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# PIRUVATO E SUPERÓXIDO DESMUTASE COMO ATENUADORES DO ESTRESSE HIDRICO NO CRESCIMENTO INICIAL DO AMENDOIM

AREIA
2022

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Tese apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal da Paraíba, como parte dos requisitos exigidos para obtenção do título de Doutor em Agronomia.

Orientadora: Prof$^{a}$. Dr$^{a}$. Roseane Cavalcanti dos Santos
Coorientador: Prof. Dr. Pedro Dantas Fernandes

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**AREIA**

**2022**

*Ao Deus da minha vida.*

*Aos meus pais, Marina Duarte e Damião Floriano (in memoriam).*

*E a todos os demais familiares e amigos.*

***Ofereço***

*Ao grande amor da minha vida, que esteve sempre presente tanto nos momentos difíceis quanto de conquistas, com sua paciência, compreensão e atenção...*

*Ao meu esposo Carlos Roberto...*

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## RESUMO GERAL

A deficiência hídrica é um dos fatores mais limitantes no desenvolvimento das plantas. Os distúrbios desencadeados durante o estresse hídrico envolvem, especialmente, redução do crescimento, devido à queda na fotossíntese em consequência da perda de água na célula, além de danos oxidativos em consequência da produção excessiva de ROS que provocam a peroxidação lipídica, degradação de proteínas e morte celular. Para evitar tais danos, as plantas desengatilham diversos mecanismos de resposta, equilibrando o desbalanço das trocas gasosas de modo a favorecer o ajustamento osmótico. Não se sabe se a suplementação com piruvato e SOD por via exógena pode mitigar o efeito do estresse hídrico em plantas sensíveis. Para testar essa hipótese, desenvolveu-se o presente trabalho que teve por objetivo investigar a atuação de dois compostos fisiológico e bioquímico, o piruvato e SOD, na atenuação dos efeitos do déficit hídrico em plantas de amendoim (*Arachis hypogaea* L.) submetidos a onze dias de estresse hídrico. As cultivares selecionadas foram BR 1 (subesp. fastigiata) e IAC Caiapó (subesp. hypogaea), tolerante e sensível a seca, respectivamente. A aplicação exógena desses compostos em diferentes concentrações foi testada em plantas jovens (fase V1) e avaliadas quanto ao crescimento, trocas gasosas, ajustamento osmótico e enzimas antioxidativas. Nos estudos com o piruvato, testados nas concentrações de 100 µM e 50 mM, verificou-se que o déficit hídrico afetou o crescimento e as funções fisiológicas das duas cultivares, porém teve ação mitigadora na sensível IAC Caiapó, a 50 mM, especialmente na recuperação da fotossíntese, condutância estomática e conteúdo relativo de água, onde as perdas foram menores, comparando com as plantas do tratamento estressado. Essa situação favoreceu o acúmulo de prolina, numa concentração menor (100 µM), que foi beneficiada pela quantidade de água disponível na célula (RWC), auxiliando no ajustamento osmótico.  Com relação as enzimas antioxidativas, não foi vista contribuição do piruvato nessa cultivar, de modo a evitar possíveis danos celulares decorrentes do estresse hídrico. Tal resposta deve estar possivelmente associada ao reduzido nível de danos, representados pelos inputs de SOD, catalase (CAT) e ascorbato peroxidase (APX) visto nas plantas estressadas. Nos estudos com a SOD exógena, testada nas concentrações de 2.5, 5.0 e 7.5 U, foi observado que o aporte da enzima promoveu benefícios em ambas cultivares, de modo diferenciado. Na BR 1, a aplicação de SOD (7.5 U) contribuiu para recuperação da taxa de fotossíntese em mais de 9%, sendo consequência da elevação do carbono interno que na menor concentração (2.5U) foi suficiente para elevar a taxa em 13%, com relação ao tratamento estressado. A ação das enzimas antioxidativas foi mais atuante nas concentrações a partir de 5 U, pois ao final do processo de neutralização de $H_2O_2$ e $O_{2,}$ o status das plantas praticamente voltaram a normalidade das plantas controle. Na IAC Caiapó, o benefício da SOD exógena foi mais expressivo, registrado na recuperação das trocas gasosas e RWC em baixa concentração (2.5 U), que foi suficiente para assegurar a recuperação das plantas sob estresse. IAC Caiapó, contudo, por ser sensível a seca, demostrou que a atividade enzimática, para recuperação do processo antioxidativo foi mais lenta, mesmo com aporte de 5 U da SOD, baseando-se nas médias do tratamento estressado. Os resultados aqui apresentados são relevantes porque demonstram o potencial atenuador do piruvato e SOD exógenos na fisiologia e bioquímica de plantas sob estresse, especialmente as sensíveis ao déficit hídrico.

**Palavras-chaves:** Enzimas antioxidantes; ajustamento osmótico; tolerância ao estresse; deficiência hídrica; trocas gasosas.

BARBOSA, D. D. **Pyruvate and superoxide desmutase exogenous metabolites in attenuation of water stress in early growth of peanut.** 75p. Universidade Federal da Paraíba (Thesis Master in Agronomy), Areia, PB. 2021.

## ABSTRACT

Water deficit is one of the most limiting factors in plant development. Plant disorders triggered during water stress involve, in particular, reduced growth, due to reduced photosynthesis as a result of water loss in the cell. Others disorders are oxidative damages that result of the excessive production of reactive oxygen species that lead to lipid peroxidation, protein degradation and cell death. SOD is the precursor enzyme of the cell detoxification process, acting in the elimination of reactive oxygen species (ROS) through the dismutation of superoxide radicals, favoring the neutralization of reactive species and reestablishment of cellular balance. In drought-tolerant plants, SOD activity is high in order to favor the action of other enzymes that precede it in the antioxidant complex. However, in sensitive plants, SOD activity is slower and, therefore, cell damage is greater, leading to unpredictable consequences during management. As tolerance to water stress is governed by genetic factors, the adoption of sensitive cultivars is restricted to favorable environments, where production can be guaranteed. It is not known whether exogenous metabolite supplementation can mitigate the effect of water stress on sensitive plants. To certify this hypothesis, the present work was developed to investigate the role of two physiological and biochemical metabolites, pyruvate and SOD, in the attenuation of the effects of water deficit in peanut plants (*Arachis hypogaea* L.) submitted to eleven days of water stress. BR 1 (subsp. fastigiata) and IAC Caiapó (subsp. hypogaea), tolerant and sensitive to drought, respectively, were used as tester. The exogenous application of these metabolites at different concentrations were tested in young plants (stage V1) and evaluated for growth, gas exchange, osmotic adjustment and antioxidant complex enzymes. In studies with pyruvate, tested at 100 µM and 50 mM, we found that water deficit affected the growth and physiological functions of both cultivars, but mitigating action was expressive in sensitive IAC Caiapó, at 50 mM, especially in recovery of photosynthesis, stomatal conductance and relative water content, where losses were lower, compared to the stressed treatment plants. This situation favored the accumulation of proline, at lower concentration (100 µM), which was benefited by the amount of water available in the cell (RWC), favored by osmotic adjustment. As to antioxidant enzymes, no expressive result was seen with exogenous pyruvate in this cultivar, in order to avoid possible cell damage caused by water stress. Such response must possibly be associated with the reduced level of damage, represented by the inputs of SOD, CAT and APX found in plants stressed in this work. In assays with exogenous SOD, tested at 2.5, 5.0 and 7.5 U, we found that the enzyme contribution promoted benefits in both cultivars, in different ways. In BR 1, the application of SOD (7.5 U) contributed to recovery the photosynthesis rate by more than 9%, as a consequence of increase in internal Carbon, which at the lowest concentration (2.5U) was enough to raise the rate by 13 %, regarding the stressed treatment. The machinery of antioxidative enzymes was more active in concentrations from 5 U, so that at the end of process of neutralizing $H_2O_2$ and $O_2$, the status of the plants practically returned to normality of the control plants. At IAC Caiapó, the benefit of exogenous SOD was more expressive, registered in the recovery of gas exchange and RWC in low concentration (2.5 U), which was sufficient to ensure the recovery of plants under stress. IAC Caiapó, however, as is sensitive to drought, demonstrated that the enzymatic activity for recovery of the antioxidative process was slower, even with contribution of SOD at 5 U, based on the means of the stressed treatment. The results presented in these works are relevant because they demonstrate the attenuating potential of exogenous pyruvate and SOD in the physiology and biochemistry of plants under stress, especially those sensitive to water deficit.

