

**UNIVERSIDADE FEDERAL DE ALFENAS**

**JOICE APARECIDA DE NOVAIS PORTUGAL**

**DERIVADOS DE TREALOSE NO SISTEMA ANTIOXIDANTE E  
FOTOSSINTÉTICO DE PLANTAS DE MILHO EXPOSTAS AO DÉFICIT HÍDRICO**

**ALFENAS/MG**

**2019**

**JOICE APARECIDA DE NOVAIS PORTUGAL**

**DERIVADOS DE TREALOSE NO SISTEMA ANTIOXIDANTE E  
FOTOSSINTÉTICO DE PLANTAS DE MILHO EXPOSTAS AO DÉFICIT HÍDRICO**

Dissertação apresentada à Universidade Federal de Alfenas como parte das exigências do Programa de Pós-Graduação em Ciências Ambientais, para a obtenção do título de "Mestre".

Área de concentração: Tecnologia Ambiental.

Orientador: Prof. Dr. Thiago Corrêa de Souza

Coorientadora: Dr<sup>a</sup>. Kamila Rezende Dázio de Souza

**ALFENAS/MG**

**2019**

Dados Internacionais de Catalogação-na-Publicação (CIP)  
Sistema de Bibliotecas da Universidade Federal de Alfenas

P853d Portugal, Joice Aparecida de Novais.  
Derivados da Trealose no sistema antioxidante e fotossintético de plantas de milho expostas ao déficit hídrico / Joice Aparecida de Novais Portugal -- Alfenas/MG, 2019.  
55 f. --

Orientador: Thiago Corrêa de Souza.  
Dissertação (Mestrado em Ciências Ambientais) - Universidade Federal de Alfenas, 2019.  
Bibliografia.

1. Milho. 2. Secas. 3. Fluorescência. 4. Clorofila. 5. Prolina. 6. Estimulantes. I. Souza, Thiago Corrêa de. II. Título.

CDD-338.14



MINISTÉRIO DA EDUCAÇÃO  
Universidade Federal de Alfenas / UNIFAL-MG  
Programa de Pós-graduação – Ciências Ambientais  
Rua Gabriel Monteiro da Silva, 714, Alfenas - MG CEP 37130-000  
Fone: (35) 3701-9685 (Coordenação) / (35) 3701-9268 (Secretaria)  
<http://www.unifal-mg.edu.br/ppgca/>



**JOICE APARECIDA DE NOVAIS PORTUGAL**

**“Derivados da trealose no sistema antioxidante e fotossintético de plantas de milho expostas ao déficit hídrico”**

A Banca julgadora, 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.

Aprovada em: 20 de dezembro de 2019.

Prof. Dr. Thiago Corrêa de Souza  
Instituição: UNIFAL-MG

Assinatura:

Prof. Dr. Adriano Bortolotti da Silva  
Instituição: UNIFENAS

Assinatura:

Dr. Luiz Carlos de Almeida Rodrigues  
Instituição: UNIFAL-MG

Assinatura:

## AGRADECIMENTOS

À minha família, minha base, pela qual luto por um mundo melhor, especialmente a meus filhos, Mariana e Mateus Henrique e o apoio incondicional de meu esposo Gleiber, necessário para que eu alcançasse meus objetivos, amo vocês. Agradeço a meus pais Ana Maria e Milton pela presença e apoio, principalmente por me ensinarem a nunca desistir diante as dificuldades impostas em meu caminho. Ao meu irmão Milton Jr. sempre presente e me apoiando e meu irmão Cleber, sempre em meu coração. Minhas cunhadas Jerusa e Luciana, pelo apoio e compreensão. Meu sobrinho Pedro Henrique e sobrinho/afilhado Pedro. Compartilho com vocês minha felicidade neste momento e espero que estejam orgulhosos.

Aos meus amigos (distantes e presentes), que sempre tiveram uma palavra de carinho e quando por diversas vezes o fardo estava pesado, dividiram-no comigo. Agradeço a todos professores que passaram por minha vida, esta conquista é semente plantada por vocês.

Agradeço a pessoa especial que me ensinou e é motivo de inspiração, Kamila Dázio, a Leticia e Valdir, pessoas admiráveis que pretendo levar pela vida, contribuíram para o meu crescimento, vocês três são especiais. Aos amigos e colegas do Laboratório BIOGEN onde risadas e lágrimas foram compartilhadas. Aos queridos Pedro, Gabriel, Marcus por toda a ajuda no processo deste trabalho e tantos outros que ajudaram nas coletas e atividades de campo. À nossa técnica Gabi por manter ordem e zelo, sempre elegante e dedicada.

Ao meu orientador, Prof. Dr. Thiago Corrêa de Souza, pessoa ímpar que me acolheu, acompanhou e aconselhou. É imenso o meu carinho e admiração, obrigada pela acolhida, paciência e consideração. A você e sua esposa o meu carinho e minhas orações.

À minha companheira de mestrado, Alexandra, você foi fundamental neste processo, obrigada pela preocupação, por estar junto nas limitações e desafios, nós vencemos. À Daniele Marques por me incentivar e me ajudar a iniciar esta caminhada.

À Universidade Federal de Alfenas e ao Programa de Pós-Graduação em Ciências Ambientais pela oportunidade.

Ao apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo financiamento do projeto de pesquisa envolvido neste estudo (Processo 423584/2016-2, chamada Universal 01/2016).

*Gratidão!*

## RESUMO

Na agricultura um dos fatores limitantes do desenvolvimento das plantas e que afeta diretamente no resultado final da produção é a disponibilidade de água. Com as constantes mudanças climáticas, faz-se necessário a realização de estudos para a descoberta de novas tecnologias visando minimizar o estresse hídrico causado nas plantas, principalmente na fase de germinação e crescimento, floração e enchimento dos grãos. Este trabalho objetivou-se a analisar a aplicação de novos dissacarídeos derivados de trealose em plantas de milho verificando a capacidade de indução a tolerância à seca. O experimento foi conduzido em casa de vegetação, utilizando o milho híbrido BRS 1030 sensível ao déficit hídrico. O estresse hídrico foi aplicado quando as plantas atingiram o estágio vegetativo V6 e foi imposto por 12 dias, com aplicação foliar da mistura dos derivados (30mM) no primeiro e décimo dia de imposição do déficit hídrico. As análises da eficiência fotossintética da clorofila a ( $ETR_{max}$ ,  $I_k$  e  $\alpha$ ), foram realizadas no primeiro e último dia de imposição do déficit hídrico e 12 horas após a reidratação, obtendo-se curvas rápidas de luz através de um fluorímetro. As coletas foliares para análise bioquímica foram realizadas no início e fim do déficit hídrico e 12 horas após a reidratação das plantas. Foram avaliados a peroxidação lipídica através dos teores de MDA e a atividade enzimática antioxidante SOD, APX, CAT e POD. Ao final do experimento a parte aérea e raízes foram coletadas para análises das concentrações de açúcares redutores, açúcares solúveis totais, amido, proteína, prolina e compostos fenólicos. Foi possível concluir que, a aplicação exógena da mistura dos derivados da trealose mostrou-se eficiente na mitigação de danos causados pelo déficit hídrico através da ativação das enzimas antioxidantes (SOD, APX e POD), além de promover o acúmulo de açúcares, prolina e compostos fenólicos e melhorando a eficiência fotossintética no híbrido de milho BRS 1030.

Palavras-chave: Milho. Secas. Fluorescência. Clorofila. Prolina. Estimulantes.

## ABSTRACT

In agriculture one of the limiting factors of plant development that directly affects the final production result is water availability. With the constant climate changes, it is necessary to carry out studies to discover new technologies to minimize water stress caused on plants, especially in the germination and growth, flowering and fruit filling phase. This work aimed to analyze the application of new trehalose-derived disaccharides in maize plants by checking the drought tolerance induction capacity. The experiment was carried out in a greenhouse using BRS 1030 hybrid corn sensitive to water deficit. Water stress was applied when the plants reached vegetative stage V6 and was imposed for 12 days, with foliar application of the mixture of derivatives (30mM) on the first and tenth day of water deficit imposition. The photosynthetic efficiency analyzes of chlorophyll a (ETR<sub>max</sub>, I<sub>k</sub> and  $\alpha$ ) were performed on the first and last day of water deficit imposition and 12 hours after rehydration, obtaining fast light curves through a fluorimeter. Leaf collections for biochemical analysis were performed at the beginning and end of water deficit and 12 hours after plant rehydration. Lipid peroxidation through MDA contents and antioxidant enzymatic activity SOD, APX, CAT and POD were evaluated. At the end of the experiment the leaves and roots were collected to analyze the concentrations of reducing sugars, total soluble sugars, starch, protein, proline and phenolic compounds. It was concluded that the exogenous application of the trehalose derivatives mixture proved to be efficient in mitigating damage caused by water deficit through the activation of antioxidant enzymes (SOD, APX and POD), besides promoting the accumulation of sugars, proline and phenolic compounds and improving photosynthetic efficiency in the BRS 1030 maize hybrid.

Keywords: *Zea mays* L.. Drought. Fluorescence. Chlorophyll. Proline. Stimulants.

## SUMÁRIO

<b>1</b>	<b>INTRODUÇÃO .....</b>	<b>9</b>
<b>2</b>	<b>REVISÃO BIBLIOGRÁFICA .....</b>	<b>11</b>
<b>2.1</b>	<b>EFEITO DO DÉFICIT HÍDRICO EM PLANTAS DE MILHO.....</b>	<b>11</b>
<b>2.2</b>	<b>ESPÉCIES REATIVAS DE OXIGÊNIO.....</b>	<b>12</b>
<b>2.3</b>	<b>SISTEMA ANTIOXIDANTE: ENZIMAS ANTIOXIDANTES E COMPOSTOS FENÓLICOS.....</b>	<b>13</b>
2.3.1	SUPERÓXIDO DISMUTASE (SOD EC 1.15.1.1) .....	13
2.3.2	CATALASE (CAT EC 1.11.1.6).....	13
2.3.3	ASCORBATO PEROXIDASE (APX EC 1.1.11.1) .....	14
2.3.4	PEROXIDASE DO GUAIACOL (POD EC 1.11.1.7).....	14
2.3.5	COMPOSTOS FENÓLICOS .....	15
<b>2.4</b>	<b>METABOLISMO PRIMÁRIO .....</b>	<b>15</b>
2.4.1	AÇÚCARES SOLÚVEIS TOTAIS E AÇÚCARES REDUTORES .....	15
2.4.2	AMIDO.....	16
2.4.3	PROLINA E PROTEÍNAS .....	17
<b>2.5</b>	<b>EFICIÊNCIA FOTOSSINTÉTICA.....</b>	<b>17</b>
<b>2.6</b>	<b>TREALOSE .....</b>	<b>18</b>
<b>2.7</b>	<b>EFEITOS DA APLICAÇÃO DA TREALOSE E DERIVADOS EM PLANTAS</b>	<b>18</b>
<b>3</b>	<b>OBJETIVOS .....</b>	<b>20</b>
<b>3.1</b>	<b>OBJETIVOS GERAIS .....</b>	<b>20</b>
<b>3.2</b>	<b>OBJETIVOS ESPECÍFICOS.....</b>	<b>20</b>
<b>4</b>	<b>JUSTIFICATIVA .....</b>	<b>21</b>
	<b>REFERENCIAL BIBLIOGRÁFICO .....</b>	<b>22</b>
	<b>ANEXO A .....</b>	<b>32</b>



## 1 INTRODUÇÃO

O milho (*Zea mays L.*) possui alto valor agregado a agricultura mundial e no território nacional ocupa o segundo lugar dentre os grãos mais produzidos atualmente (CONAB, 2018). As grandes variações climáticas afetam diretamente a produção de grãos e juntamente com a baixa disponibilidade de água e o aumento populacional impactam diretamente no resultado final das safras e na disponibilidade de recursos e bens de consumo (MCQUEEN, 2000; ZIPPER; QIU; KUCHARIK, 2016). Portanto a busca por alimentos com maior teor de nutrientes e plantas mais tolerantes são uma das vertentes de estudos na tentativa de balancear a oferta de recursos e o crescente aumento populacional, buscando soluções para a fome no mundo.

Neste contexto é primordial a promoção de estudos relacionados a tolerância à seca ou déficit hídrico, levando em consideração que este estresse abiótico é o que mais compromete os períodos de desenvolvimento e a morfologia das plantas, intensificando a atividade metabólica e gasto energético para a manutenção do desenvolvimento da planta (SARMENTO et al., 2019, ULLAH et al., 2018). A baixa disponibilidade de água no solo, juntamente com as altas temperaturas acionam mecanismos fisiológicos na tentativa de melhorar o controle da temperatura e transpiração, mas esta alteração fisiológica pode resultar em um desenvolvimento inferior ao esperado e consequentemente redução na produção final (RABÊLO et al., 2019).

Espécies mais tolerantes aos danos causados pelo déficit hídrico e com maior rendimento por área de plantio pode ser a resposta para o aumento da produção sem a necessidade do aumento da área utilizada para o plantio (WU et al., 2018). Em espécies vegetais mais tolerantes alguns mecanismos de defesas são observados, como a maior relação entre parte aérea e raiz, células com menor diâmetro, maior espessura da cutícula foliar e aumento na cerosidade, ativação do sistema de defesa antioxidante, alterações no tamanho e frequência estomática, ajuste osmótico, maior eficiência fotossintética entre outros (SOUZA et al., 2013; DE OLLAS; DODD, 2016).

Pesquisas relacionadas ao déficit hídrico ganham notoriedade no cenário nacional e internacional, abrangendo conhecimentos multidisciplinares. Para o desenvolvimento de genótipos tolerantes ao déficit hídrico faz-se necessário o uso de estudos fisiológicos e biotecnológicos. A aplicação de bioestimulantes é um exemplo e tem o intuito de induzir o mecanismo de defesa das plantas, interferindo diretamente na tolerância ao estresse causado pelo déficit hídrico (MULEY et al., 2019). Em milho, observa-se a aplicação de diversos

bioestimulantes como o ácido abscísico (SOUZA et al., 2013b), ácido fúlvico (YANG et al., 2019), ácido ascórbico (ZHANG et al., 2019), quitosana (RABÊLO et al., 2019), trealose (ZHOU et al., 2014).

Estudos como o realizado por Ali e Ashraf (2011) mostram que a aplicação exógena de trealose em milho induziu uma resposta satisfatória em relação aos danos causados pelo déficit hídrico, melhorando o sistema de defesa antioxidante e aumentando a biomassa, o que posteriormente resultou em aumento na produção de grãos. A trealose é um dissacarídeo amplamente encontrado na natureza e que não apresenta toxicidade. Diversas literaturas exploram a relação entre a trealose e a tolerância ao déficit hídrico, mas ainda se faz necessário uma maior contribuição científica sobre o assunto, principalmente envolvendo novos derivados, contribuindo para o desenvolvimento e maior produção de cereais como o milho.

## 2 REVISÃO BIBLIOGRÁFICA

### 2.1 EFEITO DO DÉFICIT HÍDRICO EM PLANTAS DE MILHO

No ambiente natural dificilmente uma planta encontrará todas as condições consideradas ideais para sua reprodução. A falta de alguns nutrientes (macro e micronutrientes), exposição excessiva a radiações, frio, excesso de sal e inclusive estresse hídrico moderado, seja por alagamento ou seca, é comum ao longo do desenvolvimento de toda espécie vegetal (ZHU, 2016; HOU; UFER; BARTELS, 2016). Porém, apenas níveis mais severos de faltas nutricionais e de estresses são prejudiciais causando danos permanentes no desenvolvimento e alterações na estrutura morfológica e fisiológica das plantas (ZHU, 2016).

