the other treatments (Fig. 5.a-b). The application of 100 µM GSNO on the 28th day of evaluation increased A and gs (Fig. 5.a-b).

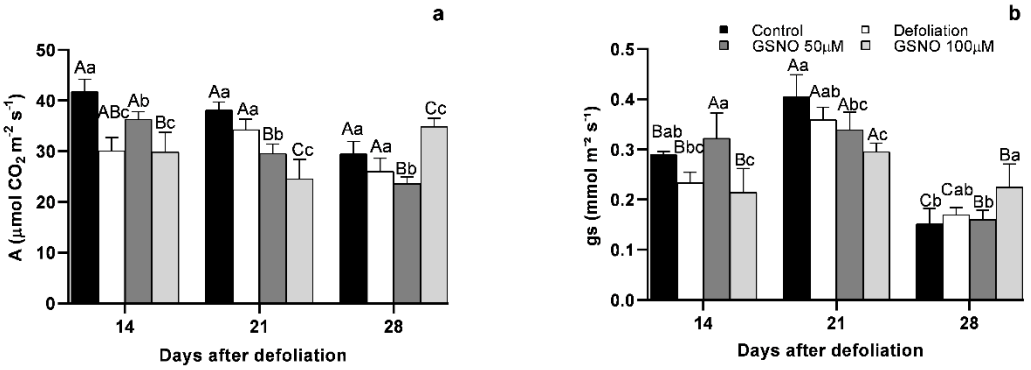

Fig. 5: Gas exchange (Photosynthesis: A (a); Stomatal Conductance: Gs (b)) of maize subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

In root morphology, the length, volume, surface area, diameter and root dry mass of maize plants in the control treatment were greater in relation to the other treatments at the three times evaluated (Table 1). Spraying 50 µM GSNO increased the surface area in the first evaluation when compared to plants under defoliation (Table 1). The same pattern was observed for root volume and diameter on the 21st day (Table 1). The application of 100 µM GSNO increased root length on the 21st day and root diameter on the 28th day, when compared to plants under defoliation (Table 1).

Table 1: Root morphology of maize plants subjected to defoliation and spraying of 50 µM and 100 µM GSNO, 14, 21 and 28 days after stress.

Days after defoliation	Root Parameters	Treatments			
		Control	Defoliation	50 µM GSNO	100 µM GSNO
14 days	Length (cm)	12144.50 Ca	2353.43 Cb	3572.59Ab	2692.84 Bb
	Volume (cm ³)	36727.00 Ca	6543.50 Bb	5965.50Bb	5528.00 Ab
	Surface area (cm ²)	2415.76 Ca	435.14 Bc	673.886Ab	432.48 Cc
	Diameter (mm)	1.20 Ba	0.51 Ab	0.52 Bb	0.53 Ab
	Dry weight (g)	2.32 Ca	0.14 Bb	0.46 Ab	0.35 Ab
21 days	Length (cm)	21699.35 Aa	4543.49Bbc	3425.88Ac	5209.39 Ab
	Volume (cm ³)	80629.0 Ba	11293.33Abc	13657.50Ab	7914.00 Ac
	Surface area (cm ²)	4950.72 Aa	819.55 Ab	659.56 Ac	667.77 Bc
	Diameter (mm)	2.40 Aa	0.52 Ac	0.66 Ab	0.44 Bc
	Dry weight (g)	4.67 Ba	0.51 Ab	0.34 Ab	0.36 Ab
28 days	Length (cm)	139464.89Ba	5932.99 Ab	4334.11Ac	4438.32 Ac
	Volume (cm ³)	96963.00 Aa	11760.00 Ab	7820.0 Bb	9339.50 Ab
	Surface area (cm ²)	4525.97 Ba	921.34 Ab	650.28 Ac	871.99 Ab
	Diameter (mm)	2.38 Aa	0.51 Abc	0.45 Bc	0.57Ab
	Dry weight (g)	5.54 Aa	0.55 Ab	0.61 Ab	0.43 Ab

Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

The specific root length (SRL) of defoliated plants was greater than that of control plants on all days evaluated (Fig. 6.a). On the 14th day after defoliation, the SRL of plants under defoliation was higher than the other treatments (Fig. 6.a). On the 21st day after defoliation, the application of 100 µM GSNO increased SRL in maize plants in relation to the other treatments (Fig. 6.a). On the 28th day, spraying 100 µM GSNO was similar to the defoliation treatment, but superior when compared to the other treatments (Fig. 6.a).