**Keywords:** Antioxidant activity; osmotic adjustment; stress tolerance; water deficiency; gas exchange.

# LISTA DE FIGURAS

# SUMÁRIO

# 1. INTRODUÇÃO GERAL

As mudanças climáticas têm sido um dos assuntos mais debatidos por autoridades políticas e comunidades cientistas mundiais, devido aos sérios prejuízos que provocam na agricultura, especialmente a seca nas regiões áridas e semiáridas.

O Brasil tem extensão continental e períodos secos ocorrem em todas regiões, porém, no Nordeste a duração e intensidade são mais expressivas, limitando a atividade agropecuária, especialmente nos municípios que se inserem no tropico semiárido, que contem atualmente 1262 municípios (IBGE, 2017).

No aspecto fisiológico, o déficit hídrico é um dos principais fatores limitantes para o desenvolvimento e crescimento das plantas. Em resposta ao estresse, os sistemas de transporte de íons e água através das membranas, funcionam para controlar as mudanças de pressão do turgor nas células guarda e estimular o fechamento estomático (Azevedo Neto et al., 2010; Osakabe et al., 2014). Contudo, o estresse hídrico induz a redução no potencial hídrico ($\Psi w$) foliar e na abertura estomática, levando à regulação negativa de genes relacionados à fotossíntese e à disponibilidade reduzida de $CO_2$ (Osakabe et al., 2014).

Quando plantas são submetidas as condições de estresse hídrico, ocorre a geração de espécies reativas de oxigeno (EROs), subprodutos do metabolismo do oxigênio, que em condições normais exercem função de sinalizadores celular, porém, a partir da imposição dos efeitos dos estresses, tornam-se substâncias altamente tóxicas para as plantas. A formação das EROs ocorre predominantemente nas mitocôndrias, cloroplastos, peroxissomos e apoplastos. As principais EROs são: radical superóxido ($O_2 \cdot^-$), oxigênio singlet ($^1O_2$), peroxido de hidrogênio ($H_2O_2$) e radical hidroxila ($HO\cdot$) (Choudhury et al., 2019).

Os processos de defesa das plantas contra os estresses bióticas e abióticas decorrente da ação das EROs, envolvem sinalização, desintoxição e ajustamento osmótico, abrangendo sistemas antioxidantes enzimáticos a partir de enzimas, como superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX), glutationa peroxidase (GPX), entre outras, além de mecanismos não enzimáticos, como alfa tocoferóis, açucares redutores, vitaminas A e C e carotenoides. Quanto ao ajustamento osmótico, o mesmo ocorre pela ação efetiva do acúmulo de compostos orgânicos e inorgânicos, como açúcares redutores, carboidratos, compostos quaternários e aminoácidos como a prolina (Sharma et al., 2012).

Estudos recentes têm investido em compostos envolvidos na linha de frente do metabolismo celular, como o piruvato, uma molécula de três carbonos é descarboxilada para a formação do acetil-Co-A, substrato do ciclo de Krebes (Taiz e Zaiger, 2017). Alguns trabalhos, têm reportado a influência do piruvato na ativação dos movimentos estomáticos, cujo fechamento é uma das principais estratégias de defesa das plantas durante o estresse hídrico (Li et al., 2014; Wang et al., 2014; Shen et al., 2017).

O papel desses compostos na linha de defesa de plantas é primordial porque, dependendo da pronta resposta diante da sinalização do estresse, a maquinaria de defesa pode atuar de forma mais eficaz permitindo o alivio do estresse, minimizando os danos oxidativos. O processo de defesa celular contra danos oxidativos é iniciado pela SOD, primeira enzima na linha de defesa contra as EROs. A SOD é responsável por dismutar o radical superóxido em peroxido de hidrogênio ($H_2O_2$), um radical menos prejudicial para planta, que em seguida é convertido em água ($H_2O$) pela catalase para ser liberada durante o processo de transpiração.

A literatura disponibiliza vários estudos demonstrando o papel da SOD na defesa celular diante de estresses ambientais em plantas de metabolismo C3, como feijão (Maia et al., 2012), arroz (Saini et al., 2018), amendoim (Pereira et al., 2012) e soja (Hao et al., 2013), e C4 como cana de-açúcar (Sales et al., 2013) e milho (Shafiq et al., 2019), entre outras. Na maioria desses estudos é reportado que durante o estresse hídrico, as plantas combatem as EROs elevando a atividade antioxidativa da SOD, como forma de minimizar danos celulares. Assim, a SOD pode ser adotada confiavelmente como um marcador de seleção analítico, para estimar plantas tolerantes ao estresse hídrico (Sharma et al., 2019).

O amendoim (*Arachis hypogaea* L.) é uma planta alotetraploide de natureza autógama, que possui larga plasticidade genética para adaptação a climas tropical e semiárido (Santos et al., 2013). Os tipos de amendoim encontrados são representados por indivíduos de porte rasteiro de ciclo longo, também conhecidos como precoce e o tipo ereto de ciclo curto. As cultivares precoces são mais adequadas ao manejo de sequeiro porque possuem habilidades para tolerar ambientes com restrição hídrica, como ocorre frequentemente nas regiões agreste e sertão do Nordeste brasileiro (Bolonhesi et al., 2013). Entre as habilidades mais citadas, especialmente nas plantas do tipo ereto, citam-se o ajustamento osmótico e fotossintético que beneficia alguns fatores morfológicos como aprofundamento das raízes de modo a garantir mais umidade para manter o crescimento das plantas (Akcay et al., 2010; Santos et al., 2012; Pereira et al., 2016). Nas cultivares sensíveis, geralmente as plantas tardias do tipo rasteiro, o ajustamento osmótico é mais lento, o que dificulta a resposta ao estresse de forma mais ágil, levando a prejuízos no crescimento e produtividade das vagens (Pereira et al., 2012; Santos et al., 2013).