No milho, segundo Magalhães e colaboradores (2009), a disponibilidade hídrica no solo faz com que ocorram mudanças no gradiente de potencial hídrico no sistema solo-planta-atmosfera, culminando na desidratação das células e tecidos fazendo com que a planta entre em período de estresse. Respostas fisiológicas e químicas, como a regulação estomática para otimizar a assimilação de CO<sub>2</sub> e reduzir a perda de água, diminuição do crescimento celular e limitação da fotossíntese, indução de sistema antioxidante enzimático e não enzimático são induzidas nas plantas para minimizar os danos causados pelo estresse (TOMBESI et al., 2015; SOUZA et al., 2013b; SOUZA et al., 2014).

A intensificação do déficit hídrico altera a turgescência celular, reduzindo o desenvolvimento sistêmico da planta, o que resulta em alterações morfológicas como o enrolamento das folhas, mudança na angulação da disposição foliar, alterações na disposição e tamanhos dos estômatos (SOUZA et al., 2013a). O déficit hídrico reduz consideravelmente a área foliar, conseqüentemente a taxa fotossintética é diminuída. Essas alterações limitam a assimilação de CO<sub>2</sub>, em fases iniciais do desenvolvimento pode resultar em plantas de milho com menor altura devido ao encurtamento dos entrenós e conseqüentemente a fase de produção e enchimento dos grãos será prejudicada, diminuindo a deposição de matéria seca, resultando em grãos menores do que o esperado (MAGALHÃES; DURÃES, 2006; MAGALHÃES et al., 2016).

A nível celular, o déficit hídrico pode facilitar o acúmulo de espécies reativas de oxigênio (EROs) que, em excesso, ativam reações peroxidativas danificando as células das plantas, pigmentos fotossintéticos, as proteínas e lipídios. As EROs são mais evidentes nas folhas por

causa dos pigmentos fotossintéticos e podem ser entendidas pelas plantas como um sinal para ativar as respostas de defesa quando em pequena quantidade (MITTLER et al., 2011).

## 2.2 ESPÉCIES REATIVAS DE OXIGÊNIO

As espécies reativas de oxigênio (EROs) são compostos formados a partir da forma reduzida ou ativada do oxigênio ( $O_2$ ) e como subprodutos do metabolismo anaeróbico. As modificações na concentração de oxigênio em organismos aeróbicos alteram os processos naturais de ativação e inibição de enzimas, aumentando a concentração de EROs que em pequenas quantidades atuam como sinalizadoras de algum distúrbio dentro das células, ativando mecanismos de defesa e de resistência aos diferentes estresses enfrentados pelas plantas ao longo de sua vida (MITTLER et al., 2011).

As EROs podem ser geradas em diferentes compartimentos celulares, mitocôndrias, apoplastos, cloroplastos, vacúolos, núcleos, peroxissomos (CHOUDHURY et al., 2017), formando uma assinatura específica de produção e remoção de EROs, dependendo do estado estacionário ou redox dos compartimentos celulares, e podem ser encontradas desde bactérias até células de mamíferos (QI et al., 2017; ZANDALINAS; MITTLER, 2018). As principais fontes de produção de EROs estão relacionadas como resultado do próprio metabolismo e como sinalização de algum estresse sofrido, geralmente são formadas pela transferência de elétrons do  $O_2$  (DEMIDCHIK, 2015). Estes compostos são energeticamente mais ativos e reagem com facilidade com outras moléculas gerando reações em cascata (KOHLI et al., 2017).

As formas mais comuns de espécies reativas encontradas nas células são representadas pelos grupos de oxigênio singleto ( $^1O_2$ ), radicais superóxidos ( $O_2^-$ ), peróxido de hidrogênio ( $H_2O_2$ ) e hidroxila ( $OH^-$ ) (CHOUDHURY et al., 2017; JAJIC; SARNA; STRZALKA, 2015). O radical superóxido ( $O_2^-$ ) é uma das formas menos reativas e não possui a capacidade de ser transportado pelas membranas celulares, por isso rapidamente é dismutado em  $H_2O_2$  (NIU; LIAO, 2016). Por sua vez o  $H_2O_2$  pode ser gerado a partir da dismutação de radicais superóxidos ou a partir de vias oxidases, NADPH-oxidases, amina oxidases, (WASZCZAK; CARMODY; KANGASJÄRVI, 2018). Embora seja muito estável, é de fácil transporte entre as membranas, sendo relatado como um importante sinalizador de estresse (JAJIC; SARNA; STRZALKA, 2015).

A  $OH^-$  é a espécie mais reativa, podendo ser formada a partir da dismutação de  $O_2^-$  ou  $H_2O_2$  na reação de Haber-Weiss e Fenton (decomposição de peróxido de hidrogênio em radicais

hidroxila altamente reativos na presença de ferro) (FISCHBACHER; VON SONNTAG; SCHMIDT, 2017). A hidroxila reage com todos os compostos celulares e o acúmulo desta espécie reativa pode levar a morte celular. A produção do oxigênio singlete causadas por estresse, assim como  $O_2^-$ , pode afetar a distribuição de energia entre os fotossistemas I e II (PSI e PSII, devido ao excesso de energia absorvida, além de causar alterações na expressão de genes nucleares nos cloroplastos, causando a clorose e conseqüentemente a morte celular (TAKAGI et al., 2016).

### **2.3 SISTEMA ANTIOXIDANTE: ENZIMAS ANTIOXIDANTES E COMPOSTOS FENÓLICOS**

Como resposta ao excesso de EROs, as plantas ativam mecanismos de defesa para manter a homeostase celular e controlar a desintoxicação celular evitando assim, a peroxidação lipídica e os danos oxidativos (HUSSAIN et al., 2019). Um dos mecanismos de defesa é o sistema de desintoxicação composto por enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT), peroxidase do ascorbato (APX) e do guaiacol (POD) (MITTLER et al., 2004; ALI; ASHRAF, 2011; SANTOS et al., 2018).

#### **2.3.1 SUPERÓXIDO DISMUTASE (SOD EC 1.15.1.1)**

A SOD é a primeira enzima da linha de defesa contra EROs e atua realizando a dismutação do radical superóxido em  $H_2O_2$ . Esta enzima está presente em todos os compartimentos celulares suscetíveis ao estresse oxidativo (GILL et al., 2015).

Classificada como uma metaloenzima, depende do seu componente metal de reação e sua localização para ser caracterizada. A Cu/Zn-SOD está localizada no citosol, cloroplastos e peroxissomos, Fe-SOD localizada principalmente nos cloroplastos e podendo ser encontrada nos apoplastos e peroxissomos e Mn-SOD na matriz das mitocôndrias (HASANUZZAMAN et al., 2012) .

#### **2.3.2 CATALASE (CAT EC 1.11.1.6)**

A CAT é uma enzima que possui diferentes isoformas e que usa o  $H_2O_2$  como substrato para a conversão em  $H_2O$  e  $O_2$  (SOFO et al., 2015). Está presente peroxissomos, glioxissomos e organelas onde  $H_2O_2$  é gerado (IANNONE; GROPPA; BENAVIDES, 2015). As catalases podem ser divididas em três classes, onde CAT1 é responsável pela remoção de  $H_2O_2$  gerado no processo de fotorrespiração em tecidos fotossintéticos; CAT2 é encontrada em tecidos vasculares e suas funções biológicas não são bem definidas, supõe-se que esta classe de CATs seja expressa durante a lignificação (ANJUM et al., 2016); e CAT3 sendo expressa em sementes e órgãos jovens, responsável pela remoção de  $H_2O_2$  resultante da degradação de ácidos graxos no ciclo glioxalato (ANJUM et al., 2016).

### 2.3.3 ASCORBATO PEROXIDASE (APX EC 1.1.11.1)

A enzima APX atua em diferentes compartimentos celulares regulando os níveis de EROs, considerada uma enzima de grande importância no controle das espécies reativas e proteção contra os estresses ambientais (HUSEYNOVA; ALIYEVA; ALIYEV, 2013). Eficiente na eliminação de  $H_2O_2$  no citosol e nos cloroplastos, a APX é uma enzima importante no ciclo ascorbato-glutationae depende do ácido ascórbico (ASA) como doador de elétrons para reduzir em água o  $H_2O_2$  formado pela SOD.

A APX apresenta várias isoformas, caracterizadas de acordo com a localização celular. Isoformas solúveis encontradas no citosol (cAPX), mitocôndrias (mitAPX) e estroma de cloroplastos (sAPX), isoformas ligadas a membrana (mAPX) e tilacóides de cloroplastos (tAPX) e cloroplastos (chlAPX) (CAVERZAN et al., 2012). A tAPX e sAPX estão envolvidas na eliminação de  $H_2O_2$  produzido na fotossíntese e a mAPX e mitAPX eliminam o  $H_2O_2$  da fotorrespiração e respiração, enquanto a cAPX está relacionada a proteção contra os danos causados pelo estresse (PANG; WANG, 2010).

### 2.3.4 PEROXIDASE DO GUAIACOL (POD EC 1.11.1.7)

A peroxidase (POD) possui grande afinidade com o guaiacol e utiliza-o como doador de elétrons para catalisar o  $H_2O_2$ . Amplamente distribuída entre as plantas, a POD está presente em diversos tecidos, nos vegetais é encontrada na forma solúvel. Além de ser uma importante enzima no controle de EROs, regulando o  $H_2O_2$  e oxidando diversos substratos, as PODs ainda estão envolvidas em diversos processos fisiológicos, como a síntese de lignina e outros

polifenóis, alongamento celular e cicatrização de danos sofridos pelo estresse (MARCHAND; GREBENSHCHYKOVA; MENCH, 2016; ASTHIR, 2015).

### 2.3.5 COMPOSTOS FENÓLICOS

Os compostos fenólicos possuem em sua estrutura anéis aromáticos ou grupos hidroxilas e desempenham importante papel no combate ao estresse oxidativo (VAN HUNG, 2016). Este grupo de compostos possui variadas estruturas moleculares, as principais são ácidos fenólicos, flavonoides e derivados da cumarina.

As características antioxidantes dos compostos fenólicos e as diferentes propriedades químicas, atuam de maneira sinérgica contribuindo para a proteção celular no sequestro de radicais livres (BLOMHOFF et al., 2006).

## 2.4 METABOLISMO PRIMÁRIO

O metabolismo primário das plantas está associado aos processos fotossintéticos responsáveis pela assimilação de carboidratos e estão envolvidos nos processos comuns e vitais das plantas. Através da assimilação do carbono e em resposta a estresses abióticos, há a formação e acúmulo de amido, proteínas, prolina, açúcar solúveis e açúcares redutores a fim de evitar danos a planta (KOSAR et al., 2018).

### 2.4.1 AÇÚCARES SOLÚVEIS TOTAIS E AÇÚCARES REDUTORES

Os açúcares acumulados em altas concentrações são sacarose, frutose e glicose. Os açúcares estão presentes no metabolismo primário das plantas, na germinação de sementes e na fotossíntese onde o carbono fixado é convertido em açúcares através de células fotossintéticas especializadas (KOSAR et al., 2019).

O açúcar fotossintetizado imediatamente está disponível para transporte e alocação em vacúolos e tecidos de armazenamento de diversos órgãos da planta, servindo como reservatório para períodos onde a fotossíntese é reduzida ou comprometida. O transporte ocorre por meio do floema e a forma mais comum do açúcar transportado é a sacarose (PAGLIARANI et al., 2019).

O metabolismo, transporte e armazenamento de açúcares é constante e controlado por diversos processos de regulação. Durante períodos de estresse a reserva de amido pode ser hidrolisada em açúcares solúveis e ser translocada para o apoplasto mantendo o funcionamento de atividades essenciais para a sobrevivência celular (SECCHI; ZWIENIECKI, 2012).

O transporte de açúcares entre as células e o apoplasto é mediado pelos cotransportadores de açúcar/prótons da membrana plasmática, esta regulação evita o colapso celular por falta de nutrientes e controla o fluxo de açúcares através da membrana plasmática. Plantas expostas a longos períodos de seca podem alterar o pH e a concentração de solutos na seiva do floema. O acúmulo de fontes de energia como os açúcares é fundamental para o enfrentamento de danos causados pelo déficit hídrico, como a manutenção do turgor celular, baixas pressões hidrostáticas são mantidas durante o acúmulo de açúcares (KOSAR et al., 2018; SECCHI; ZWIENIECKI, 2012).

Os monossacarídeos também conhecidos como açúcares redutores (AR) possuem em sua estrutura um grupo cetona ou aldeído e são capazes de reduzir íons oxidantes. Em cana de açúcar constatou-se que condições de estresse podem reduzir a concentração de amido e sacarose nas folhas e aumentar a concentração de açúcares redutores (GARCIA et al., 2020), portanto pode-se dizer que a concentração de açúcares redutores ajudam no controle osmótico de plantas submetidas ao déficit hídrico.

#### 2.4.2 AMIDO

O amido é a principal polissacarídeo de reserva na maioria das plantas, nas folhas é considerado transitório e em raízes, tubérculos, frutos e sementes, como amido de reserva. O amido é formado por cadeias  $\alpha$ -D-glucose, composto por cadeias lineares conhecidas como amilose e cadeias ramificadas como amilopectinas (LIN et al., 2016).

Durante estresse osmóticos e em resposta a altas temperaturas, as plantas mobilizam amido, resultando no acúmulo de maltose. A hidrólise de maltose por  $\beta$ -amilase é a principal via de degradação do amido (THALMANN et al., 2016).

O amido é sintetizado nos plastídeos/cloroplastos nas folhas e em amiloplastos especializados nos órgãos e tecidos de armazenamento. A síntese de amido envolve três etapas, sendo a primeira o alongamento das extremidades não redutoras das cadeias de glicose pela ADPglucose (adenosina 5'-difosfato-glicose), segunda etapa ramificações das cadeias existentes através das reações de glucanotransferase e a terceira etapa enzimas de degradação



hidrolisam novamente alguns ramos das cadeias existentes. Há uma complexidade na síntese do amido, conferindo características específicas aos grânulos de amido (PFISTER; ZEEMAN, 2016).

#### 2.4.3 PROLINA E PROTEÍNAS

O acúmulo de solutos osmoprotetores, ativação de vias alternativas que estimulam os mecanismos de defesa celular, o fechamento estomático, o controle fotossintético, na tentativa de regular a atividade metabólica e a cadeia de transporte de elétrons, são alguns dos recursos utilizados na tentativa de minimizar os danos causados pelo déficit hídrico (SELLO et al., 2019).

A presença da prolina em células vegetais está associada ao mecanismos de ajuste osmótico (NOUNJAN et al., 2018), o que pode favorecer a manutenção do turgor celular, estabelecendo o controle de atividades celulares. A prolina atua diretamente no controle osmótico em plantas submetidas ao déficit hídrico, contribuindo para a estabilidade de membranas celulares e mantendo adequando as concentrações de  $\text{NADP}^+/\text{NADPH}$  (HAYAT et al., 2012).

Em seus estudos Laloum et al. (2018) e Farooq et al. (2018) mostram que diante de situações de estresse vias alternativas de codificação de proteínas são estimuladas, facilitando o acúmulo de grupos de proteínas osmoprotetoras responsáveis por proteger as membranas, evitando a desnaturação de proteínas e facilitando a tolerância ao estresse causado pelo déficit hídrico.

#### 2.5 EFICIÊNCIA FOTOSSINTÉTICA

Sob déficit hídrico as plantas ativam diferentes mecanismos para manter o equilíbrio de todo o aparato fotossintético. Entre estes mecanismos a fotoaclimação e o fechamento estomático, regulam a cadeia de transporte de elétrons entre os fotossistemas (PSI e PSII) permitindo que danos causados pelo desequilíbrio de energia não afetem o metabolismo vegetal (SELLO et al., 2019).