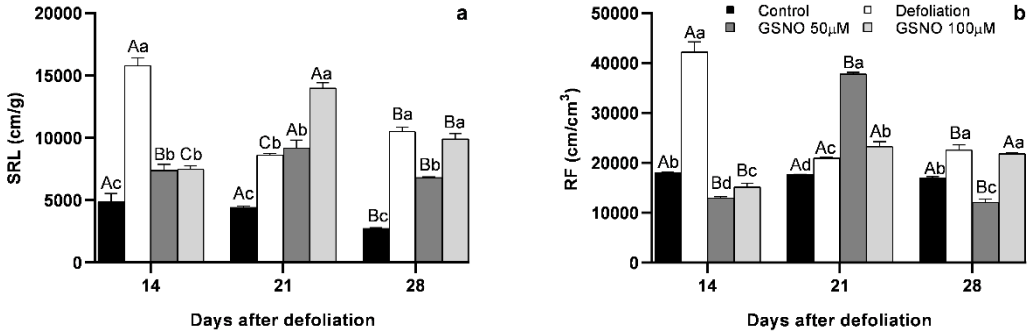

Fig. 6: Root morphological attributes (Specific Root Length: SRL (a); Root Fineness: RF (b)) of maize subjected to defoliation and treatment with GSNO at 50 µM and 100 µM, 14, 21 and 28 days after stress. Means followed by the same letter do not differ statistically by the Tukey test at a 5% probability ($p \leq 0.05$). Uppercase letters compare treatments over time. Lowercase letters compare treatments at each time point. Each value indicates the treatment mean ± standard deviation (SD).

In root fineness (RF), on the 14th day after the imposition of treatments, plants under defoliation

showed higher RF when compared to other treatments (Fig. 6.b). However, the application of 50 µM GSNO to plants increased RF when compared to the other treatments (Fig. 6.b). On the 28th day, plants under defoliation and spraying of 100 µM GSNO were similar in RF and superior to the other treatments (Fig. 6.b).

Discussion

Defoliation caused a reduction in sugars and starch in the leaves and roots of maize plants, especially in the first days after stress imposition. This may be associated with the importance of these metabolites for plant growth and development. The fact is that plants have to modulate their metabolism and sugar transport in order to mitigate the harmful effects caused by environmental stress, such as defoliation (Saddhe et al. 2021).

When subjected to defoliation, maize plants suffered a reduction in protein content in the leaves and amino acids in the shoot and roots. Furthermore, there is a clear decrease in amino acid content over time, in accordance with leaf development and senescence in all treatments, with the highest concentration of amino acids observed on the fourteenth day after defoliation. In general, growing leaves require a greater reallocation of carbon and nitrogen compounds between roots and leaves, as is the case with defoliated maize plants (Meuriot et al. 2018). It is still possible to relocate from older to younger tissues due to source-sink relationships and due to their mobility (Lefevre et al. 1991). This explains, in part, the decline in amino acid content in plants subjected to defoliation, as well as their reduction in all treatments over time. Thus, after defoliation, maize plants can regulate the amino acid content and their transport throughout the plant to adapt the plant carbon and nitrogen status for the development of new leaves (Arnold et al. 2015; Zeier 2013). Finally, several secondary metabolites are synthesized from amino acids (Jan et al. 2021), which also explains the reduction in amino acids in plants subjected to defoliation.

Defoliation induced a rapid reduction in amino acids in the root and an increase in the leaves, mainly asparagine, alanine and glycine. This indicates that this type of stress causes a reallocation of resources, inducing a relative increase in N allocation to growing leaves at the expense of roots. In other words, the nitrogen reserve of the roots contributed to the recovery of nitrogen content in growing leaves (Meuriot et al. 2018).