Os trabalhos sobre aplicação exógena de compostos orgânicos em plantas C3 submetidas a estresse hídrico têm sido reportados na literatura e demonstram o benefício de alguns deles na mitigação dos efeitos deletérios da seca, em algumas plantas cultivadas (Li et al., 2014; Wang et al., 2014; Karimian et al., 2015; Shen et al., 2017). Visando aprofundar o tema nos aspectos biológicos do amendoim desenvolveu-se o presente trabalho, que teve por objetivo investigar as respostas fisiológicas e bioquímicas do piruvato e da SOD, como possíveis atenuadores dos efeitos do estresse hídrico, tomando-se como referência duas cultivares contrastantes para tolerância a seca, baseando-se em dados disponíveis na literatura.

# 2. OBJETIVOS

## 2.1. OBJETIVO GERAL

Identificar a resposta de duas cultivares contrastantes de amendoim submetidas a déficit hídrico na fase vegetativa e suplementados com piruvato e superóxido dismutase - SOD.

## 2.2. OBJETIVOS ESPECIFICOS

- Avaliar os possíveis efeitos do piruvato e da SOD aplicados em plantas de amendoim sob déficit hídrico.
- Verificar se as doses de superóxido dismutase influenciam na tolerância a seca das cultivares contrastantes de amendoim.
- Analisar as respostas fisiológicas e bioquímicas das cultivares de amendoim sob déficit hídrico e com aplicação de atenuadores do déficit hídrico.
- Identificar se cultivares sensíveis a seca de amendoim podem suportar o déficit hídrico após receberem a aplicação de compostos com potencial de atenuadores de déficit hídrico.
- Selecionar dentre as doses aplicadas de piruvato e SOD aquela com melhor potencial de resposta ao déficit hídrico.

# 3. REFERENCIAL TÉORICO

## 3.1. A seca e as implicações para agricultura

A seca é um fenômeno mundial que ocorre em vários países, inclusive nos que detém climas árido e semiárido. Nos ambientes agricultáveis, a seca provoca prejuízos consideráveis na produção das lavouras, além de depreciação na qualidade do produto. Entre todos os estresses de origem abiótico, a seca é considerada a mais danosa sobre a biota do solo e das plantas, justamente por ser responsável pelo complexo desencadeamento fisio-bioquímico que incorre nas plantas, afetando o agrossistema que as contêm (Zhu, 2016; Zia et al., 2021).

Estima-se que as mudanças climáticas causadas pelo efeito estufa, aumentarão a temperatura do planeta em até 2 ºC graus (ONU, 2020), o que será suficiente para alterar o regime hidrológico de várias regiões do mundo, afetando principalmente as áreas cultivadas. Além das mudanças ambientais, a demanda por alimento será maior, pois a população mundial, que atualmente é de 7,7 bilhões de habitantes, passará 9,7 bilhões em 2050, segundo estimativas populacionais da ONU (ONU, 2020; FAO, 2020).

De acordo com o relatório da FAO (2020), cerca de 3 bilhões de pessoas vivem em áreas agrícolas com níveis altos ou muito altos de déficit e escassez de água, e quase metade dessas pessoas enfrenta severas limitações hídricas. Além disso, a disponibilidade de recursos de água potável por pessoa diminuiu em mais de 20% durante as duas últimas décadas, ressaltando a importância de produzir mais com menos, especialmente na agricultura, o maior usuário de água no mundo.

Atualmente, cerca de 128 milhões de hectares no planeta enfrentam secas frequentes, correspondendo a 11% das terras agrícolas (ANA, 2020). Nas áreas irrigadas, aproximadamente 171 milhões de hectares são afetadas pelo déficit hídrico, representando 60% do total (ANA, 2020).

Em 2018, uma onda de seca e calor na Europa, provocou redução de 8% na produção de cereais, suficiente para causar a escassez de forragem para o gado, além de elevar o valor de commodities, como trigo (*Triticum aestivum*) e cevada (*Hordeum vulgare*) na ordem de 34 e 48%, respectivamente (Brás et al., 2021).Segundo a Agência Nacional de Água - ANA (2020), as áreas agricultáveis brasileiras enfrentam cerca de 37% de déficit hídrico ao ano; desse percentual 30% incidem na fase vegetativa, justificando-se ai o motivo de frustação das safras em ambientes que manejam lavoura de sequeiro. Na região Nordeste, especialmente nos municípios que englobam o trópico semiárido, a situação é mais drástica devido aos frequentes períodos de seca prolongada ou precipitações fluviais irregulares, especialmente no estabelecimento de lavouras de subsistência. Nessa região, a agricultura de sequeiro é a mais predominante, onde o manejo é praticamente dependente das chuvas e as frustações de safra são frequentes. As lavouras do milho, feijão e mandioca são constantes para atender a subsistência familiar, enquanto a da palma atende a alimentação animal (Lima et al., 2016).

Além da seca influenciar diretamente a dinâmica da produção agrícola, também causa prejuízos econômicos, expondo muitas vezes as pessoas que dependem da agricultura de base familiar, a vulnerabilidade social e ao êxodo rural (Lima et al., 2016). Para minimizar os agravos agrícolas/econômicos decorrentes da seca, programas do governo foram instituídos com o objetivo de subsidiar os pequenos agricultores durante o período de seca, como é o caso do Seguro da Agricultura Familiar (SEAF) criado dentro Programa Nacional de Fortalecimento da Agricultura Familiar (PRONAF), com o objetivo garantir a cobertura total do financiamento, para a família produtora que perder a safra em razão de fenômenos climáticos (CONTRAF BRASIL, 2021).

Observa-se, que a seca afeta não somente o micro (planta) mas o macro (fatores socioeconômicos) também. Então, para diminuir esses danos, é necessário a promoção de políticas públicas, direcionadas para o custeio da produção agrícola desses pequenos produtores, através da concessão de incentivos financeiros e acesso a tecnologias agrícolas, voltados para o enfrentamento da seca no semiárido brasileiro. Acredita-se que através de investimentos e manejo adequados, seja possível produzir bem na região semiárida, desde que sejam ofertadas ferramentas e alternativas de produção, pois embora a seca seja característica intrínseca da região, responsável por gerar perdas nas lavouras ao longo dos anos, também é possível produzir em áreas de seca, desde que seja observado e considerado a heterogeneidade do semiárido, respeitando e explorando as especificidades de cada área e/ou município que integra o semiárido brasileiro (Lima et al., 2016).