Os efeitos do déficit hídrico no aparato fotossintético podem ser investigados com o auxílio de curvas rápidas de luz, onde parâmetros como a máxima eficiência do uso da luz ( $\alpha$ ), taxa máxima de transporte de elétrons ( $\text{ETR}_{\text{max}}$ ) e irradiância mínima de saturação ( $I_k$ ) são

mensuradas (WU et al., 2013). Trabalhos apresentados por Wu et al. (2013) e Lobos et al. (2019) indicam que durante períodos de estresse as plantas tendem a reduzir a taxa de transporte de elétrons passando do PSII para o PSI alterando a quantidade de luz que satura os fotossistemas, e a eficiência fotossintética da clorofila a, reduzindo assim, a eficiência do uso da luz, contribuindo para baixo rendimento fotossintético e assimilação de carbono.

## **2.6 TREALOSE**

A aplicação de bioestimulantes em plantas (substâncias capazes de promover a tolerância frente a estresses bióticos e abióticos) é relatada em diversos trabalhos mostrando a eficiência na promoção a tolerância à seca em diversas culturas como milho (SOUZA et al., 2014), arroz (GARG et al., 2002), batata (MULEY et al., 2019) e rabanete (SHAFIQ; AKRAM; ASHRAF, 2015a).

A trealose é um dissacarídeo não redutor constituído por duas moléculas de D-glicose numa ligação  $\alpha$ - $\alpha$ , ( $\alpha$ -D-glicopiranosil-[1,1]- $\alpha$ -D-glicopiranosídeo). É amplamente distribuída na natureza, fonte de energia na maioria dos organismos vivos, encontrada em insetos, fungos, invertebrados e plantas (HIGASHIYAMA, 2002; SATOH-NAGASAWA et al., 2006). Está associada à capacidade de proteção e estabilização de diferentes organismos a estresses bióticos e abióticos. Nas plantas a trealose desempenha um importante papel como bioestimulante na tolerância ao déficit hídrico por ser considerada por alguns autores como osmoprotetora, quando acumulada (AVONCE et al., 2006).

São registradas cinco diferentes rotas metabólicas da trealose em fungos, bactérias e leveduras. Nas plantas a biossíntese da trealose ocorre a partir de moléculas de UDP- glicose e glicose-6-fosfato que pela ação de duas enzimas principais, trealose-6-fosfato sintase (TPS) e trealose-6-fosfato fosfatase (TPP), que atuam sobre a trealose-6-fosfato (T6P), desfosforilando em trealose (AVONCE et al., 2006; FERNANDEZ et al., 2010).

## **2.7 EFEITOS DA APLICAÇÃO DA TREALOSE E DERIVADOS EM PLANTAS**

A trealose constantemente é associada à capacidade de conferir aos vegetais tolerância a diferentes condições de estresse, devido a sua ação osmoprotetora (ZHOU et al., 2014). Os mecanismos de ação mostrando a eficácia da trealose podem incluir a vitrificação e formação

de cristais de anidro, que após períodos de seca são capazes de reabsorver água (STREETER, 2003). Podendo ainda gerar ligações de hidrogênio estabilizando as membranas celulares em condição de déficit hídrico (ITURRIAGA et al., 2009).

Em plantas superiores a presença da trealose era questionada, até a identificação em espécies como *Arabidopsis thaliana* (WINGLER et al., 2000). No cultivo de arroz a aplicação de trealose elevou os teores de açúcares solúveis aumentando a tolerância ao déficit hídrico e ao estresse salino (FERNANDEZ et al., 2010; GARG et al., 2002). A trealose ainda pode influenciar na formação da inflorescência do milho, e aumentar a tolerância a estresses como salino e ao déficit hídrico (SATO-H-NAGASAWA et al., 2006; ZHOU et al., 2014). Em nabo forrageiro a aplicação com sprays de trealose nas folhas aumentou a biomassa (AKRAM et al., 2015). A trealose em trigo aumentou a tolerância a seca e reduziu o acúmulo de EROs (LUO et al., 2018).

Diversas pesquisas demonstram que algumas culturas têm obtido ganhos significativos com a aplicação da trealose. A manipulação das vias bioquímicas e as enzimas envolvidas no processo de metabolização da trealose, aliado ao melhoramento genético, trouxe ganhos na área da produção mundial, aumentando a resistência e a tolerância dos cultivares a diversos tipos de estresse (LUNN et al., 2014; PAUL et al., 2015; ZHAO et al., 2019).

A maioria dos trabalhos citados ao longo deste estudo envolvem a aplicação da mistura de trealose e outros reguladores de crescimento em espécies como milho, soja, cana-de-açúcar, nabo (ALI; ASHRAF, 2011; AVONCE et al., 2006; SHAFIQ; AKRAM; ASHRAF, 2015), porém não foi encontrado na literatura o uso de derivados da trealose na tentativa de inibir estresses bióticos e abióticos, ou como estimulantes no desenvolvimento de espécies vegetais. Neste estudo analisamos o uso da trealose e uma mistura de seus derivados trealose tosílada (6,6'-didesoxi-6,6'-di-O-(p-toluenossulfonil)- $\alpha,\alpha$ -trealose) e trealose azídico (6,6'-diazido-6,6'-didesoxi- $\alpha,\alpha$ -trealose).

### **3 OBJETIVOS**

#### **3.1 OBJETIVOS GERAIS**

Comparar o efeito mitigador da trealose e da mistura de seus derivados (tosila e azídica) sobre o déficit hídrico em plantas de milho.

#### **3.2 OBJETIVOS ESPECÍFICOS**

- a) Verificar os mecanismos de defesa antioxidante de plantas de milho sob estresse hídrico, em casa de vegetação, com e sem aplicação foliar de trealose e seus derivados, pela avaliação do sistema antioxidante enzimático e não enzimático (compostos fenólicos);
- b) avaliar as modificações no metabolismo primário e na eficiência fotossintética após a aplicação da trealose e dos seus derivados de em milho submetido ao estresse hídrico.

#### 4 JUSTIFICATIVA

O milho é um dos cereais mais produzidos no mundo, servindo como base de alimentação para grande parte da população mundial e inclusive para alimentação de muitos animais. De acordo com a Companhia Nacional de Abastecimento (CONAB), a produção de grãos na primeira safra de 2018/2019 sofreu decréscimo em torno de 10% em relação à safra anterior. Podemos atribuir esta baixa na produção a dois fatores, o primeiro é o baixo preço praticado do produto e o segundo fator está relacionado às alterações climáticas dos últimos anos, fazendo com que o excesso ou falta de chuvas interfira diretamente no cultivo das plantas (CONAB, 2019).

No Brasil a plantação de milho estende-se em quase todo o território nacional, sendo a região centro-oeste onde as lavouras estão mais concentradas. As condições climáticas, disponibilidade de nutrientes e de água no solo são características que devem ser observadas para o plantio, porém as variações climáticas tornam-se um desafio para a produção da cultura (SANS *et al.*, 2001).

A descoberta de novas tecnologias possibilitando a indução de tolerância ao déficit hídrico, como o proposto com a aplicação de uma mistura de derivados de trealose que nunca foram testados em plantas traz novas possibilidades de produção e estudos de bioestimulantes baseados em trealose para o mercado brasileiro.

## REFERÊNCIAS

- AKRAM, N. A. et al. Exogenous application of trehalose alters growth, physiology and nutrient composition in radish (*Raphanus sativus* L.) plants under water-deficit conditions. **Revista brasileira de botânica**, v. 38, n. 3, p. 431–439, 2015.
- AKRAM, N. A. et al. Trehalose pretreatment induces drought tolerance in radish (*Raphanus sativus* L.) plants: some key physio-biochemical traits. **Acta physiologiae plantarum**, v. 38, n. 1, p. 1–10, 2016.
- ALDESUQUY, H. Exogenous salicylic acid and trehalose ameliorate short term drought stress in wheat cultivars by up-regulating membrane characteristics and antioxidant defense system. **Journal of horticulture**, v. 2, n. 2, 2015.
- ALI, Q.; ASHRAF, M. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. **Journal of sgronomy and crop science**, v. 197, n. 4, p. 258–271, 2011.
- ALVES DA COSTA, P. H. et al. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. **Brazilian journal of plant physiology**, v.17, n.4, p.353-362, 2005.
- AMBRÓSIO, A. S. **Avaliação em milho sob déficit hídrico com aplicação de derivados da trealose**. 2019. Dissertação (Mestrado em Ciências Ambientais) - Uniniversidade Federal de Alfenas, Alfenas, MG, 2019.
- ANJUM, N. A. et al. Catalase and ascorbate peroxidase—representative H<sub>2</sub>O<sub>2</sub>-detoxifying heme enzymes in plants. **Environmental science and pollution research**, v. 23, n. 19, p. 19002–19029, out. 2016.
- ASTHIR, B. Mechanisms of heat tolerance in crop plants. **Biologia plantarum**, v. 59, n. 4, p. 620–628, dez. 2015.
- AVONCE, N. et al. Insights on the evolution of trehalose biosynthesis. **BMC evolutionary biology**, v. 6, n. 1, p. 109, 2006.
- BARBOSA, M. R. et al. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência rural**, v.44, n.3, p.453-460, 2014.
- BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and soil**, v.39, n.1, p.205-207, 1973.
- BERGONCI, J. I. et al. Potencial da água na folha como um indicador de déficit hídrico em milho. **Pesquisa agropecuaria brasileira**, v. 35, n. 8, p. 1531–1540, 2000.
- BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-aeration following hypoxia or anoxia

- leads to activation of the antioxidative defense system in roots of wheat seedlings. **Plant physiology**, v. 116, n. 2, p. 651–658, 1998.
- BLOMHOFF, R. et al. Health benefits of nuts: potential role of antioxidants. **British journal of nutrition**, v. 96, n. S2, p. S52-S60, 2006.
- BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry**, v. 72, n. 1-2, p. 248-254, 1976.
- BRODRIBB, T. J.; MCADAM, S. A. M. Evolution of the stomatal regulation of plant water content. **Plant physiology**, v. 174, n. 2, p. 639-649, 2017.
- BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. **Methods in enzymology**, v. 52, p. 303-310, 1978.
- CAMPOS, C. N. et al. Melatonin reduces oxidative stress and promotes drought tolerance in young coffea arabica L. plants. **Agricultural water management**, v. 211, p. 37-47, 2019.
- CAVERZAN, A. et al. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Genetics and molecular biology**, v. 35, n. 4, p. 1011-1019, 2012.
- CHOUDHURY, F. K. et al. Reactive oxygen species, abiotic stress and stress combination. **The plant journal**, v. 90, n. 5, p. 856–867, jun. 2017.
- COLLA, E. et al. Optimization of trehalose production by rhodotorula dairenensis following a sequential strategy of experimental design. **Food and bioprocess technology**, v.3, n.2, p. 265-275, 2010.
- Companhia Nacional de Abastecimento. CONAB. Acompanhamento da safra brasileira: grãos. **Monitoramento agrícola- safra 2017/18**, v. 5, n. 11, p. 1-148, ago. 2018.
- Companhia Nacional de Abastecimento. CONAB. Acompanhamento da safra brasileira: grãos. **Monitoramento agrícola- safra 2019/20**, v. 7, n.1, p. 1-114, out. 2019.
- DARYANTO, S.; WANG, L.; JACINTHE, P. A. Global synthesis of drought effects on maize and wheat production. **Plos one**, v. 11, n. 5, p. 1–15, 2016.
- DE OLLAS, C.; DODD, I. C. Physiological impacts of ABA–JA interactions under water-limitation. **Plant molecular biology**, v. 91, n. 6, p. 641–650, ago. 2016.
- DE SOUZA, T. B. et al. Synthesis and antimicrobial activity of 6-triazolo-6-deoxy eugenol glucosides. **Carbohydrate research**, v. 410, p. 1-8, 2015.
- DEMIDCHIK, V. Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. **Environmental and experimental botany**, v. 109, p. 212-228, jan. 2015.
- ELEUTHERIO, E. et al. Revisiting yeast trehalose metabolism. **Current genetics**, v. 61, n. 3,

p. 263–274, ago. 2015.

FAROOQ, M. et al. Desi chickpea genotypes tolerate drought stress better than kabuli types by modulating germination metabolism, trehalose accumulation, and carbon assimilation.

**Plant physiology and biochemistry**, v. 126, p. 47–54, 2018.

FERNANDEZ, O. et al. Trehalose and plant stress responses: friend or foe? **Trends in plant science**, v. 15, n. 7, p. 409–417, 2010.

FISCHBACHER, A.; VON SONNTAG, C.; SCHMIDT, T. C. Hydroxyl radical yields in the fenton process under various pH, ligand concentrations and hydrogen peroxide/Fe(II) ratios.

**Chemosphere**, v. 182, p. 738–744, 2017.

GARCÍA-LIMONES, C. et al. Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea

(*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. *ciceris*. **Physiological and molecular plant pathology**, v. 61, n. 6, p. 325–337, 2002.

GARCIA, F. H. S. et al. Water deficit tolerance in sugarcane is dependent on the accumulation of sugar in the leaf. **Annals of applied biology**, v. 17, n. 1, p. 65-74, 2020.

GARG, A. K. et al. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. **Proceedings of the national academy of sciences**, v. 99, n. 25, p. 15898–15903, 2002.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: i. occurrence in higher plants.

**Plant physiology**, v. 59, n. 2, p. 309–314, 1977.

GILL, S. S. et al. Superoxide dismutase - mentor of abiotic stress tolerance in crop plants.

**Environmental science and pollution research**, v. 22, n. 14, p. 10375-10394, 2015.

GUNATHILAKA, R. P. D.; SMART, J. C. R.; FLEMING, C. M. Adaptation to climate change in perennial cropping systems: Options, barriers and policy implications.

**Environmental science and policy**, v. 82, p. 108-116, 2018.

HAN, B. et al. Interspecies and intraspecies analysis of trehalose contents and the biosynthesis pathway gene family reveals crucial roles of trehalose in osmotic-stress tolerance in cassava. **International journal of molecular sciences**, v. 17, n. 7, 2016.

HASANUZZAMAN, M. et al. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. **Crop stress and its management: perspectives and strategies**, p. 261–315, ago. 2012.

HAVIR, E. A.; MCHALE, N. A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. **Plant physiology**, v. 84, n. 2, p. 450–455, 1987.

HAYAT, S. et al. Role of proline under changing environments: A review. **Plant signaling**



- and behavior**, v. 7, n. 11, p. 1456-1466, 2012.
- HIGASHIYAMA, T. Novel functions and applications of trehalose. **Pure and applied chemistry**, v. 74, n. 7, p. 1263–1269, 2002.
- HOU, Q.; UFER, G.; BARTELS, D. Lipid signalling in plant responses to abiotic stress. **Plant cell and environment**, v. 39, n. 5, p. 1029-1048, 2016.
- HURA, T. et al. Water stress-induced flag leaf senescence may be accelerated by rehydration. **Journal of plant physiology**, v. 236, p. 109-116, 2019.
- HUSEYNOVA, I. M.; ALIYEVA, D. R.; ALIYEV, J. A. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Peroxidases: biochemical characteristics, functions and potential applications**. Nova science publishers, p. 141-158, 2013.
- HUSSAIN, S. et al. Oxidative stress and antioxidant defense in plants under drought conditions. In: **Plant abiotic stress tolerance**. Springer, Cham, p. 207-219, 2019.
- IANNONE, M. F.; GROPPA, M. D.; BENAVIDES, M. P. Cadmium induces different biochemical responses in wild type and catalase-deficient tobacco plants. **Environmental and experimental botany**, v. 109, p. 201–211, jan. 2015.
- IBRAHIM, H. A.; ABDELLATIF, Y. M. R. Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. **Annals of Agricultural Sciences**, v. 61, n.2, p. 267-274, 2016.
- ILHAN, S.; OZDEMIR, F.; BOR, M. Contribution of trehalose biosynthetic pathway to drought stress tolerance of capparid ovata desf. **Plant biology**, v.17, n. 2, p. 402-407, 2015.
- IODACHESCU, M.; IMAI, R. Trehalose biosynthesis in response to abiotic stresses. **Journal of integrative plant biology**, v. 50, n. 10, p. 1223–1229, out. 2008.
- ITURRIAGA, G. et al. Trehalose metabolism: from osmoprotection to signaling. **International Journal of molecular sciences**, v. 10, n. 9, p. 3793–3810, set. 2009.
- JAJIC, I.; SARNA, T.; STRZALKA, K. Senescence, stress, and reactive oxygen species. **Plants**, v. 4, n. 3, p. 393-411, 2015.
- JASSBY, A. D.; PLATT, T. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. **Limnology and oceanography**, v. 21, n. 4, p. 540-547, 1976.
- JIANG, B. et al. Detection of maize drought based on texture and morphological features. **Computers and electronics in agriculture**, v.151, p. 50-60, 2018.
- KOHLI, S. K. et al. ROS signaling in plants under heavy metal stress. In: **Reactive oxygen**

**species and antioxidant systems in plants: role and regulation under abiotic stress.**

Springer, Singapore, p. 185–214, 2017.