Defoliation led to a reduction in aspartate levels in roots and leaves on the first day evaluated. This amino acid is derived from oxaloacetate via transamination of glutamate in the cytosol. In C4 plants, such as maize, aspartate can be transported to the cells of the vascular sheath, where it can be converted into oxaloacetate and alanine, increasing CO_2 around the rubisco active site, favoring C fixation. Moreover, this amino acid can be converted into asparagine, through the action of asparagine synthetase (Kerbauy 2004; Taiz et al. 2017). This fact matches the results found in this study, where aspartate was reduced at the same time as there was an increase in asparagine on the first day of evaluation.

GSNO led to an increase in Asp in leaves above that found in defoliation and control treatments at both concentrations. At 100 µM, the photosynthetic rate was increased, as well as stomatal conductance on the 28th day after defoliation. This result may indicate that GSNO acted as a biostimulant, favoring the recovery of defoliated corn plants through mechanisms that induce improvements in photosynthesis under stress.

Defoliation led to a reduction in glutamate (Glu) levels in leaves and roots on the fourteenth day after stress. This event can be explained by the fact that Glu connects carbon and nitrogen metabolism and serves as a N donor for the biosynthesis of other amino acids such as glutamine, arginine and proline, as well as other nitrogen-containing compounds (Hu et al. 2023; Kerbauy 2004). Under stress, this conversion of glutamate into other compounds can occur quickly, in order to quickly mitigate the deleterious effects. Furthermore, glutamate

can trigger mechanical stress signals based on calcium channels, which are essential for the transport of proteins that precede responses (Kouhen et al. 2023; Lian et al. 2022). Such characteristics may explain the reduction in Glu, especially in the initial recovery phases of maize plants under defoliation.

In relation to glutamine, defoliation showed a reduction in both leaves and roots compared to the control. However, the reduction was more pronounced in the roots. Gln is formed from glutamate and ammonium; its synthesis is catalyzed by glutamine synthetase (GS), serving as a more stable reserve of glutamate and also a source of nitrogen transport (Kerbauy 2004). GS is related to the regulation of nitrogen levels in plants, and can directly affect several photosynthetic and metabolic aspects, in addition to being strongly responsive to abiotic stress and being involved in processes related to tolerance increase. Besides, its expression is altered and regulated by carbohydrate levels and light (Miflin and Habash 2002), indicating a relationship between loss of leaf area and mobilization of carbohydrates in plants that have undergone mechanical damage, with the reduction in glutamine in the treatment with defoliation.

With defoliation, maize may have sought to increase N content and transport, since there was an increase in asparagine in the leaves and roots of maize plants subjected to stress. This amino acid has great stability due to its high C:N ratio (4:2), being highly assimilated under stress conditions due to this characteristic (Hildebrandt et al. 2015), which justifies its increase with the imposition of defoliation. Asparagine is associated with the GS/GOGAT pathway, whereas changes in its content can cause changes in the concentrations of glutamine and glutamate (Hildebrandt et al. 2015; Miflin and Habash 2002). Therefore, the increase in asparagine may be associated with a reduction in the content of both amino acids in plants subjected to defoliation, thus showing the relationship between these pathways for the assimilation, transport and storage of nitrogen under adverse conditions, such as mechanical damage by defoliation

In leaves from the control treatment, arginine was the amino acid with the highest content. There was also a drastic reduction in its content in roots and leaves subjected to defoliation. However, in treatments that underwent defoliation and received the application of GSNO at both concentrations, there was an increase in its content in the roots in relation to defoliation. It is known that there is a close relationship between the availability of carbohydrates and the use of arginine. Therefore, the reduction in sugar levels causes a substantial increase in the activity levels of the enzymes arginase, urease and arginine decarboxylase, in order to synthesize polyamines and NO (Winter et al. 2015). These are important stress signals and development regulators and may explain the drastic reduction in arginine content in the defoliation treatments compared to the control in both the aerial and root parts. In addition to being directly related to the reduction in sugar (reducing and total soluble) and starch content. The fact that Arg is a precursor of NO and polyamines may explain, in part, its increase in roots in treatments with GSNO in relation to defoliation (Winter et al. 2015) and both may be related in their signaling pathways and formation under abiotic stress conditions (Tun et al. 2006; Wimalasekera et al. 2011).