## 3.2. A batalha celular para combate ao estresse oxidativo

O déficit hídrico acarreta diversas alterações no crescimento das plantas em função das respostas fisiológicas e bioquímicas que elas expressam. Em condições normais o conteúdo de água nos tecidos é mantido a partir do equilíbrio entre as taxas de perda e captação de água. Isso ocorre principalmente por meio do movimento estomático que se adequa de modo a estabilizar o balanço hídrico da célula. A capacidade de acumular água nos tecidos, o espessamento da cutícula e a permeabilidade também são fatores diretamente relacionados com a manutenção do equilíbrio e quantidade de água nas células (Verslues et al., 2006). No aspecto morfológico, a redução da área foliar leva a efeitos drásticos nas plantas submetidas a déficit hídrico, uma vez que reduz a fotossíntese e, consequentemente, o crescimento vegetativo (Taiz e Zeiger, 2017). Essa redução nas folhas ocorre devido a pressão de turgescência das células, que é determinada pelo conteúdo de água na planta (Araújo Junior, 2019). O sistema radicular, que é o sitio motor de todo entendimento do status hídrico da planta, induz a sinalização dos processos fisiológicos e bioquímicos para promover o fechamento rápido dos estômatos, de modo a limitar a perda excessiva de água pela da transpiração. A taxa de fotossíntese é afetada porque todo esse processo afeta as trocas gasosas, uma vez que reduz significativamente a entrada de $CO_2$ pela abertura estomática (Dutra et al., 2018).

O aparato dos estômatos é o diferencial para permitir que a planta enfrente o estresse sem maiores danos a maquinaria celular. O fechamento estomático é uma resposta primaria ao sinal do estresse que, ao provocar redução da absorção de $CO_2$, limita as trocas gasosas e redução da fotossíntese para evitar perda de água por transpiração (Farooq et al., 2019). A troca de vapor d'água por $CO_2$ na superfície das folhas das plantas é a base para a assimilação de carbono, na qual o $CO_2$ atua como um receptor para o fluxo de elétrons sustentado pela fotossíntese (Sperlich et al., 2015).

Os estômatos são as estruturas responsáveis pela absorção de $CO_2$ para realização da fase química da fotossíntese. O processo de abertura dos estômatos envolve a saída de moléculas de água e por isso o fechamento estomático é primeira resposta fisiológica da planta ao déficit hídrico, como consequência da desordem dos fotossistemas induzido pela desidratação. Com a redução da transpiração, a habilidade de manter o equilíbrio entre a captação e perda de água é diminuída, já que os níveis de água no solo e nas células são baixos. Nesse caso, como mecanismo de tolerância, as plantas evitam a dessecação celular, impedindo o efluxo através do espessamento da parece celular ou promovem o influxo de água a partir do acúmulo de solutos na célula, para reduzir o potencial osmótico ($\Psi s$) e favorecer o ajustamento osmótico (Zhang et al., 1999).

O metabolismo da planta sob estresse envolve o acúmulo de compostos orgânicos para manter o equilíbrio das trocas gasosas, de modo a manter a turgescência celular sob condições adversas ou durante a recuperação após o período de deficiência hídrica (Padmavathi e Rao, 2013). Prolina, trealose, glicina betaina, inositol, sorbitol e manitol, estão entre os osmolitos mais reportados durante o estresse hídrico. Trata-se de moléculas de baixo peso molecular, atóxicos e solúveis em água (Sharma et al., 2019).

A prolina é um dos osmolitos mais reportados na literatura, em trabalhos envolvendo estresses ambientais. A capacidade da planta sob estresse em acumular prolina está relacionada a tolerância, uma vez que ela contribui na eliminação das EROs (Espécies Reativas de Oxigênio), na supressão do oxigênio singleto e no aumento da atividade de enzimas antioxidantes (Sharma et al., 2019). Adicionalmente, a prolina exerce função de proteção dos fotossistemas e atua na sinalização para expressão de genes envolvidos com a tolerância (Islam et al, 2009; Szabados e Savoure, 2010).

A geração de EROs, formadas a partir do metabolismo do $O_2$ durante os processos de respiração e fotossíntese, é um processo frequente diante da percepção de estresse sinalizado por receptores celulares estabelecido nas membranas. Na maquinaria celular, as EROs funcionam principalmente como moléculas de transdução de sinal, regulando diferentes vias durante o processo de aclimatação da planta ao estresse. No entanto, quando o estresse se estabelece, a geração excessiva de EROs torna-se tóxica levando a danos variados ou morte celular. Como cada compartimento subcelular contém seu próprio mecanismo de produção e eliminação das EROs, o nível de estado estacionário, bem como o estado redox de cada compartimento é diferente no aparato celular (Noctor et al., 2018).

O excesso de EROs na célula provoca peroxidação lipídica, oxidação de proteínas, inibição enzimática, danos aos ácidos nucleicos, entre outros (Breusegem et al., 2001; Sharma et al., 2019). Dependendo do nível de tolerância da espécie, esses danos podem ser mitigados nas células pela ação de moléculas envolvidas no sistema de defesa, enzimáticas e não enzimáticas, ativadas para combater as EROs. No processo enzimático de defesa, a Superóxido dismutase (SOD) atua como enzima catalisadora da dismutação do radical superóxido ($O_2^{\bullet -}$), convertido para peróxido de hidrogênio ($H_2O_2$) e oxigênio molecular ($O^2$). Nessa ocasião, a atividade da SOD se eleva rapidamente para manter o equilíbrio celular, eliminando o excesso de $O_2^{\bullet -}$. As moléculas de $H_2O_2$ remanescentes são inativadas e convertidas em água ($H_2O$) e oxigênio molecular ($O_2$) pela ação das enzimas Catalase (CAT) e as peroxidases (POXs) (Gil e Tuteja, 2010; Sharma et al., 2019).

A SOD foi primeiro reportada em 1969, por Joe McCord e Irwin Fridovich em eritrócitos bovinos, constituindo-se um marco para os estudos envolvendo ação dos radicais livres oriundos do metabolismo do oxigênio (Del Río et al., 2018). Trata-se de uma enzima intracelular, pertencente à família das metaloenzimas, com três isoformas (Cu/Zn-SOD, Mn-SOD e Fe-SOD), localizados em diferentes compartimentos subcelulares propensos a oxidação mediada por EROs, como cloroplastos, mitocôndrias, citosol e peroxissomos (Pilon et al., 2011;

Huseynova et al., 2014). Em plantas sob estresse, a elevação de SOD está associada a tolerância ao estresse ambiental (Maia et al., 2012; Pereira et al., 2012; Hao et al., 2013; Barbosa et al., 2018).

Com relação as moléculas de defesa não enzimáticas, várias se agregam no processo biosintético para auxiliar na redução danos oxidativos, como ácido ascórbico (AS), flavonoides, ácido salicílico (AS), α-tocoferóis, carotenoides, entre outras (Jin e Daniell, 2014; Nakabayashi et al., 2014; Kaur e Nayyar, 2014). Cada uma tem papel fundamental no processo de defesa celular e atuam de forma integrada de modo a minimizar os danos em decorrência do estresse ambiental.

Com α-tocoferóis, Sadiq et al., (2017) testaram as doses de 100, 200 e 300 mg $L^{-1}$ em plantas de feijão mungo (*Vigna radiata*) submetidas a 60% da capacidade de campo por 28 dias, e observaram que aplicação foliar de α-tocoferol na dose de 300 mg $L^{-1}$ promoveu aumento da SOD, CAT e POX em 233%, 100% e 40%, respectivamente, comparados com as médias das plantas estressadas.