KOSAR, F. et al. Trehalose-induced improvement in growth, photosynthetic characteristics and levels of some key osmoprotectants in sunflower (*Helianthus annuus* L.) under drought stress. **Pakistan journal of botany**, v. 50, n. 3, p. 955-961, 2018.

KOSAR, F. et al. Trehalose: A key organic osmolyte effectively involved in plant abiotic stress tolerance. **Journal of plant growth regulation**, v. 38, n. 2, p. 606-618, 2019.

LALOUM, T.; MARTÍN, G.; DUQUE, P. Alternative splicing control of abiotic stress responses. **Trends in plant science**, v. 23, n. 2, p. 140-150, 2018.

LI, Z. G.; LUO, L. J.; ZHU, L. P. Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings.

**Botanical Studies**, v. 55, n. 1, p. 20, 2014.

LIN, L. et al. Comparative structure of starches from high-amylose maize inbred lines and their hybrids. **Food hydrocolloids**, v. 52, p. 19-28, 2016.

LOBOS, G. A. et al. Spectral reflectance modeling by wavelength selection: studying the scope for blueberry physiological breeding under contrasting water supply and heat conditions. **Remote sensing**, v. 11, n. 3, fev. 2019.

LUNN, J. E. et al. Trehalose metabolism in plants. **Plant journal**, v. 79, n. 4, p. 544–567, 2014.

LUO, Y. et al. Exogenously-supplied trehalose provides better protection for d1 protein in winter wheat under heat stress. **Russian journal of plant physiology**, v. 65, n.1, p. 115-122, 2018.

MAGALHÃES, P. C. et al. Caracterização ecofisiológica de linhagens de milho submetidas a baixa disponibilidade hídrica durante o florescimento. **Revista brasileira de milho e sorgo**, v. 8, n. 3, p. 223–232, 2009.

MAGALHÃES, P. C. et al. Phenotypic plasticity of root system and shoots of sorghum bicolor under different soil water levels during pre-flowering stage. **Australian journal of crop science**, v. 10, n. 1, p. 81–87, 2016.

MAGALHÃES, P. C.; DURÃES, F. O. M. Circular Técnica 76 - Fisiologia da produção de milho. **Circulares técnicas Embrapa**, p. 1-10, 2006.

MARCHAND, L.; GREBENSHCHYKOVA, Z.; MENCH, M. Intra-specific variability of the guaiacol peroxidase (GPOD) activity in roots of *phragmites australis* exposed to copper excess. **Ecological engineering**, v. 90, p. 57–62, mai. 2016.

- MARTINEZ, V. et al. Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. **Molecules**, v. 23, n. 3, p. 535, 2018.
- MCQUEEN, R. E. World population growth, distribution and demographics and their implications on food production. **Canadian journal of animal science**, v. 80, n. 2, p. 229-234, 2000.
- MENGER, F. M.; MBADUGHA, B. N. A. A. Gemini surfactants with a disaccharide spacer. **Journal of the american chemical society**, v. 123, n. 5, p. 875–885, fev. 2001.
- MILLER, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**, v. 31, n. 3, p. 426-428, 1959.
- MINUZZI, R. B.; LOPES, F. Z. Desempenho agrônômico do milho em diferentes cenários climáticos no Centro-Oeste do Brasil. **Revista brasileira de engenharia agrícola e ambiental**, v. 19, n. 8, p. 734–740, 2015.
- MITTLER, R. et al. Reactive oxygen gene network of plants. **Trends in plant science**, v. 9, n. 10, p. 490–498, 2004.
- MITTLER, R. et al. ROS signaling: the new wave?. **Trends in plant science**, v. 16, n.6, p. 300-309, 2011.
- MU, M. et al. Genome-wide Identification and analysis of the stress-resistance function of the TPS (Trehalose-6-Phosphate Synthase) gene family in cotton. **BMC genetics**, v. 17, n. 1, p. 54, 2016.
- MULEY, A. B. et al. Gamma radiation degradation of chitosan for application in growth promotion and induction of stress tolerance in potato (*Solanum tuberosum* L.). **Carbohydrate polymers**, v. 210, p. 289–301, 2019.
- NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. **Plant e cell physiology**, v. 22, n. 5, p. 867-880, 1981.
- NIU, L.; LIAO, W. Hydrogen peroxide signaling in plant development and abiotic responses: Crosstalk with nitric oxide and calcium. **Frontiers in plant science**, v. 7, p. 230, 2016.
- NOUNJAN, N. et al. High performance of photosynthesis and osmotic adjustment are associated with salt tolerance ability in rice carrying drought tolerance QTL: Physiological and co-expression network analysis. **Frontiers in plant science**, v. 9, p. 1135, 2018.
- PAGLIARANI, C. et al. Priming xylem for stress recovery depends on coordinated activity of sugar metabolic pathways and changes in xylem sap pH. **Plant, cell e environment**, v. 42, n. 6, p. 1775–1787, 2019.
- PANG, C. H.; WANG, B. S. Role of ascorbate peroxidase and glutathione reductase in

- ascorbate-glutathione cycle and stress tolerance in plants. In: **Ascorbate-glutathione pathway and stress tolerance in plants**. Springer, Dordrecht, p. 91–113, 2010.
- PAUL, M. J. et al. Differential Role for Trehalose Metabolism in Salt-Stressed Maize. **Plant physiology**, v. 169, n. 2, p. 1072–1089, 2015.
- PELLNY, T. K. et al. Genetic modification of photosynthesis with E. coli genes for trehalose synthesis. **Plant biotechnology journal**, v. 2, n. 1, p. 71–82, 2004.
- PETROV, V. et al. ROS-mediated abiotic stress-induced programmed cell death in plants. **Frontiers in plant science**, v. 6, p. 69, 2015.
- PFISTER, B.; ZEEMAN, S. C. Formation of starch in plant cells. **Cellular and molecular life sciences**, v. 73, n. 14, p. 2781–2807, 2016.
- POURCEL, L. et al. Flavonoid oxidation in plants: from biochemical properties to physiological functions. **Trends in plant science**, v. 12, n. 1, p. 29-36, 2007.
- QI, J. et al. Apoplastic ROS signaling in plant immunity. **Current opinion in plant biology**, v. 38, p. 92–100, 2017.
- RABÊLO, V. M. et al. The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. **Scientific reports**, v. 9, n. 1, p. 1-13, 2019.
- RAIJ, B. V. ET AL. Recomendações de adubação e calagem para o estado de São Paulo. **Instituto agrônomo/fundação IAC**, v. 100, n. Boletim técnico, p. 285, 1997.
- REIS, C. O. et al. Action of n-succinyl and n,o-dicarboxymethyl chitosan derivatives on chlorophyll photosynthesis and fluorescence in drought-sensitive maize. **Journal of plant growth regulation**, v. 38, n. 2, p. 619–630, 2019.
- REZAYIAN, M.; NIKNAM, V.; EBRAHIMZADEH, H. Differential responses of phenolic compounds of Brassica napus under drought stress. **Iranian journal of plant physiology**, v. 8, n. 3, p. 2417-2425, 2018.
- SACCON, P. Water for agriculture, irrigation management. **Applied soil ecology**, v. 123, p. 793-796, 2018.
- SÁNCHEZ-REINOSO, A. D.; LIGARRETO-MORENO, G. A.; RESTREPO-DÍAZ, H. Chlorophyll  $\alpha$  fluorescence parameters as an indicator to identify drought susceptibility in common bush bean. **Agronomy**, v.9, n. 9, p. 526, 2019.
- SANTOS, M. O. et al. Antioxidant system differential regulation is involved in coffee ripening time at different altitudes. **Tropical plant biology**, v. 11, n. 3-4, p. 131-140, 2018.
- SARMENTO, E. C. S. et al. Water deficit on germination and vigour in seeds of the jambu.

- Bioscience journal**, v. 35, n. 4, p. 1013–1021, 2019.
- SATOH-NAGASAWA, N. et al. A trehalose metabolic enzyme controls inflorescence architecture in maize. **Nature**, v. 441, n. 7090, p. 227–230, 2006.
- SECCHI, F.; ZWIENIECKI, M. A. Analysis of xylem sap from functional (nonembolized) and nonfunctional (embolized) vessels of *populus nigra*: chemistry of refilling. **Plant physiology**, v. 160, n. 2, p. 955–964, 2012.
- SELLO, S. et al. Plant biodiversity and regulation of photosynthesis in the natural environment. **Planta**, v. 249, n. 4, p. 1217-1228, 2019.
- SHAFIQ, S.; AKRAM, N. A.; ASHRAF, M. Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions?. **Scientia horticulturae**, v. 185, p. 68–75, 2015.
- SHAHBAZ, M. et al. Foliar-applied trehalose modulates growth, mineral nutrition, photosynthetic ability, and oxidative defense system of rice (*Oryza sativa* L.) under saline stress. **Journal of plant nutrition**, v. 40, n. 4, p. 584-599, 2017.
- SHARMA, A. et al. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. **Molecules**, v.24, n.13, p. 2452, 2019.
- SHIM, J. S. et al. Heterologous expression of bacterial trehalose biosynthetic genes enhances trehalose accumulation in potato plants without adverse growth effects. **Plant biotechnology reports**, v. 13, n. 4, p. 409–418, 2019.
- SINGLETON, V. L.; ORTHOFER, R.; LAMUELA-RAVENTÓS, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. **Methods in enzymology**, v. 299, p. 152-178, 1999.
- SOFO, A. et al. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. **International journal of molecular sciences**, v. 16, n. 6, p. 13561-13578, 2015.
- SOUZA, T. C. et al. Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. **Acta physiologiae plantarum**, v. 35, n. 11, p. 3201–3211, 2013a.
- SOUZA, T. C. et al. The influence of ABA on water relation, photosynthesis parameters, and chlorophyll fluorescence under drought conditions in two maize hybrids with contrasting drought resistance. **Acta physiologiae plantarum**, v. 35, n. 2, p. 515–527, 2013b.
- SOUZA, T. C. et al. ABA application to maize hybrids contrasting for drought tolerance: Changes in water parameters and in antioxidant enzyme activity. **Plant growth regulation**, v.

73, n. 3, p. 205-217, 2014.

STREETER, J. G. Effect of trehalose on survival of bradyrhizobium japonicum during desiccation. **Journal of applied microbiology**, v. 95, n. 3, p. 484–491, 2003.

TAKAGI, D. et al. Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition. **Plant physiology**, v. 171, n. 3, p. 1626–1634, 2016.

THALMANN, M. et al. Regulation of leaf starch degradation by abscisic acid is important for osmotic stress tolerance in plants. **Plant cell**, v. 28, n. 8, p. 1860–1878, 2016.

TOMBESI, S. et al. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. **Scientific reports**, v. 5, n. 1, p. 1-12, 2015.

ULLAH, H. et al. Growth, yield and silicon uptake of rice (*Oryza sativa*) as influenced by dose and timing of silicon application under water-deficit stress. **Archives of agronomy and soil science**, v. 64, n. 3, p. 318–330, 2018.

VAN HUNG, P. Phenolic compounds of cereals and their antioxidant capacity. **Critical reviews in food science and nutrition**, v. 56, n. 1, p. 25–35, 2016.

VERONEZE-JÚNIOR, V. et al. Leaf application of chitosan and physiological evaluation of maize hybrids contrasting for drought tolerance under water restriction. **Brazilian journal of biology**, 2019.

WASZCZAK, C.; CARMODY, M.; KANGASJÄRVI, J. Reactive oxygen species in plant signaling. **Annual review of plant biology**, v. 69, p. 209-236, 2018.

WINGLER, A. et al. Photorespiration: Metabolic pathways and their role in stress protection. **Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences**, v. 355, n. 1402, p. 1517–1529, 2000.

WU, W. et al. Global cropping intensity gaps: Increasing food production without cropland expansion. **Land use policy**, v. 76, p. 515–525, 2018.

WU, W. M. et al. Effects of nitrogen fertilization on chlorophyll fluorescence change in maize (*Zea mays* L.) under waterlogging at seedling stage. **Journal of Food, Agriculture and Environment**, v. 11, n. 1, p. 545-552, 2013.

YANG, W. et al. Co-application of soil superabsorbent polymer and foliar fulvic acid to increase tolerance to water deficit maize: photosynthesis, water parameters, and proline. **Chilean journal of agricultural research**, v. 79, n. 3, p. 435–446, 2019.

YEMM, E. W.; WILLIS, A. J. The estimation of carbohydrates in plant extracts by anthrone. **The biochemical journal**, v. 57, n. 3, p. 508, 1954.

ZANDALINAS, S. I.; MITTLER, R. ROS-induced ROS release in plant and animal cells. **Free Radical Biology and Medicine**, v. 122, p. 21-27, 2018.

ZHANG, K. et al. Exogenous application of ascorbic acid mitigates cadmium toxicity and uptake in Maize (*Zea mays* L.). **Environmental Science and Pollution Research**, v. 26, n. 19, p. 19261–19271, 2019.

ZHAO, M. L. et al. Ectopic expression of *Jatropha curcas* TREHALOSE-6-PHOSPHATE PHOSPHATASE *jc* causes late-flowering and heterostylous phenotypes in *Arabidopsis* but not in *Jatropha*. **International journal of molecular sciences**, v. 20, n. 9, p. 2165, 2019.

ZHOU, M. L. et al. Trehalose metabolism-related genes in maize. **Journal of plant growth regulation**, v. 33, n. 2, p. 256–271, 2014.

ZHU, J. K. Abiotic stress signaling and responses in plants. **Cell**, v. 167, n. 2, p. 313–324, 2016.

ZIPPER, S. C.; QIU, J.; KUCHARIK, C. J. Drought effects on US maize and soybean production: spatiotemporal patterns and historical changes. **Environmental research letters**, v.11, n. 9, p. 094021, 2016.

**ANEXO A**

**Mixture of trehalose derivatives stimulates the antioxidant system and improves photosynthetic efficiency in maize under water deficit.**

Joice Aparecida de Novais Portugal<sup>1</sup>, Thiago Corrêa de Souza<sup>1</sup>

<sup>1</sup>Federal University of Alfenas – UNIFAL-MG, Institute of Natural Sciences- ICN,700, Gabriel Monteiro Street, P. O. Box 37130-001, Alfenas, MG, Brazil

**Corresponding author:** Federal University of Alfenas – UNIFAL-MG, Institute of Natural Sciences- ICN,700, Gabriel Monteiro Street. Phone: +553 532 991 419, FAX: +553 532 991 419, P. O. Box 37130-000, Alfenas-MG, Brazil. Email: thiagonepre@hotmail.com. ORCID: 0000-0002-4991-7704 \*Corresponding author



**Abstract**

So far, studies on synthesis of trehalose derivatives and their effects on plants have not been explored. Therefore, the purpose of this study has been to evaluate antioxidant activity, primary metabolism and photosynthetic efficiency in maize plants under water deficit sprayed with a mixture of trehalose derivatives (tosyl and azidic). The experiment was conducted in greenhouses, using a maize hybrid sensitive to water deficit. Water deficit was imposed for 12 days, with foliar application of the mixture of derivatives (30mM) on the first day and on the fifth day of stress imposition. The analysis of photosynthetic efficiency of the antioxidant enzymatic system and of lipid peroxidation through rapid light curves were conducted on the first and on the last day of water deficit imposition and 12 hours after rehydration. Reducing sugars, total soluble sugars, starch, protein, proline, and phenolic compounds were analyzed at the end of the stress. Both trehalose and the mixture of trehalose derivatives influenced the response in the plants and the effects and possible reasons were analyzed. It can be concluded that the mixture of trehalose derivatives can contribute to increase tolerance to water deficit in maize through the stimulus of superoxide dismutase enzyme, ascorbate peroxidase, guaiacol peroxidase, through the accumulation of sugars, proteins, proline and phenolic compounds and through the improvement in the maximum electron transport rate.