There was an increase in serine in treatments that received GSNO application, as well as a reduction in carbohydrate content in leaves and roots. One of the ways in which serine is formed is through the phosphorylation of 3-phosphoglycerate, which is produced from sucrose and starch via glycolysis (Waditee et al. 2007). This fact links serine directly to the metabolism of C and N since, while carbohydrate levels were reduced, their levels increased, indicating that the formation of this amino acid may have occurred through them. Furthermore, Ser had its content increased in the presence of the NO donor (GSNO). This fact may be related to the role that NO plays on hydrolytic enzymes or terminal glucose phosphorylation pathways that mobilize starch,

with an increase in β-amylase activity being observed in plants that received the application of the NO donor solution (Zhang et al. 2005).

In the case of defoliation, there was a reduction in the photosynthetic area of the plant, decreasing photosynthesis (A) and, as a result, negatively impacting sugar levels. In addition, when analyzing gas exchange, A and stomatal conductance (gs) were higher in maize plants with the application of 50 µM GSNO. This result may suggest that spraying with GSNO maximized CO_2 availability in the intercellular spaces of maize leaves (increased gs), mitigating possible photosynthetic limitations, favoring the increase in A and the resumption of leaf growth immediately after mechanical damage. This fact is also observed with the spraying of 100 µM GSNO on the 28th day. Therefore, it is possible to infer that higher concentrations of GSNO present later beneficial responses in maize plants. This is related to valine concentrations, where its degradation or synthesis can favor the maintenance of gs.

When analyzing root morphology, it is evident that defoliation reduces the development of maize roots, due to the relocation of assimilates (sugars and starch) and nitrogenous compounds (proteins and amino acids) to the shoot under reconstruction. The results found in this research confirm that all resources were initially directed to the shoot, aiming to restore the leaves and causing remodeling in the root system of maize plants. Moreover, defoliation resulted in a decrease in all morphological variables, which reflected in a reduction in root biomass.

It is worth highlighting that the application of 50 µM GSNO increased the root surface area of maize plants on the 14th day, suggesting that nitric oxide is favoring greater absorption of nutrients and water by the roots (Imada et al. 2008; Marques et al. 2018). Besides, it was observed that the same concentration increased root fineness (RF) on the 21st day. Plants can produce longer roots by increasing biomass allocation or root fineness and/or reducing root tissue density, leaving biomass allocation unchanged (Abenavoli et al. 2016). Thus, nitric oxide induces remodeling of the root system, preserving finer maize roots under defoliation.

Defoliation induced greater specific root growth (SRL). The increase in SRL by defoliated plants is possibly a survival strategy, since a greater SRL reflects in greater exploration and acquisition of water and nutrients in the soil per unit of carbon invested (Bouma et al. 2001). This may also be related to the increase in starch concentration in the roots on the 21st and 28th day after defoliation in plants from the defoliation treatment and those from the treatment with 50 µM GSNO since, under certain stress conditions, starch can be stored in the roots for later remobilization to support root growth when favorable conditions are restored (Fabre et al. 2019).

Conclusion

Defoliation in maize causes a mobilization of soluble reducing sugars, starch, proteins and amino acids from the roots for shoot recovery, especially on the first days after stress imposition. Maize plants undergoing defoliation reestablish their metabolism on the twenty-eighth day after defoliation.

Nitric oxide (50 and 100 µM) is capable of causing changes in the amino acid pools related to nitrogen transport, such as asparagine. Furthermore, it modifies root morphology with an increase in length, surface area, volume, specific length and root fineness, as well as in carbon compounds that favor the recovery of the plant under defoliation. Thus, GSNO can play a role in regulating the homeostasis of plants subjected to this mechanical stress. However, it is still necessary to further explore its mechanisms of action.

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4 CONSIDERAÇÕES FINAIS

A desfolha tem efeitos significativos nos metabólitos primários das plantas de milho, afetando aminoácidos, açúcares e amido. No entanto, a aplicação de GSNO como bioestimulante pode mitigar esses efeitos negativos, promovendo respostas que beneficiam a recuperação das plantas após a desfolha. Isso sugere o potencial do GSNO como uma ferramenta para melhorar a resistência das plantas de milho ao estresse e promover um crescimento mais saudável.

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