Há mais outros relatos disponíveis na literatura sobre a ação de compostos orgânicos na mitigação do estresse hídrico. Como esses metabolitos se envolvem direta ou indiretamente nas rotas de defesa celular, o aporte oferecido nas plantas sob estresse geralmente traz benefícios fisio-bioquímicos, embora a natureza genética do acesso deva ser considerada, uma vez que a maquinaria nas plantas tolerantes é diferenciada.

## 3.3. O papel do piruvato no ajustamento estomático

O fechamento estomático é um dos principais mecanismos de defesa ligado diretamente a via do ácido abscisico (ABA), que sob suspensão hídrica rapidamente se eleva para reduzir o turgor das células-guardas e promover o fechamento dos estômatos (Zelicourt et al., 2016). O piruvato, substrato usado no ciclo ácido tricarboxílico (TCA) ou ciclo de Krebs, pode atuar como um sinalizador celular envolvido na ativação do canal aniônico ao promover o fechamento estomático de forma dependente das EROs, que aumentam para promover a acumulação do ABA (Shen et al., 2017). Adicionalmente, participa da biossíntese de compostos orgânicos como glicose, lactato e ácidos graxos.

A produção do piruvato ocorre a partir da transformação da glicose em ATP pelo metabolismo aeróbio, na presença de oxigênio, pela via da glicólise. Nesse processo, o piruvato é convertido irreversivelmente em acetil-CoA, para ser usado no TCA, ou seja, na síntese dos ácidos de três carbonos (Taiz e Zaiger, 2017). Uma outra via metabólica de atuação do piruvato, é a respiratória alternativa, onde o piruvato atua como ativador da enzima oxidase alternativa (AOX), responsável por limitar a produção de EROs nas mitocôndrias, decorrentes de mudanças ambientais (Juszczuk e Rychter, 2003).

A literatura reporta vastamente sobre o envolvimento do ABA e alguns precursores na rota de defesa contra danos ambientais. Shen et al. (2017) reportaram o papel de proteínas mitocondriais carreadoras de piruvato (MPCs), que estão envolvidas na ativação dos movimentos estomáticos através da sinalização celular, acionando canais aniônicos que ativam o fechamento dos estômatos em resposta a seca. O trabalho desses autores teve como base a pesquisa de Wang et al. (2014), que ao usar piruvato exógeno em plantas de *Arabidopsis* submetidas a estresse hídrico, descobriram uma proteína de regulação negativa atuando na abertura estomática das plantas e na sinalização das células-guarda induzida por ABA. Segundo esses autores, a proteína NRGA1 (Negative Regulator of Guard cell ABA signaling 1) seria um fator de transcrição responsável por ativar o acúmulo de piruvato e sinalizar para a ação do ABA desencadeando o fechamento estomático.

As MPCs, que modulam a capacidade respiratória auxiliando na tolerância ao estresse, transportam o piruvato até a mitocôndria, onde ele entra no ciclo do TCA (Shen et al., 2017). Em leveduras, as MPC1 e MPC2 estão envolvidas com a produção de aminoácidos e ácido lipóico, enquanto a MPC3 está envolvida na taxa respiratória e na resistência ao estresse oxidativo (Timon-Gomez et al., 2013).

## 3.4. Características gerais da cultura do amendoim

O amendoim é uma planta nativa da América do Sul, com ampla adaptação ambiental, que permite o cultivo em várias partes do mundo. Atualmente, é considerada a quinta oleaginosa mais cultivada, liderada pelos países China, Índia, Nigéria, Sudão e Estados Unidos (FAO, 2021). Em 2020, a produção mundial de amendoim alcançou 49 milhões de toneladas, ficando atrás apenas da soja, girassol, colza e algodão (USDA, 2020).

No Brasil, a produção do amendoim na safra de 2020 foi de 1,6 milhão de toneladas, representada pelos estados de São Paulo, Minas Gerais e Paraná, que possuem clima tropical. Trata-se de uma leguminosa herbácea, com hábito de crescimento rasteiro ou ereto, ciclo anual podendo variar de 85 a 160 e altura entre 12 a 60 cm, dependendo do tipo botânico (Nogueira et al., 2013; Santos et al., 2013).

Pertencente à família fabaceae, o gênero *Arachis* comporta 80 espécies, dentre estas são encontradas, principalmente espécies diploides ($2n = 2x = 20$), como também indivíduos com aneuploidia (2n = 2x = 18), além de dois tetraploides (2n = 4x = 40). A distribuição natural do gênero é restrita aos países da América do Sul (Brasil, Bolívia, Paraguai, Uruguai e Argentina). Contudo, o cultivo do amendoim estende-se internacionalmente graças a ampla variabilidade genética, que permite através dos programas de melhoramento genético, o desenvolvimento de cultivares que atendem a necessidade do mercado. A espécie *A. hypogaea*, que representa o amendoim cultivado, é possivelmente originária do cruzamento entre as espécies diploides *A. duranensis* e *A. ipaënsis*, que resultando em um híbrido estéril, cujos cromossomos foram duplicados, levando à restauração da fertilidade e provável seleção de controle genético da meiose (Valls, 2013).

No aspecto comercial, existem três tipos de amendoim disponíveis nos mercados mundiais de óleo e grãos. Os tipos Valência e Spanish pertencem a subespécie *fastigiata*, cujos acessos apresentam crescimento ereto ou semi-ereto, ciclo curto variando de 85 a 110 dias, haste principal com flores e ramificação esparsa (poucos ramos secundários e raros terciários). No geral, as vagens do tipo Spanish são pequenas, com constrição moderada, detendo duas sementes de coloração bege; as do tipo Valência são de tamanho médio, geralmente sem contrição, contendo entre 3 a quatro sementes de coloração vermelha (Santos et al., 2013). Os tipos rasteiro (Runner) pertencem a subespécie *hypogaea*, possuem ramificação profusa, ausência de flores na haste principal e ciclo longo de 120 a 160 dias. As sementes são largas ou extra largas, com moderada constrição, detendo duas sementes por vagens, de coloração bege (Santos et al., 2013).

No Brasil, o mercado de sementes é predominado pelo tipo Runner, com 24 cultivares registradas no Ministério de Agricultura (http://sistemas.agricultura.gov.br/snpc/cultivarweb/cultivares_registradas.php), desenvolvidas pelas empresas Embrapa, Instituto Agronômico de Campinas, El Carmen Sementes do Brasil, Wilco do Brasil LTDA e Instituto de Desenvolvimento Rural do Paraná. Essas cultivares possuem como característica principal a elevada produtividade e rendimento das sementes, além de apresentar excelente qualidade de óleo, rico em ácidos graxos como o ácido oleico, que confere maior estabilidade oxidativa e mais tempo de prateleira.