**Key words:** *Zea mays* L.; oxidative stress; disaccharide; natural substance; proline; electron transport rate; phenolic compounds.

## 1. Introduction

The constant climatic changes have been causing substantial variations on temperature, altering the seasons and sometimes increasing the period of drought [1]. Thereby, one of the environmental factors which are most limiting to development and to plant production is the poor availability of water, or water deficit [2].

Water deficit can enable the maize plant to enter a period of stress and cause cell dehydration. Thereby, changes such as osmotic control and photosynthesis reduction occur, altering the harvesting and dissipation of light energy, causing modifications to the whole photosynthetic apparatus [3, 4].

Once photosynthesis (carboxylation) is reduced, an excess of energy in the leaves occurs, excess that can be transferred to O<sub>2</sub> and that, consequently, increases the formation of oxygen reactive (EROs) [5, 6, 7, 4, 8]. The accumulation of EROs may cause enzymatic and DNA degradation, modifications to the plasma membrane, such as lipid peroxidation, and even cause cellular apoptosi [9].

Maize is demanding with regard to the amount of water available and studies have shown that water deficit can cause irreversible damage to the cultivation, when it occurs: (1) at the vegetative stage, mainly at V6 stage, in which the production point is fixed and the result of the final production may be affected; (2) in flowering and in (3) grain filling, affecting floral synchrony and grain formation and filling [10, 11].

Water-deficit tolerant maize plants can activate the antioxidant protective system by increasing enzymatic activity as dismutase superoxide (SOD), and peroxidases such as ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (POD) [12, 13, 7, 14].

In the fight against the oxidative stress caused by the excess of EROs, maize plants can activate the non-enzymatic antioxidant system as polyphenols in an attempt to combat the damage [12]. In addition, plants can mitigate water stress by activating the primary methabolism, altering sugar content, proline, or even by controlling photosynthesis and improving its efficiency through biochemical ways (enzymatic activity) and through biophysical ways (photosynthetic apparatus and electron transport rate control) [15, 16].

Trehalose ( $\alpha$ D-glucopyranosyl-[1,1]- $\alpha$ -D-glucopyranose) is a natural substance, a non-reducing dissaccharide with osmoprotective properties [17], formed by a catalyzed reaction through trehalose phosphate synthase (TPS), obtaining trehalose-6-phosphate (T6P) and uridine diphosphate (UDP). Subsequently, the trehalose phosphate phosphatase enzyme (TPP) converts T6P in free trehalose [18]. Trehalose is widely found in insects, fungi, bacteria and plants, in

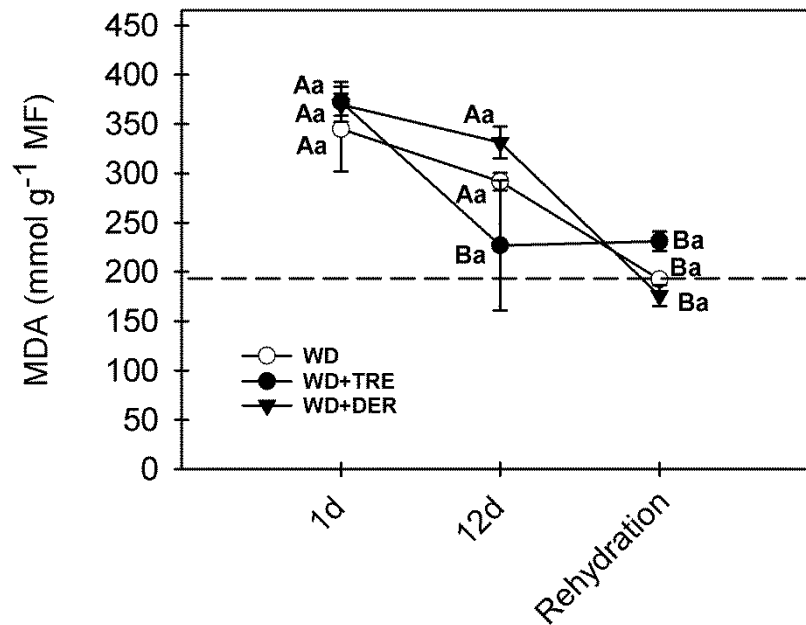
which there are reports of its functions as osmoprotector and osmoregulator toward stress conditions [19, 20, 21]. In agriculture, trehalose is used as a biostimulator, acting on tolerance increase to abiotic stress in plants, including water deficit, being a sustainable alternative to synthetic products [22].

Trehalose has been identified as a growth and development (productivity) promoter and also associated with osmotic, photosynthetic and antioxidant regulation in plants such as radish [23, 24], rice [25], cassava [26], maize [5, 27], wheat [28] and sunflower [28] cultivated under stress conditions. However, it is still necessary to explore the technologies that contribute to the mitigation of the effects of water deficit on vegetables, reducing losses, mainly in productivity [29, 30, 31]. Although many studies have widely shown the efficiency of trehalose usage in agriculture (including maize under water stress), there are no reports in the literature of studies showing results for the synthesis and use of trehalose derivatives, thus justifying the importance of researches that analyze the effect of trehalose derivatives on cultivations like maize when submitted to water deficit. Therefore, the goal of this study has been to verify the potential effect of the application of a mixture of trehalose derivatives on maize under water deficit, on the antioxidant system activation, on primary metabolism and on photosynthetic efficiency.

## **2. Results**

### *2.1 Quantification of lipid peroxidation (MDA) and antioxidant system*

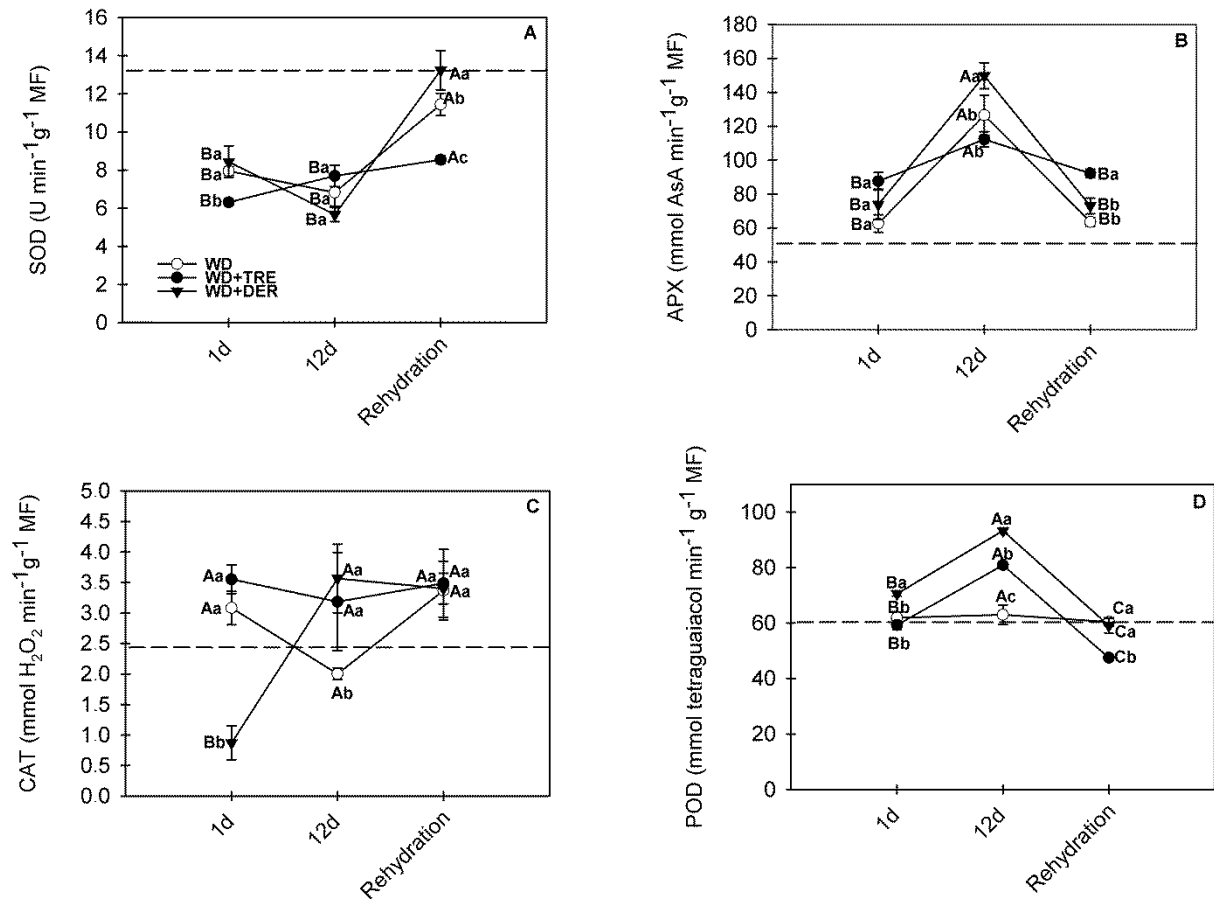
Comparing the treatments both on a water deficit day (1d) and after 12 days (12d) it was noted that the level of MDA did not show significant difference between Water Deficit (WD), Water Deficit and Trehalose (WD+TRE) and Water Deficit and Mixture of Trehalose Derivatives (WD+ DER) (Fig. 1). However, throughout water deficit (harvest season 1 d e 12 d) it was observed that the level of MDA decreased more considerably during (WD+TRE) treatment. And after 12 hours from rehydration, there was a decrease in the concentration of MDA during all the treatments, with values similar to the ones for irrigated plants (Fig. 1).



**Figure 1.** Lipid peroxidation (MDA) in maize leaves sprayed with trehalose and its derivatives during the imposition of water deficit and rehydration. Means followed by the same uppercase letter between the harvest seasons and lowercase letter between treatments do not differ by Scott-Knott test at 5% of probability ( $P \leq 0.05$ ). Treatments: WD = water deficit; WD+TRE= water deficit with foliar application of trehalose; and WD+DER= water deficit with foliar application of a mixture of trehalose derivatives. Harvest seasons: 1d = one day of water deficit; 12d= 12 days of water deficit; and Rehydration = 12 hours after rehydration. The bars correspond to standard error of the mean (four repetitions). The dotted line represents the average activity of irrigated control enzymes during the experiment.

In the evaluation of the antioxidant system it was observed that during water deficit (1d and 12d) there was a decrease in the superoxide dismutase enzymatic activity (SOD) (Fig. 2A). However, during rehydration there was an increase in the enzymatic activity in all the tested treatments. Among the treatments, it should be stressed that, during rehydration, WD+DER enzymatic activity was the highest and the closest to the value found for irrigated control (dotted line).

Throughout the water deficit, the ascorbate peroxidase enzyme (APX) showed greater activity after 12 days of stress for the three treatments (Fig. 2B). In assessing the treatments, we could highlight greater activity in the plants that were sprayed with the mixture of derivatives (WD+DER) after 12d and that were sprayed with trehalose (WD+TRE) on rehydration.



**Figure 2.** Superoxide dismutase enzymatic activity (SOD) (A), ascorbate peroxidase enzyme (APX) (B), catalase (CAT) (C) and guaiacol peroxidase (POD) (D) in maize leaves sprayed with trehalose and its derivatives during the imposition of water deficit and rehydration. Means followed by the same uppercase letter between harvest seasons and lowercase between the treatments do not differ by Scott-Knott test at 5% of probability ( $P \leq 0.05$ ). Treatments: WD= water deficit; WD+TRE= water deficit with foliar application of trehalose; and WD+DER= water deficit with foliar application of a mixture of trehalose derivatives. Harvest seasons: 1d = one day of water deficit; 12d= 12 days of water deficit; and Rehydration= 12 hours after rehydration. The bars correspond to standard error of the mean (four repetitions). The dotted line represents the activity of irrigated control enzymes during the experiment.

For Catalase enzyme (CAT) no difference between the harvest seasons was observed (1d, 12d, rehydration) (Fig. 2C). However, comparing the treatments, on the first day of water deficit there was greater activity in WD and WD+TRE and, on the twelfth day of water deficit, both the plants that received trehalose and the plants that received the mixture of derivatives increased their activity. On the other hand, on rehydration, there was no difference between the treatments.

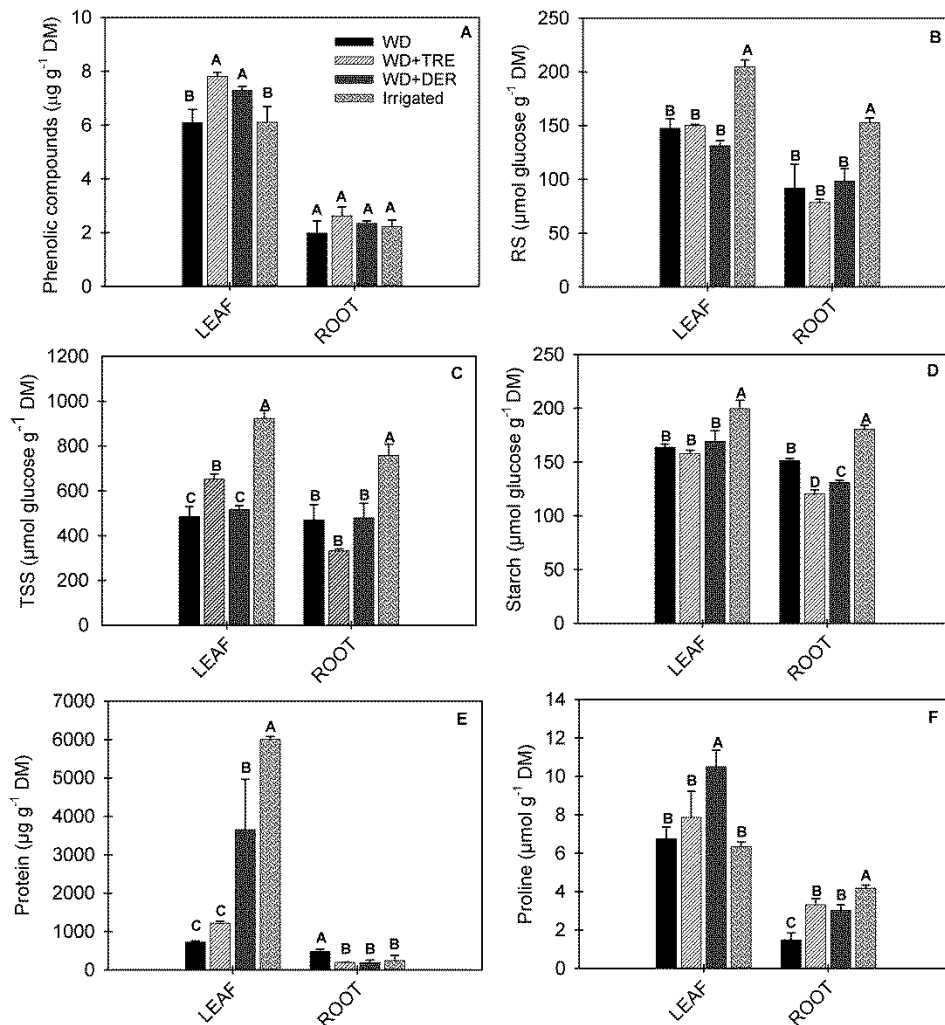
At 1 day and 12 days of water deficit we could notice, in all the treatments, an increase in the activity of guaiacol peroxidase enzyme (POD) followed by a reduction on Rehydration (Fig. 2D). Comparing the treatments, it was observed that POD activity was higher in the plants that received the mixture of derivatives (WD+DER), both on the first and on the twelfth day of

water deficit.