Apesar de ser cultivado largamente em regiões de clima semiárido, a planta do amendoim, especialmente a de porte ereto, possui larga adaptação para manejo em ambientes com baixa disponibilidade hídrica devido a habilidade do sistema radicular para se aprofundar em busca de umidade, quando a seca se estabelece (Nogueira e Távora, 2013); Pereira et al., 2012; Santos et al., 2013). Independentemente do tipo botânico, a planta do amendoim é mais afetada pela deficiência hídrica na fase reprodutiva, principalmente, durante o período de florescimento e enchimento das vagens (Nogueira e Távora, 2013). As cultivares rasteiras, por terem esse período mais largo, são mais vulneráveis, porém respondem adequadamente em ambiente semiárido quando o fluxo hídrico é regularmente estabelecido (Santos et al., 2012). Como as irregularidades pluviométricas ocorrem em várias regiões brasileiras, o melhoramento genético do amendoim, conduzido por empresas de pesquisa no Brasil, tem envidado esforços para identificar novos genótipos de amendoim rasteiro que apresentem adaptação a tal situação, mantendo a produção em níveis razoáveis para evitar frustação de safra. Um exemplo desse esforço é cultivar Perola Branca, desenvolvida pela Embrapa, rasteira precoce, com ciclo de apenas 115 dias. Essa precocidade é resultado de um trabalho de cruzamento envolvendo a precoce ereta BR 1 e a tardia rasteira LViPE06, resultando em um material de adaptação ao manejo em ambiente semiárido (Santos et al., 2012).

As mudanças climáticas causam sérios danos ao sistema agrícola e a necessidade de ajuste a essas alterações são requeridas com a máxima rapidez. Contudo, as plantas precisam de tempo para se reorganizarem e responderem adequadamente ao clima. Por isso alguns programas de melhoramento de plantas buscam através das pesquisas no campo da biotecnologia, alternativas no desenvolvimento de tecnologias que correspondam a essas mudanças, de maneira a minimizar as perdas da produção agrícola, através de subsídios para o cultivo sob escassez hídrica. A aplicação de compostos orgânicos pode ser auxiliar nessa corrida.

Ainda não se sabe se a suplementação via exógena é capaz de minimizar os efeitos deletérios do déficit hídrico em plantas de amendoim sensíveis a seca. Para obter a resposta a esse questionamento, o presente trabalho foi desenvolvido, com o objetivo de investigar a atuação do piruvato e da SOD, na atenuação dos efeitos do déficit hídrico em cultivares sensíveis e tolerantes de amendoim submetidas a seca.

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# CAPÍTULO I

## EXOGENOUS PYRUVATE MITIGATES THE DETRIMENTAL EFFECTS OF WATER STRESS IN CONTRASTING PEANUT GENOTYPES

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# 5. Exogenous pyruvate mitigates the detrimental effects of water stress in contrasting peanut genotypes

## Running title: Pyruvate mitigates drought in water-stressed peanut

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**ABSTRACT.** Water stress triggers various cellular responses in plants, altering the normality of metabolic flow. Pyruvate, an important component of the glycolysis pathway, is directly involved in cell processes, triggering genes that influence the drought tolerance. Researches with *Arabidopsis* have shown that synthetic pyruvate reliefs the damages of drought. Here, we evaluated the effects of exogenous pyruvate on mitigation of drought in two peanut cultivars submitted to water suppression, and further treated with product at 100 and 50,000 µM. The evaluations were based on growth, gas exchange, and photosynthesis rate analyses. In addition, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) enzymes, and free proline assays were also tested. In general, the exogenous application of pyruvate contributed to mitigate the effects of water stress in IAC Caiapó (sensitive to drought), based on gas exchange and instantaneous efficiency of water use. In biochemical aspect, exogenous pyruvate contributed to restore totally the action of antioxidative enzymes in BR 1 (tolerant to drought), based on inputs of SOD (45%), CAT (129%) and APX (60%) in stressed plants, and full recovery at 50,000 µM. As to proline, stress attenuation was found only at 100 µM in both cultivars; the excess seemed to have negative interaction in stressed plants probably affecting the cell environment.

**Key words:** *Arachis hypogaea*; Drought tolerance; Gas exchange; Proline, Antioxidative enzymes

## 5.1 INTRODUCTION

Water scarcity is a climatic factor that limits crop production, causing several physiological and biochemical disturbances in plants. When the cell detects the water stress signal, a strategic adjustment in defense scheme usually begins, whose quick physiological response indicates the differences in tolerant and sensitive genotypes (Osakabe et al, 2014; Dutra et al, 2018).

The complex signaling pathways in response to environmental stress, leads to various cellular changes, resulting in decrease of turgor pressure in guard cells and stomatal closure. In response to drought, plants synthesize abscisic acid (ABA) that induces stomatal closure, in order to reduce the transpirational water loss. This strong increase of plant ABA levels is accompanied by a major change in gene expression and in adaptive responses arising from many novel functional genes and transcription factors acting in molecular mechanisms of ABA signaling and stomatal movement (Munemasa et al, 2015). These elements activate the ion channels of the guard cells and external potassium channels in order to act on efflux of solutes and promote stomatal closure (Osakabe et al, 2014).

All signaling, adjustment and adaptation processes demand energy for synthesis of phytohormones and others cell metabolites. Glycolysis is inserted in this context, through a sequence of reactions mediated by different enzymes in the respiratory flow of plants (Plaxton and Podestá, 2006; Rich and Maréchal, 2010). Pyruvate, a final product of glycolysis in the cytosol, is an important component of cellular metabolism, supplier of ATP and NADH in aerobic conditions. NADP-malic enzyme and pyruvate phosphate dikinase are present in $C_3$ and $C_4$ plants and are important under stress conditions. NADP- malic enzyme catalyzes the oxidative decarboxylation of L-malate to produce pyruvate, NADPH and $CO_2$ (Drincovich et al, 2001; Hýsková et al, 2014). In the mitochondria, each pyruvate molecule loses one carbon atom and becomes an acetyl molecule to enter the tricarboxylic acid cycle (TCA Cycle - Krebs cycle) (Plaxton and Podestá, 2006). It is a branching point for the syntheses of glucose, lactate, fatty acids, and amino acids (Rich and Maréchal, 2010; Li et al, 2014).

The import of pyruvate into mitochondria is a crucial step for both biosynthesis of organic compounds and for oxidative energy metabolism and MPC, a protein complex that negatively regulates ABA-induced guard cell signaling, are necessary for the uptake of pyruvate in the inner mitochondrial membrane (Timon-Gomez et al, 2013; Li et al, 2014; Wang et al, 2014).

Stomatal closure is mediated by MPCs, which favor plant survival, avoiding the loss of water by transpiration (Wang et al, 2014; Shen et al, 2017). In leaves, roots and flowers of *Arabidopsis*, Li et al. (2014) found robust expression of a transcript in guard cells, named *NRGA1*, that is involved in maintenance of stomatal aperture during drought stress, avoiding discontinuity of $CO_2$ during photosynthesis process. As to authors, the NRGA1/MPC is a negatively regulated protein, that contributes to alleviating the ABA effect in both inhibition of inward $K^+$ channels during stomatal opening, favoring the pathway of anionic channels during stomatal closing. Further genetics and cellular analysis carried out by authors using mutant and *Arabidopsis* transgenic plants, suggested a likely function for the MPC protein NRGA1 in plant stomatal movements and drought stress response.

Stomatal closure is one of the important responses in drought stress tolerance to reduce water loss. ABA and ROS are essential signals for these stomatal movements. Although overproduction of ROS is related to oxidative damage, these species also act benefiting the cell system providing protection against environmental stresses (Karuppanapandian et al, 2011). NADH is required for the synthesis of ROS and consequent guard cell $Ca^{2+}$ channel activation and stomatal closure (Timon-Gomez et al, 2013; Wang et al, 2014).