The quantification of Phenolic Compounds showed a difference only in the leaves, proving to be higher in WD+TRE and WD+DER treatments (Fig. 3A).

## 2.2 Quantification of Primary Metabolism

In analyzing reducing sugars level (Fig. 3B), it became apparent that the values found in WD, WD+TRE, WD+DER treatments were similar and lower than those found in the irrigated treatment, both in the leaves and in the maize roots.



**Figure 3.** Phenolic compounds concentration (A), reducing sugars (RS) (B); total soluble sugars (TSS) (C); starch (D); protein (E); proline (F) in maize leaves and roots sprayed with trehalose and its derivatives after 12 days of water deficit imposition. Means followed by the same letter do not differ by *Skott-Knott* test at 5% of probability ( $P < 0.05$ ). Treatments: WD= water deficit; WD+TRE= water deficit with foliar application of trehalose; WD+DER= water deficit with foliar application of a mixture of trehalose derivatives; and Irrigated= irrigated control. The bars correspond to standard error of the mean (four repetitions).

The total soluble sugars (Fig. 3C) showed higher concentration in the Irrigated treatment, followed by WD+TRE when analyzed in the leaves, and showed higher

concentration in the Irrigated treatment when analyzed in the roots. The starch level (Fig. 3D) in the leaves was higher in the irrigated treatment and in the roots was lower in WD+TRE and WD+DER treatments.

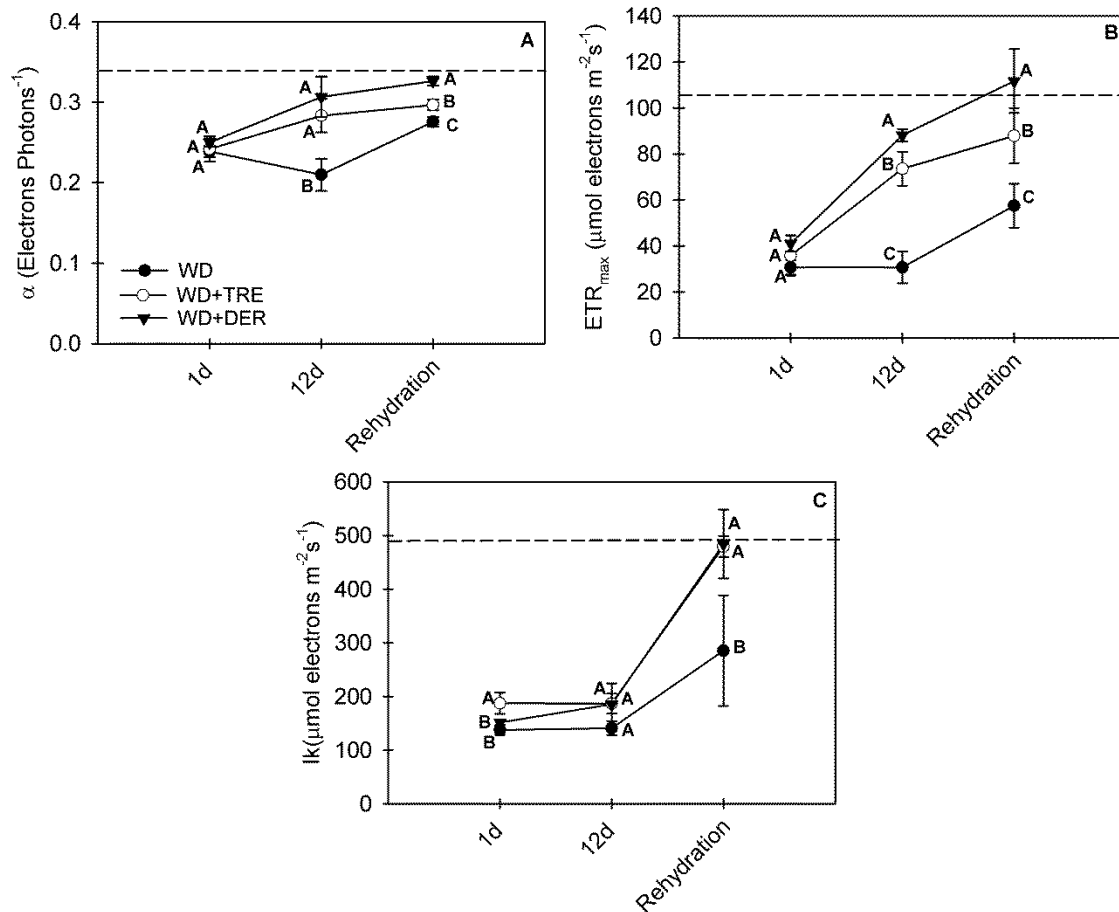
The protein concentration in the leaves was higher in the Irrigated treatment followed by WD+DER (Fig. 3E). And, in the roots, the WD treatment showed the highest concentration of proteins with respect to the other treatments which did not differ from each other. The proline level in the leaves (Fig. 3F) was higher in the WD+DER treatment, while in the other treatments the levels were lower and did not differ from each other. In the roots there was higher concentration of proline level in the Irrigated treatment, followed by the treatments with application of trehalose and derivatives (WD+TRE e WD+DER). In addition, the WD treatment showed lower average of proline levels.

### *2.3 Analysis of photosynthetic efficiency*

Throughout the water deficit (1d e 12d) and on rehydration we could observe that the maximum light use efficiency ( $\alpha$ ) (Fig. 4A), maximum electron transport rate ( $ETR_{max}$ ) (Fig. 4B) and the minimum saturating irradiance ( $I_k$ ) (Fig.4C) parameters increased, with the exception of WD treatment which reported a decrease in these parameters on water stress days.

In comparing the treatments, it was observed that at 1d there was no difference in any of the parameters analyzed. However, at 12d it was observed that the trehalose application (WD+TRE) and the mixture of derivatives (WD+DER) increased  $\alpha$  and  $ETR_{max}$ . This emphasized that the values of the treatment with application of the mixture of derivatives were much higher in these two parameters compared with the values of the treatment with trehalose.

On rehydration, and in all the three parameters analyzed, the WD+TRE and WD+DER treatments showed higher averages than the WD treatment, thus highlighting for  $\alpha$  that the application of the mixture of derivatives resulted in higher averages compared with the application of only trehalose (FIG. 4A).



**Figure 4.** Analysis of photosynthetic efficiency through the measurement of chlorophyll *a* fluorescence parameters through rapid light curves in maize leaves sprayed with trehalose and its derivatives during the imposition of water deficit and rehydration. (A) maximum light use efficiency ( $\alpha$ ), (B) maximum electron transport rate ( $ETR_{max}$ ), (C) Minimum saturating irradiance ( $I_k$ ). Means followed by the same letter do not differ by *Skott-Knott* test at 5% of probability ( $P < 0.05$ ). Treatments: WD= water deficit; WD+TRE= water deficit with foliar application of trehalose; e WD+DER= water deficit with foliar application of a mixture of trehalose derivatives. Harvest seasons: 1d= one day of water deficit; 12d= 12 days of water deficit; and Rehydration= 12 hours after rehydration. The bars correspond to standard error of the mean (three repetitions). The dotted line represents the average activity of irrigated control enzymes during the experiment.

### 3. DISCUSSION

Water deficit is one of the limiting factors to plant development. In maize, the increase in the production of oxygen reactive species (ERO) and the imbalance in the antioxidant defense system result in oxidative damage in the whole plant system [7, 32, 12]. The increase in EROs may cause changes in the cellular membrane structure leading to malonaldehyde increase (MDA), that is, lipid peroxidation, causing alterations in its functions [33].

Souza et al.[8] demonstrated that during water deficit maize presented higher levels of MDA, confirming the result found in this study with BRS 1030 genotype, which is sensitive to water deficit. The foliar application of trehalose and its derivatives did not interfere with the concentration of MDA, compared with the stressed treatment (WD), unlike the study presented



by Ali and Ashraf [5], in which, after foliar application of trehalose there was stimulation of the reduction in concentration of MDA in maize under water deficit. A possible explanation for obtaining no changes in MDA levels is that this mixture of trehalose derivatives, when applied on maize leaves, stimulates a regulated dissipation of energy in photosystems [34] that may reduce the excessive formation of EROs and, consequently, not change MDA levels significantly.

The foliar application of trehalose and its derivatives did not alter lipid peroxidation under water deficit, but modified the antioxidant enzymatic activity, raising the hypothesis that, although trehalose and its derivatives are not responsible for the reduction of cellular damage, they act as stress markers. Both trehalose and the mixture increased the antioxidant enzymatic activity. SOD, when activated, is responsible for O<sub>2</sub>•-free radicals sequestration, dismuting in H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> [35], reducing the risk of OH•- formation and controlling the accumulation of H<sub>2</sub>O<sub>2</sub> in chloroplast, mitochondria, cytosol and peroxisomes [36]. Subsequently, the antioxidant enzymatic activity of APX, CAT and POD is stimulated for H<sub>2</sub>O<sub>2</sub> elimination [32].

In some studies, the foliar application of trehalose has increased SOD [5]; APX [32], CAT [37] and POD activity [28]. This higher activity could be explained by the endogenous increase of trehalose in the cytoplasm of cells [38, 39, 20]. The foliar application of trehalose induces the endogenous accumulation of sugar, characterized by different studies as a compatible solute, that is, non-toxic, highly stable due to its high binding stability ( $\alpha$ - $\alpha$ ) and low binding energy spending (1 kcal mol<sup>-1</sup>), which gives trehalose an important role in the maintenance and cellular protection of plants under different kinds of abiotic stress, as water deficit [40, 41].

After analyzing the mixture of derivatives, the latter was more efficient in stimulating antioxidant activity than trehalose itself (there was an increase in SOD on rehydration, APX and POD during water deficit), which might demonstrate higher influence of these new derivatives in the induction of trehalose-6-phosphate synthase genes (TPS). These genes are associated with the production of enzymes that synthesize trehalose [42]. Maize plants tolerant to water deficit tend to have a higher antioxidant enzyme system activity [8, 12] and the mixture of derivatives seems to enhance this activity.

It is important to stress the considerable activity of guaiacol peroxidase (POD), both at the beginning (1d), and at the end of stress (12d), when the mixture of derivatives was applied. POD in plants under water deficit, besides acting on hydrogen peroxide sequestration, can be associated with other enzymes acting on diphenols and phenols for the production of other

phenolic compounds relevant to mitigate the damage caused by oxidative stress through the sweeping of free radicals [43, 44, 45]. This increase in POD could explain the higher level of phenolic compounds in maize treatments that received the disaccharides.

With regard to carbohydrates metabolism, several studies have shown its modulation by trehalose. Our study did not reveal great modifications, but the application of trehalose increased the levels of soluble sugars in the leaves. In rice transgenic plants that were added to the fusion of 2 trehalose biosynthetic genes, increases in the levels of soluble sugars were reported as well [46].

However, the mixture of derivatives, as well as trehalose, reduced the starch in the roots. This reduction can be associated with starch degradation and sugar transport via xylem corroborating the increase of soluble sugars in the leaves. These adjustments are important for plant tolerance, since soluble sugars can work as antioxidant compounds or as osmotically active solutes in plants under stress [47]. In contrast to this, in *Arabidopsis* the application of trehalose in leaves stimulated starch biosynthesis, as the applied sugar induced an *ApL3* gene expression that codifies the great subunit of Adenosine Diphosphate (ADP)-glucose pyrophosphorylase [48, 49].

The exogenous application of trehalose and the mixture of its derivatives can activate a cascade of reactions in plants, in the attempt to mitigate the effect caused by water deficit. Among the processes activated, the accumulation of proline in plant cells is associated with the osmotic adjustment mechanism [50], which may favour the maintenance of cell turgor, establishing cellular activity control. Studies as presented by Kosar [21] corroborate the data found in this study, in which the application of trehalose was able to increase the concentration of proline. In our results, the application of tozyl and azidic trehalose derivatives proved to be more efficient for the accumulation of proline, mitigating water deficit to a greater extent.

The mixture of trehalose derivatives promoted the accumulation of proteins. Studies as the one presented by Laloum et al. [51] and Farooq et al. [52] show that, in the face of stress situations, alternative ways of protein coding are stimulated, facilitating the accumulation of osmoprotective protein groups responsible for protecting the membranes, avoiding protein denaturation and facilitating tolerance to stress caused by water deficit. Since an increase in photosynthetic efficiency was also reported in this study, this increase in the total proteins of the leaves due to the mixture of trehalose derivatives could also be explained by the increase of Rubisco content (photosynthetic enzyme), one of the most plentiful proteins in the leaves. This because the exogenous application of trehalose induces genes involved in the production of

precursors such as trehalose-6-phosphate [53, 54] that increase Rubisco level [55, 49]. Thus, the mixture of trehalose derivatives could be more active in this genetic induction than trehalose alone.

With regard to photosynthetic efficiency in plants, tolerant genotypes can be discriminated with chlorophyll fluorescence parameters  $a$  through rapid light curves (RLC) [56].

Maize plants sensitive to drought (and other species as well) tend, under water deficit, to reduce the electron transport rate (ETR) going from PSII to PSI, altering the amount of light that saturates the photosystems, thus also reducing light use efficiency ( $\alpha$ ) [57, 58, 59]. However, trehalose, and more intensively the mixture, seems to mitigate the stress and induce tolerance to drought, since these sprayed substances increased these parameters at 12d of stress and on rehydration. Moreover, this higher  $ETR_{max}$  in the mixture occurs because these derivatives increase leaf gas exchanges (photosynthesis) and photochemical dissipation (qP) in maize under water deficit [34], so that a higher electron flow in the photosystems is necessary in order to supply the production of photoassimilates at the biochemical stage.

High values of maximum light use efficiency ( $\alpha$ ) in maize plants that received the mixture of derivatives may indicate a stable apparatus and alleviation in photoinhibition, since trehalose itself was identified as photosystem II [54] D1 Protein protector.

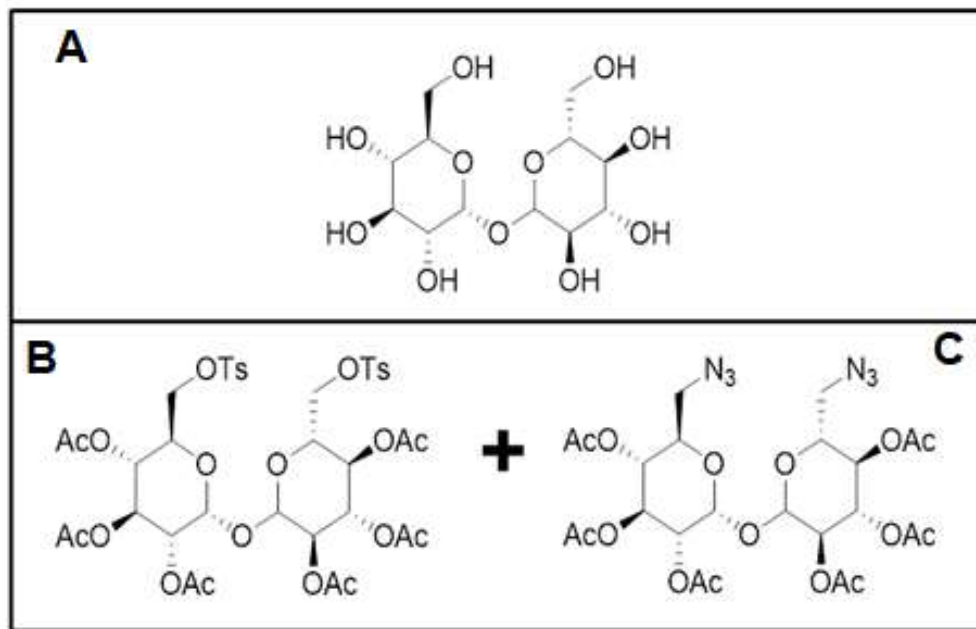
The possible justification for the mixture of trehalose derivatives to increase photosynthetic efficiency more than trehalose is a better action on specific genes. The application of trehalose may lead to an overexpression of the *OTsA* gene involved in the production of trehalose-6-phosphate and to the increase of photosynthetic efficiency [27, 55, 60].