ROS production is controlled by various enzymatic and nonenzymatic antioxidative systems. Enzymatic antioxidants include CAT, POX, SOD, among others, whose compounds react with free radicals and neutralize them, overcoming the damage caused by stress (Karuppanapandian et al, 2011; Timon-Gomez et al, 2013). The accumulating of osmolytes as an adjuvant in the cellular defense processes is also widely reported. Proline is one of the most common compatible osmolytes in drought stressed plants and the accumulation in plant tissues is reliable marker for environmental stress, particularly in plants under drought stress (Hare et al, 1997; Hayat et al, 2012). However, as all defense process requires the involvement of mitochondria, it is believed that high production of endogenous pyruvate is necessary in order to minimize cell damages, which will imply in increased unfolding glucose molecules (glycolysis). Based on this hypothesis, it is possible that supplementation of exogenous pyruvate may be a substrate to supply energy in stressed plants.

Although these studies are limited, results found in literature have demonstrated pyruvate functions as a negative regulator of guard cell ABA signaling, and involvement in regulation of stomatal closure in *Commelina benghalensis* and *Arabidopsis* (Raghavendra et al, 1976; Li et al, 2014; Shen et al, 2017). In stressed detached leaves of *Arabidopsis*, submitted to pyruvate at 10 to 1000 μM, Shen et al, (2017) reported that the induction of stomatal closure, favored by increased pyruvate content, was dependent on the function of NADPH oxidases and ROS concentrations in guard cells. NADPH oxidases are membrane proteins that may produce ROS in plasma membrane ion channels (Keller et al., 1998). In a study carried out by Kwak et al (2003) with *Arabidopsis*, involving functions of NADPH oxidase in ROS-dependent ABA signaling, authors provided molecular and biological evidences that ROS are rate-limiting second messengers in ABA signaling, and that NADPH oxidases function in guard cell ABA signal transduction.Peanut (*Arachis hypogaea* L.) is a highly appreciated oilseed and has been widely grown in both tropical and semiarid climates, to attend grain and oil markets. In dry environments, plants are constantly exposed to water stress during the cycle, affecting the yield and quality of pods, due to changes in the content of phenolic compounds and other bioactive constituents of the grains (Juliano et al, 2021). Several upright cultivars (*A. hypogaea* subsp. fastigiata) are short cycle and suitable for rainfed management because have tolerate to drought environments (Pereira et al, 2016; Dutra et al, 2018); the runner types (*A. hypogaea* subsp. hypohaea), although are widely grown at worldwide because are high oil, plants are very vulnerable to water-restricted environments (Pereira et al, 2015; Santos et al, 2012). This limits the adoption these cultivars for management in semiarid climate.

Several works available in literature have reported on exogenous application of organic compounds in plants submitted to water stress, and the benefits on mitigating of deleterious effects of drought (Karimian et al., 2015; Sadiq et al., 2017; Aziz et al, 2018). Here, we focus our research on evaluating the effects of pyruvate in peanut genotypes, hypothesizing that exogenous application could mitigate the biological damages in drought sensitive plants. In order to strengthen this hypothesis, we chose a reference concentration, 100 uM, based on the findings of Shen et al, (2017) in *Arabidopsis*, and a broader (50,000 uM), to ensure greater absorption of pyruvate by the cell.

## 5.2. MATERIAL AND METHODS

### 5.2.1. Germplasm and growth analysis

The assay was carried out in greenhouse (Campina Grande, PB, Brazil, 07°13‘S; 53°31‘W), during 20 d, at dry season (Dec, 2019). Ten seeds of each BR 1 (subsp. *fastigiata*, upright and tolerant to drought) and IAC Caiapó (subsp. *hypogaea*, runner and sensitive to drought), were sown in pots (750 mL, height: 13 cm, diameter: 10 cm) containing organic substrate (340 g, Basaplant Base Agro, Brazil). Each pot contained two plants. Seedlings were daily watered, keeping the substrate humidity at the level corresponding to the field capacity (Whitehead, 1966; Dane and Topp, 2002). To monitor the humidity level, we added 5 additional pots (750 mL, 340 g) to the experiment, with mean retention of 240 mL of water, corresponding to the level of field capacity. The substrate was moistened before twilight, with enough water to drain the excess overnight. In next morning, the amount of water retained was estimated, based on the difference between the weight of the dry and wet substrate contained in the pots. On the third day of stress, and the first day of pyruvate application, we estimated the volume of water retained in the substrate that was 35% representing 65% of deficit to reach field capacity.

After 11 d of emergence (phase V1), treatments were then differentiated: Control (C- normal watering), Stress (S- total suppression of watering), P1 and P2 (application of synthetic pyruvate (Pyruvate acid, Merck, 8.20170.0500) at 100 µM and 50,000 µM, respectively, in leaves of stressed plants. The completely randomized experimental design was adopted, with 5 replications. The water suppression lasted 9 d, and pyruvate applications started at 3 d after the beginning of water stress. In this occasion, stomatal closure in stressed plants was estimated in 19.05% (*gs*: 0.42 mol $H_2O$ $m^2$ $s^{-1}$ in control, and 0.34 mol $H_2O$ $m^2s^1$ in stressed plants), obtained through Infrared gas analyzer (IRGA, LCPro SD, ADC Bioscientific, UK) equipment.

The water content in substrate was estimated in 86.4% and 28.8% for control and stress treatments, respectively, based on methodology described in Uhland et al, (1951). The control of soil moisture was maintained through a daily application of 20 ml of water. This value was based on the differences between the pot weight at field capacity and after water loss during the day. The stressed treatment was achieved based on the differences between the weight of control (583 g) and stressed substrates (378 g), after 11 d of water suspension. In this occasion, the water deficit corresponded to a reduction of 65% in soil moisture, in relation to the field capacity estimated at the beginning of assay.

Pyruvate applications were performed using a cotton swab dipped in solutions (pyruvate and distilled water, v/v), sliding on a set of tetrafoliolated during 6 d, on the same leaves. During the assay, the means of temperature and relative humidity of the air (minimum and maximum) were 21.5 °C to 43 °C, and 40% to 86%.

Total number of leaves, height of main stem, dry mass of canopy and dry mass of roots were recorded for growth analysis. The tissues were dried in oven with forced air circulation, at 65 ºC during 72 h.

### 5.2.2. Gas exchange and relative water content

The physiological data were collected at final of water stress, during 9:00 h to 11:00 h, using IRGA, without artificial Carbon source and with artificial light (1,200 µmol $m^{-2}$ $m^{-1}$). The soil moisture recorded in control and stress treatments were 86% and 28%, respectively. The following traits were registered: net photosynthesis rate ($A$), intercellular $CO_2$ concentration ($Ci$), stomatal conductance ($g_s$) and transpiration rate ($E$). From these data, we estimated the instantaneous efficiency of carboxylation (*IEC*), based on $A/Ci$ ratio, and the instantaneous efficiency of water use (*IEWU*), based on $A/E$ ratio.