## 4. Material and Methods

### 4.1 Synthesis of trehalose derivatives mixture

The trehalose derivatives used were tosylate trehalose, 6-*O*-[(4-methylphenyl) sulfonyl]- $\alpha$ -D-glucopyranoside of 6-*O*-[(4-methylphenyl) sulfonyl]- $\alpha$ -D-glucopyranosyl with molecular weight of 650,13 g mol<sup>-1</sup> and azidic trehalose, 6-azide-6-deoxy- $\alpha$ -D-glucopyranoside of 6-azide-6-deoxy-D-glucopyranosyl with molecular weight of 392,13 g mol<sup>-1</sup>. The molecular structures of trehalose, tosylate trehalose and azidic trehalose are presented in Figure 5. These trehalose derivatives used in the mixture were synthesized from pure trehalose (Sigma-Aldrich®). Trehalose reacted with tosyl chloride, pyridine, acetic anhydride resulting in the intermediate derivative 2,3,4-tri-*O*-acetyl-6-*O*-[(4-methylphenyl)sulfonyl]- $\alpha$ -D-

glucopyranoside of 2,3,4-tri-*O*-acetyl-6-*O*-[(4-methylphenyl) sulfonyl]- $\alpha$ -D-glucopyranosyl. Tosylate trehalose was then produced by basic hydrolysis of the intermediate derivative acetyl esters with NaOH in methanol. The intermediate derivative also reacted with sodium azide in pyridine, generating a new intermediate derivate that, by basic hydrolysis of acetyl esters with NaOH in methanol, produced azidic trehalose [61, 62]. The chemical characterization of the derivatives was detailed by [34].



**Figure 5** Molecular structure of trehalose (A), of the mixture of tosylate trehalose derivatives (B) and of azidic trehalose (C).

#### 4.2 Plant material and growth conditions

The experiment was conducted in greenhouse at Unidade Educacional Santa Clara of Universidade Federal de Alfenas (UNIFAL-MG), located in the city of Alfenas, South of Minas Gerais state (21°25' S, 45°58' W, 818 meters above mean sea level ). Air relative humidity and temperature were registered daily inside the greenhouse, being 30°C the maximum average temperature, 25°C the minimum average temperature and 74,4% the relative humidity average. The global radiation incident inside the greenhouse was measured by radiometer (Instrutherm/MES-100, São Paulo - SP) with values of 900 W m<sup>-2</sup> at midday.

8-liter capacity pots filled with 6 kg of dry, pounded to break up clods, penerated top layer soil were used (0 – 20cm). The soil was classified as dystrophyc Red Latosol soil, very

clayey. Subsequently, further fertilizations were made, according to the recommended for maize cultivation [63]. After the fertilization, four seeds of BRS 1030 sensitive to water deficit maize seeds, coming from Embrapa Maize and Sorghum Program, were sown in each pot [4].

Soil moisture was kept at about 70% of field capacity (CC) for all plants, through daily irrigation, until the plants reached V6 stage, with 6 fully-expanded leaves. Water replacement was made according to the weight of the pots (in the morning and in the afternoon), examined with the aid of a digital weight scale (Blackbull 200SS, São Paulo -SP).

#### *4.3 Imposition of water deficit and application of trehalose and derivatives*

Once the plants reached V6 stage, irrigation was suspended in order to reach 55% of field capacity (CC), considered to be the maximum allowable stress value for the execution of the experiment.

In addition, to typify the soil water deficit, the base water potential (pre-dawn,  $\psi_{pd}$ ) of the plants, determined before dawn (5 a.m.), through a pressure chamber of Scholander type (Soil Moisture Equipment Corp., Modelo SEC-3015G2, Santa Barbara CA, USA) was used in four full-expanded by treatment leaves. The base water potential reflects the water level of the plants and of the soil (equilibrium) [64]. The plants under water deficit (55% CC) reached a water potential value of -1,7 MPa, while, under 70% CC, a water potential of -0,31 MPa was verified. In all, it was 12 days of water deficit; the stress imposition effectively started in plants on the V6 stage, one of the stages that report higher susceptibility to abiotic stresses, including water deficiency [10].

The experiment consisted of three treatments: water deficit (WD), water deficit with foliar application of trehalose (WD+TRE) in concentration of 30 mM and water deficit with foliar application of the mixture of (azide + tosyl) trehalose derivatives (WD+DER) in the concentration of 15 mM each, totaling 30 mM as recommended by Ali e Ashraf [5].

After obtaining 55% of field capacity in the pots, two foliar applications were made on the 1<sup>st</sup> and 5<sup>th</sup> day of water deficit, with trehalose and the mixture having a final concentration of 3,75 mmol plant<sup>-1</sup>, using a mechanical hand-compression sprayer PCP1P with capacity of 1,5L (Guarany®). This sprayer allowed the application of a fine mist of treatment syrup on each plant. The molecules were diluted in ethylene glycol 20%. The applications were made at dusk, the treatments were disposed in straight lines and out of the greenhouse, in order to cover the entire abaxial and adaxial foliar surface (V1 Vídeo in Supplementary Material).

Three collections were made, the first on the 1<sup>st</sup> day of water deficit (1d), the second on the 12<sup>th</sup> day of water deficit (12d) and the third collection 12 hours after Rehydration, that is, when all treatments returned to 70% of soil CC. In each collection, samples of leaves for biochemical analyses (lipid peroxidation and antioxidant enzymes) were taken and photosynthetic efficiency parameters were measured (Chlorophyll Fluorescence *a*). Instead, the quantification of phenolic compounds and primary metabolism was made at 12 days of water deficit in leaves and roots. Irrigated plants (under condition of 70% CC throughout the entire experiment) were also collected and/or analyzed and named as “Irrigated Control”.

#### *4.4 Extraction and quantification of Lipid Peroxidation (malonaldehyde - MDA)*

200 mg of fresh plant material were macerated in liquid nitrogen with 50% PVPP until a fine powder was obtained. After that, 1500 $\mu$ L of trichloroacetic acid (TCA) at 0,1% were added. The material was centrifuged at 12.000rpm for 15 minutes at 4°C, followed by the supernatant collection.

The quantification of lipid peroxidase (MDA level) was made according to the methodology proposed by Buege and Aust [65]. The level of lipid peroxidation was measured in values read in absorbance of 535 to 600 nm, the higher the color presented, the higher the level of MDA concentration, using the molar extinction coefficient of 1,56 mM<sup>-1</sup>cm<sup>-1</sup>.

#### *4.5 Antioxidant system: Extraction and quantification of antioxidant enzymes and phenolic compounds*

For enzyme extraction, 200 mg of fresh plant material, macerated in liquid nitrogen and 50% PVPP until a fine powder was obtained, were used. The extraction was made with an extraction buffer composed of a mixture of potassium phosphate at 400 mM, pH 7.8, 10 mM EDTA, 200 mM ascorbic acid and water, with a final volume of 1500 $\mu$ L/reaction. Subsequently, the enzymes were transferred to micro centrifuge tubes. The samples were centrifuged for 10 minutes at 13.000rpm, at the temperature of 4°C and the supernatants collected for quantification, according to the methodology proposed by Biemelt et al. [66].

All the enzymes had their activity analyzed using Elisa spectrophotometer reader (anthos Zenyth 200rt, Austria). Superoxide dismutase activity (SOD, EC 1.15.1.1) was measured by the capacity of inhibition of nitrotetrazole blue (NBT) photoreduction, according to Giannopolitis and Ries methodology [67]; ascorbate peroxidase activity (APX, EC 1.11.1.11) was determined by kinetic characterization of the decrease in absorbance of ascorbate oxidation

at 290 nm for 3 minutes, with  $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$  extinction coefficient. The methodology used was the one proposed by Nakano, Y.; Asada [68]; catalase activity (CAT, EC 1.11.1.6) was determined by decrease in absorbance due to the consumption of  $\text{H}_2\text{O}_2$  at 240 nm for 3 minutes, with molar extinction coefficient of  $36 \text{ mM}^{-1} \text{ cm}^{-1}$  according to Havir and McHale [69]; guaiacol peroxidase activity (POD, EC 1.11.1.7) was determined by guaiacol oxidation with increase in absorbance at 470 nm, molar extinction coefficient of  $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ . The methodology for the observation of POD activity followed the one proposed by Nakano, Y.; Asada [68] with García-Limones et al. [70] modifications.

For phenolic compounds extraction, 500 mg of dry plant material were macerated and 4 mL of ethanol were added. The material rested overnight and was subsequently centrifuged at 4.000 rpm for 30 minutes at the temperature of  $20^\circ\text{C}$ . The supernatant was collected and the above described procedure repeated. The quantification of phenolic compounds followed Singleton et al. [71] methodology.

#### *4.6 Primary metabolism: Extraction and quantification of reducing sugars, total soluble sugars, starch, protein, proline*

For the extraction of reducing sugars, total soluble sugars, proteins and starch, 400 mg of dry plant material were macerated, adding the extractor solution of 3 mL of methanol, 1,25 mL of chloroform and 0,75ml of water. The samples were left to rest overnight and after 24 hours were centrifuged for 30 minutos at 1300 rpm. The supernatant was collected for analyses. 3 mL of 30% perchloric acid were added to the pellet, which rested overnight again for the extraction and quantification of starch.

The quantification of reducing sugars was conducted according to Miller methodology [72], and for total soluble sugars the methodology described by Yemm e Willis [73] was followed. The quantification of proteins followed the method proposed by Bradford [74] and the quantification of starch was conducted according to Yemm and Willis [73] methodology.

For the extraction of proline, 100 mg of dry plant material were macerated with 10 mL of sulfosalicylic acid at 3%. The solution rested in tubes and was mixed by agitation for 60 minutes at 255 rpm. After separating the material, the sample was filtered through filter paper and analyzed according to Bates et al. methodology [75].

#### *4.7 Analyses of photosynthetic efficiency: Clorophyll a Fluorescence*

For evaluation of photosynthetic efficiency, the measurement of chlorophyll *a* fluorescence parameters was taken through a Mini-PAM II modulated fluorometer (Heinz Walz, Effeltrich, Germany Heinz). Rapid Light Curves (RLC) were obtained using the referred fluorometer. Photosynthetic active radiation (PAR) ranged from 0 to 1150  $\mu\text{mol f\acute{o}tons m}^{-2} \text{ s}^{-1}$  in 10 levels every 20 seconds in order to determine the electron transport rate (*ETR*) versus PAR. *ETR* was calculated as  $[(F_m' - F_s / F_m') \times \text{PAR} \times 0,5 \times 0,84]$ . Using the software supplied with the equipment used (WinControl-3) it was possible, from these curves, to calculate the maximum electron rate ( $ETR_{\text{max}}$ ) through the equation  $ETR = ETR_{\text{max}} \times \tanh(\alpha \times \text{PAR} / ETR_{\text{max}})$  [76]. It was also possible to calculate a maximum light use efficiency, which corresponds to *ETR/PAR* curve inclination and the  $I_k$  Minimum saturating irradiance, which corresponds to  $ETR_{\text{max}}/\alpha$ . 3 curves were made for each treatment (3 repetitions).

#### 4.8 Trial design and Data analysis

Trial design was completely randomized (DIC) in 3x3 factorial design, through three treatments (WD, WD+TRE e WD+DER), three harvest seasons (1d, 12d and Rehydration) and four repetitions, totaling 36 experimental units. Changes in pots placement on the greenhouse benches were made periodically in order to minimize environmental interferences.

Means and  $\pm$  standard error (SE) were calculated for each parameter. For statistical analysis of the results, variance analysis (ANAVA) and *Scott-Knott* means comparison test at 5% of probability ( $p \leq 0.05$ ) were used, on Sisvar version 5.7 software (Universidade Federal de Lavras - UFLA, Lavras, Brasil).

## 5. Conclusion

The foliar application of the mixture of trehalose derivatives promoted a better response in the main characteristics analyzed compared with trehalose alone. Therefore, it can be concluded that the mixture of trehalose derivatives can contribute with studies to the formulation of bioestimulants, since it stimulates the antioxidant system and improves the photosynthetic efficiency in maize under water deficit.

**Supplementary Materials.** Video V1: Application of a mix of chitosan derivatives in maize plants under water deficit.



Author Contributions. T.C.S. Conceptualization; Methodology, T.C.S, K.R.D.S., D.F.D., P.R.S-F, T.B.S., M.H.S and A.C.D.; Validation J.A.N.P and A.S.A.; Formal Analysis, J.A.N.P, A.S.A, and K.R.D.S.; Investigation, P.C.M. and T.C.S.; Resources, T.C.S.; Data Curation, J.A.N.P, A.S.A., K.R.D.S and T.C.S; Writing – Original Draft Preparation, J.A.N.P. and K.R.D.S.; Writing – Review & Editing, T.C.S., D.F.D., K.R.D.S.; Visualization, X.X.; Supervision, T.C.S.; Project Administration, T.C.S.; Funding Acquisition, T.C.S.

**Acknowledgments.** The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding the research project involved in this study (Processo 423584/2016-2, chamada Universal 01/2016). The present study was also accomplished with the scholarship support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001. We also thank for the research productivity scholarship of the CNPq (modalidade PQ, categoria 2) granted to Souza, TC (Processo: 304421/2018-9).

**Conflicts of Interest.** The authors declare no conflict of interest.

## Referências

1. Gunathilaka, R.P.D.; Smart, J.C.R.; Fleming, C.M. Adaptation to climate change in perennial cropping systems: Options, barriers and policy implications. *Environ. Sci. Policy* **2018**.
2. Saccon, P. Water for agriculture, irrigation management. *Appl. Soil Ecol.* **2018**.
3. Brodribb, T.J.; McAdam, S.A.M. Evolution of the Stomatal Regulation of Plant Water Content. *Plant Physiol.* **2017**.
4. Souza, T.C.; Castro, E.M.; César Magalhães, P.; Oliveira Lino, L.; Trindade Alves, E., Albuquerque, P.E.P. Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. *Acta Physiol. Plant.* **2013**, *35*, 3201–3211.
5. Ali, Q.; Ashraf, M. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: Growth, Photosynthesis, Water Relations and Oxidative Defence Mechanism. *J. Agron. Crop Sci.* **2011**, *197*, 258–271.
6. Campos, C.N.; Ávila, R.G.; Souza, K.R.D.; Azevedo, L.M.; Alves, J.D. Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* L. plants. *Agric. Water Manag.* **2019**.
7. Barbosa, M.R.; Silva, M.M. de A.; Willadino, L.; Ulisses, C.; Camara, T.R. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. *Ciência Rural* **2014**.
8. Souza, T.C.; Magalhães, P.C.; Castro, E.M.; Carneiro, N.P.; Padilha, F.A.; Júnior, C.C.G. ABA application to maize hybrids contrasting for drought tolerance: Changes in water parameters and in antioxidant enzyme activity. *Plant Growth Regul.* **2014**.
9. Martinez, V.; Nieves-Cordones, M.; Lopez-Delacalle, M.; Rodenas, R.; Mestre, T.C.; Garcia-Sanchez, F.; Rubio, F.; Nortes, P.A.; Mittler, R.; Rivero, R.M. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules* **2018**.
10. Magalhães, P.C.; Durães, F.O.M. Circular Técnica 76 - Fisiologia da Produção de Milho. *Circ. Técnicas Embrapa* **2006**.
11. Jiang, B.; Wang, P.; Zhuang, S.; Li, M.; Li, Z.; Gong, Z. Detection of maize drought based on texture and morphological features. *Comput. Electron. Agric.* **2018**.
12. Rabêlo, V.M.; Magalhães, P.C.; Bressanin, L.A.; Carvalho, D.T.; Reis, C.O. dos; Karam, D.; Doriguetto, A.C.; Santos, M.H. dos; Santos Filho, P.R. dos S.; Souza, T.C. de The foliar application of a mixture of semisynthetic chitosan derivatives induces tolerance to water deficit in maize, improving the antioxidant system and increasing photosynthesis and grain yield. *Sci. Rep.* **2019**.
13. Alves Da Costa, P.H.; Azevedo Neto, A.D.; Bezerra, M.A.; Prisco, J.T.; Gomes-Filho, E. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. *Brazilian J. Plant Physiol.* **2005**.
14. Santos, M.O.; Oliveira Silveira, H.R.; Souza, K.R.D.; Lima, A.A.; Boas, L.V.V.;

- Barbosa, B.C.F.; Barreto, H.G.; Alves, J.D.; Chalfun-Junior, A. Antioxidant System Differential Regulation is Involved in Coffee Ripening Time at Different Altitudes. *Trop. Plant Biol.* **2018**.
15. Reis, C.O.; Magalhães, P.C.; Avila, R.G.; Almeida, L.G.; Rabelo, V.M.; Carvalho, D.T.; Cabral, D.F.; Karam, D.; de Souza, T.C. Action of N-Succinyl and N,O-Dicarboxymethyl Chitosan Derivatives on Chlorophyll Photosynthesis and Fluorescence in Drought-Sensitive Maize. *J. Plant Growth Regul.* **2019**, *38*, 619–630.
  16. Sello, S.; Meneghesso, A.; Alboresi, A.; Baldan, B.; Morosinotto, T. Plant biodiversity and regulation of photosynthesis in the natural environment. *Planta* **2019**.
  17. Paul, M.J.; Kollman, A.; Sakr, S.; Lagrimini, L.M.; Henry, C.; Griffiths, C.A.; Bledsoe, S.W. Differential Role for Trehalose Metabolism in Salt-Stressed Maize. *Plant Physiol.* **2015**, *169*, 1072–1089.
  18. İlhan, S.; Özdemir, F.; Bor, M. Contribution of trehalose biosynthetic pathway to drought stress tolerance of *Capparis ovata* Desf. *Plant Biol.* **2015**.
  19. Colla, E.; Pereira, A.B.; Hernalsteens, S.; Maugeri, F.; Rodrigues, M.I. Optimization of trehalose production by *Rhodotorula dairenensis* following a sequential strategy of experimental design. *Food Bioprocess Technol.* **2010**.
  20. Fernandez, O.; Béthencourt, L.; Quero, A.; Sangwan, R.S.; Clément Christophe, C. Trehalose and plant stress responses: Friend or foe? *Trends Plant Sci.* **2010**, *15*, 409–417.
  21. Kosar, F.; Akram, N.A.; Ashraf, M.; Sadiq, M.; Al-Qurainy, F. Trehalose-induced improvement in growth, photosynthetic characteristics and levels of some key osmoprotectants in sunflower (*Helianthus annuus* L.) under drought stress. *Pakistan J. Bot.* **2018**.
  22. Akram, N.A.; Noreen, S.; Noreen, T.; Ashraf, M. Exogenous application of trehalose alters growth, physiology and nutrient composition in radish (*Raphanus sativus* L.) plants under water-deficit conditions. *Rev. Bras. Bot.* **2015**, *38*, 431–439.
  23. Akram, N.A.; Waseem, M.; Ameen, R.; Ashraf, M. Trehalose pretreatment induces drought tolerance in radish (*Raphanus sativus* L.) plants: some key physio-biochemical traits. *Acta Physiol. Plant.* **2016**, *38*, 1–10.
  24. Shafiq, S.; Akram, N.A.; Ashraf, M. Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? *Sci. Hortic. (Amsterdam)*. **2015**, *185*, 68–75.
  25. Shahbaz, M.; Abid, A.; Masood, A.; Waraich, E.A. Foliar-applied trehalose modulates growth, mineral nutrition, photosynthetic ability, and oxidative defense system of rice (*Oryza sativa* L.) under saline stress. *J. Plant Nutr.* **2017**.
  26. Han, B.; Fu, L.; Zhang, D.; He, X.; Chen, Q.; Peng, M.; Zhang, J. Interspecies and intraspecies analysis of trehalose contents and the biosynthesis pathway gene family reveals crucial roles of trehalose in osmotic-stress tolerance in cassava. *Int. J. Mol. Sci.* **2016**, *17*.
  27. Li, Z.G.; Luo, L.J.; Zhu, L.P. Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings.

- Bot. Stud.* **2014**, *55*.
28. Aldesuquy, H. Exogenous Salicylic Acid and Trehalose Ameliorate Short Term Drought Stress in Wheat Cultivars by Up-regulating Membrane Characteristics and Antioxidant Defense System. *J. Hortic.* **2015**, *2*.
  29. Hura, T.; Hura, K.; Ostrowska, A.; Gadzinowska, J.; Fiust, A. Water stress-induced flag leaf senescence may be accelerated by rehydration. *J. Plant Physiol.* **2019**.
  30. Minuzzi, R.B.; Lopes, F.Z. Desempenho agrônômico do milho em diferentes cenários climáticos no Centro-Oeste do Brasil. *Rev. Bras. Eng. Agrícola e Ambient.* **2015**, *19*, 734–740.
  31. Daryanto, S.; Wang, L.; Jacinthe, P.A. Global synthesis of drought effects on maize and wheat production. *PLoS One* **2016**, *11*, 1–15.
  32. Liu, Z.; Shi, S.; Zhang, C.; Yin, G.; Yang, F. Drought tolerance in alfalfa (*Medicago sativa* L.) varieties is associated with enhanced antioxidative protection and declined lipid peroxidation. *J. Plant Physiol.* **2018**, *232*, 226–240.
  33. Petrov, V.; Hille, J.; Mueller-Roeber, B.; Gechev, T.S. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.* **2015**.
  34. Ambrósio, A. dos S. Avaliação em milho sob déficit hídrico com aplicação de derivados da trealose, UNIFAL, 2019.
  35. Lunn, J.E.; Delorge, I.; Figueroa, C.M.; Van Dijck, P.; Stitt, M. Trehalose metabolism in plants. *Plant J.* **2014**, *79*, 544–567.
  36. Mittler, R.; Vanderauwera, S.; Gollery, M.; Van Breusegem, F. Reactive oxygen gene network of plants. *Trends Plant Sci.* **2004**, *9*, 490–498.
  37. Shafiq, S.; Akram, N.A.; Ashraf, M. Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? *Sci. Hortic. (Amsterdam)*. **2015**, *185*, 68–75.
  38. Shim, J.S.; Seo, J.S.S.; Seo, J.S.S.; Kim, Y.; Koo, Y.; Do Choi, Y.; Jung, C. Heterologous expression of bacterial trehalose biosynthetic genes enhances trehalose accumulation in potato plants without adverse growth effects. *Plant Biotechnol. Rep.* **2019**, *13*, 409–418.
  39. Eleutherio, E.; Panek, A.; De Mesquita, J.F.; Trevisol, E.; Magalhães, R. Revisiting yeast trehalose metabolism. *Curr. Genet.* **2015**, *61*, 263–274.
  40. Kosar, F.; Akram, N.A.; Sadiq, M.; Al-Qurainy, F.; Ashraf, M. Trehalose: A Key Organic Osmolyte Effectively Involved in Plant Abiotic Stress Tolerance. *J. Plant Growth Regul.* 2018, 1–13.
  41. Ibrahim, H.A.; Abdellatif, Y.M.R. Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. *Ann. Agric. Sci.* **2016**.
  42. Mu, M.; Lu, X.K.; Wang, J.J.; Wang, D.L.; Yin, Z.J.; Wang, S.; Fan, W.L.; Ye, W.W. Genome-wide Identification and analysis of the stress-resistance function of the TPS (Trehalose-6-Phosphate Synthase) gene family in cotton. *BMC Genet.* **2016**.
  43. Pourcel, L.; Routaboul, J.M.; Cheynier, V.; Lepiniec, L.; Debeaujon, I. Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends Plant Sci.* 2007.

44. Rezayian, M.; Niknam, V.; Ebrahimzadeh, H. Differential responses of phenolic compounds of *Brassica napus* under drought stress. *Iran. J. Plant Physiol.* **2018**.
45. Sharma, A.; Shahzad, B.; Rehman, A.; Bhardwaj, R.; Landi, M.; Zheng, B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* **2019**, *24*.
46. Garg, A.K.; Kim, J.-K.; Owens, T.G.; Ranwala, A.P.; Choi, Y.D.; Kochian, L. V.; Wu, R.J. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci.* **2002**, *99*, 15898–15903.
47. Sami, F.; Yusuf, M.; Faizan, M.; Faraz, A.; Hayat, S. Role of sugars under abiotic stress. *Plant Physiol. Biochem.* **2016**.
48. Wingler, A.; Lea, P.J.; Quick, W.P.; Leegood, R.C. Photorespiration: Metabolic pathways and their role in stress protection. *Philos. Trans. R. Soc. B Biol. Sci.* **2000**, *355*, 1517–1529.
49. Iordachescu, M.; Imai, R. Trehalose Biosynthesis in Response to Abiotic Stresses. *J. Integr. Plant Biol.* **2008**, *50*, 1223–1229.
50. Nounjan, N.; Chansongkrow, P.; Charoensawan, V.; Siangliw, J.L.; Toojinda, T.; Chadchawan, S.; Theerakulpisut, P. High performance of photosynthesis and osmotic adjustment are associated with salt tolerance ability in rice carrying drought tolerance QTL: Physiological and co-expression network analysis. *Front. Plant Sci.* **2018**.
51. Laloum, T.; Martín, G.; Duque, P. Alternative Splicing Control of Abiotic Stress Responses. *Trends Plant Sci.* **2018**, *23*, 140–150.
52. Farooq, M.; Ullah, A.; Lee, D.J.; Alghamdi, S.S.; Siddique, K.H.M. Desi chickpea genotypes tolerate drought stress better than kabuli types by modulating germination metabolism, trehalose accumulation, and carbon assimilation. *Plant Physiol. Biochem.* **2018**, *126*, 47–54.
53. Zhao, M.L.; Ni, J.; Chen, M.S.; Xu, Z.F. Ectopic expression of *jatropha curcas* TREHALOSE-6-PHOSPHATE PHOSPHATASE *j* causes late-flowering and heterostylous phenotypes in *Arabidopsis* but not in *Jatropha*. *Int. J. Mol. Sci.* **2019**.
54. Luo, Y.; Wang, W.; Fan, Y.Z.; Gao, Y.M.; Wang, D. Exogenously-Supplied Trehalose Provides Better Protection for D1 Protein in Winter Wheat under Heat Stress. *Russ. J. Plant Physiol.* **2018**.
55. Pellny, T.K.; Ghannoum, O.; Conroy, J.P.; Schluepmann, H.; Smeekens, S.; Andralojc, J.; Krause, K.P.; Goddijn, O.; Paul, M.J. Genetic modification of photosynthesis with *E. coli* genes for trehalose synthesis. *Plant Biotechnol. J.* **2004**, *2*, 71–82.
56. Sánchez-Reinoso, A.D.; Ligarreto-Moreno, G.A.; Restrepo-Díaz, H. Chlorophyll  $\alpha$  fluorescence parameters as an indicator to identify drought susceptibility in common bush bean. *Agronomy* **2019**.
57. Veroneze-Júnior, V.; Martins, M.; Mc Leod, L.; Souza, K.R.D.; Santos-Filho, P.R.; Magalhães, P.C.; Carvalho, D.T.; Santos, M.H.; Souza, T.C. Leaf application of chitosan and physiological evaluation of maize hybrids contrasting for drought tolerance under water restriction. *Brazilian J. Biol.* **2019**.
58. Wu, W.M.; Li, J.C.; Chen, H.J.; Wang, S.J.; Wei, F.Z.; Wang, C.Y.; Wang, Y.H.; Wu,

- J.D.; Zhang, Y. Effects of nitrogen fertilization on chlorophyll fluorescence change in maize (*Zea mays* L.) under waterlogging at seedling stage. *J. Food, Agric. Environ.* **2013**.
59. Lobos, G.A.; Escobar-Opazo, A.; Estrada, F.; Romero-Bravo, S.; Garriga, M.; del Pozo, A.; Poblete-Echeverría, C.; Gonzalez-Talice, J.; González-Martinez, L.; Caligari, P. Spectral reflectance modeling by wavelength selection: Studying the scope for blueberry physiological breeding under contrasting water supply and heat conditions. *Remote Sens.* **2019**, *11*.
  60. Zhou, M.L.; Zhang, Q.; Sun, Z.M.; Chen, L.H.; Liu, B.X.; Zhang, K.X.; Zhu, X.M.; Shao, J.R.; Tang, Y.X.; Wu, Y.M. Trehalose Metabolism-Related Genes in Maize. *J. Plant Growth Regul.* **2014**, *33*, 256–271.
  61. Menger, F.M.; Mbadugha, B.N.A.A. Gemini surfactants with a disaccharide spacer. *J. Am. Chem. Soc.* **2001**, *123*, 875–885.
  62. De Souza, T.B.; Raimundo, P.O.B.; Andrade, S.F.; Hipólito, T.M.M.; Silva, N.C.; Dias, A.L.T.; Ikegaki, M.; Rocha, R.P.; Coelho, L.F.L.; Veloso, M.P.; et al. Synthesis and antimicrobial activity of 6-triazolo-6-deoxy eugenol glucosides. *Carbohydr. Res.* **2015**.
  63. Raij, B.V. et al. Recomendações de adubação e calagem para o estado de São Paulo. *Inst. Agrônomo/Fundação IAC* **1997**, *100*, 285.
  64. Bergonci, J.I.; Bergamaschi, H.; Berlato, M.A.; Santos, A.O. Potencial da água na folha como um indicador de déficit hídrico em milho. *Pesqui. Agropecu. Bras.* **2000**, *35*, 1531–1540.
  65. Buege, J.A.; Aust, S.D. Microsomal Lipid Peroxidation. *Methods Enzymol.* **1978**.
  66. Biemelt, S.; Keetman, U.; Albrecht, G. Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings. *Plant Physiol.* **1998**, *116*, 651–658.
  67. Giannopolitis, C.N.; Ries, S.K. Superoxide Dismutases: I. Occurrence in Higher Plants. *Plant Physiol.* **1977**, *59*, 309–314.
  68. Nakano, Y.; Asada, K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.* **1981**.
  69. Havir, E.A.; McHale, N.A. Biochemical and Developmental Characterization of Multiple Forms of Catalase in Tobacco Leaves. *Plant Physiol.* **1987**, *84*, 450–455.
  70. García-Limones, C.; Hervás, A.; Navas-Cortés, J.A.; Jiménez-Díaz, R.M.; Tena, M. Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. *ciceris*. *Physiol. Mol. Plant Pathol.* **2002**, *61*, 325–337.
  71. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**.
  72. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Anal. Chem.* **1959**.
  73. YEMM, E.W.; WILLIS, A.J. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **1954**.

74. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**.
75. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* **1973**.
76. Jassby, A.D.; Platt, T. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.* **1976**.