The relative water content (RWC) was estimated from six leaf discs collected from mature leaves located at canopy top: RWC= [(FM - DM)/(TM- DM)]x100, where FM- fresh matter, TM- turgid matter, DM- dry matter.

### 5.2.3. Antioxidant activity and free proline

A crude extract (25%) of total protein from leaves was prepared in phosphate monobasic buffer (100 mM) and EDTA (0.1 mM), pH 7.0 (Pereira et al, 2015), and quantified through Bradford (1976) method, at 595 nm. The activity of antioxidative enzymes was estimated according methodologies described in Bulbovas et al. (2005) for SOD, Azevedo et al. (1998) for CAT, and Nakano and Asada, (1981) for APX, at 560 nm, 240 nm and 290 nm, respectively. Free proline was estimated according to methodology described in Bates et al. (1973), at 520 nm.

### 5.2.4. Statistical analysis

Data were submitted to analysis of variance (ANOVA, F test) and mean classifications (Scott and Knott, 1974). The SISVAR software, version 5.6 (Ferreira, 2010) was adopted to analyses.

## 5.3. RESULTS AND DISCUSSION

### 5.3.1. Growth and gas exchange of peanut plants submitted to water stress with application of exogenous pyruvate

Based on the phenotypical aspect, peanut cultivars were affected differently by water stress, with visible reduction in canopy and plant height, especially in BR 1 that is an upright material and the difference becomes more visible (Figure 1). However, based on the means of growth traits, no expressive contribution was found by exogenous application of pyruvate in both cultivars. The number of leaves (Figure 2A), dry mass of canopy (Figure 2C), and dry mass of roots (Figure 2D) decreased only due to the water stress effect. The exception was found to main stem height of IAC Caiapó, that grew up approximately 37% above the mean of stressed treatment (S) (Figure 2B), whose plants was reduced by 44%, compared to height of control plants.

**Figure 1.** Aspect of peanut plants submitted to 9 d-water stress and treated with exogenous pyruvate. A- BR 1, B- IAC Caiapó. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Pyruvate application lasted 6 days on leaves of stressed plants.

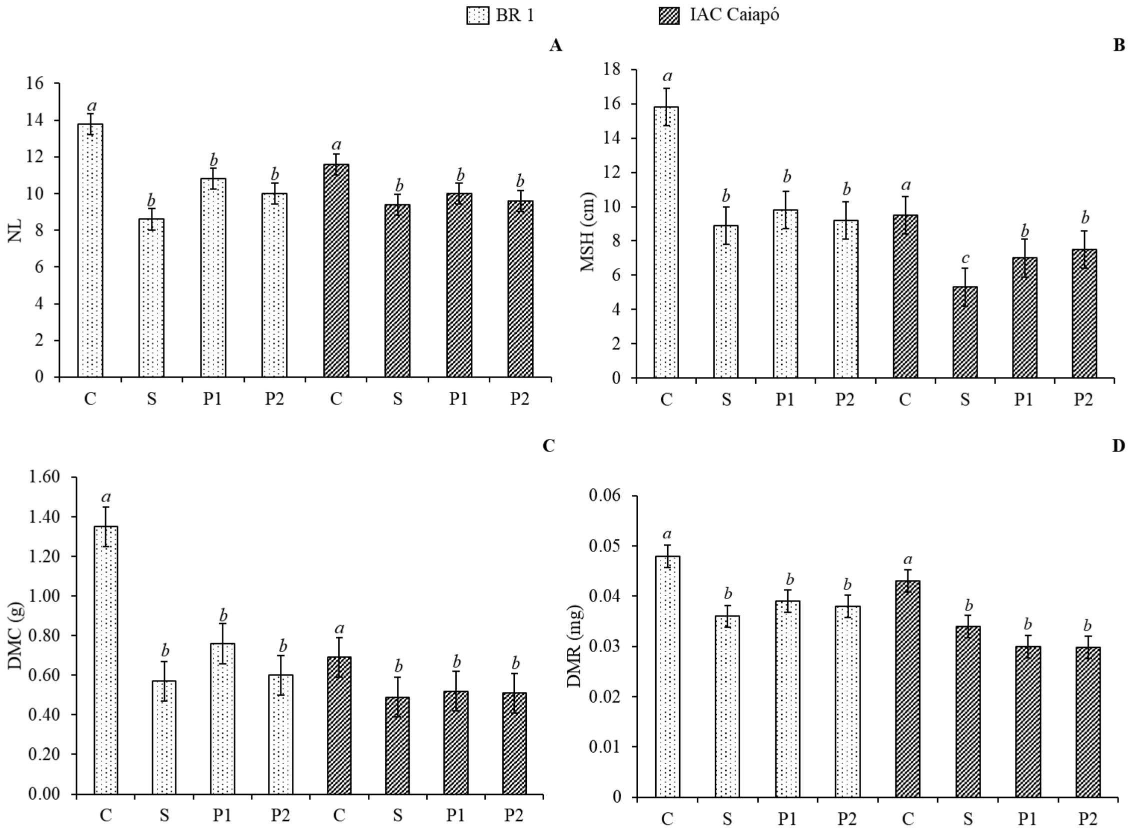

**Figure 2.** Growth traits of two peanut cultivars submitted to water stress and treated with exogenous pyruvate. NL- number of leaves (A), MSH- Main stem height (B), DMC- Dry mass of canopy (C), DMR- dry mass of roots (D). C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with same letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

At physiological aspect, the application of exogenous pyruvate was genotype-dependent, so that in BR 1, no relief of water stress was found in plants (Figure 3). Instead, in an overview, the application of exogenous pyruvate appeared to be harmful to stressed plants. In IAC Caiapó, however, exogenous pyruvate mitigated the physiological disorders, especially at highest concentration (P2, 50,000 µM). In these plants, we found an increase of 56% in *gs* (Figure 3A), and 46% in *E* (Figure 3B), compared to mean of stressed treatment, indicating attenuating effect of pyruvate in stressed plants. Compared to control (C), *gs* and *E* were reduced to 59% and 44%, respectively, in plants 9 d- water stressed, confirming the sensibility of IAC Caiapó to dry environment (Santos et al, 2012).

Because of attenuation of water stress on *gs* and *E* in stressed IAC Caiapó plants, the liquid photosynthesis *A* (Figure 3C) was also benefited, with an increase of 93%, in relation to stressed treatment. Based on means of control plants, we found large reductions (66%) in stressed plants, and slowed in P2 (44%).

The intercellular $CO_2$ concentration (*Ci*) is another relevant physiological parameter, since it is directly related to photosynthesis. Here we find the same behavior of *Ci* in P2 treatment for both cultivars, but the physiological interpretations are different, considering the values of *gs*, *E* and *A* previously reported. In IAC Caiapó, the reduction in *Ci* is a consequence of the input on carbon flow in order to favor the photosynthetic machinery, benefited by increase of *gs* (Figure 3A) and *E* (Figure 3B). Thus, the cost of *Ci* in cells of P2 plants was spent due to elevation of *A* (Figure 3C), demonstrating efficiency for $CO_2$ fixation, even under conditions of water deficit. In BR 1, the reduction in *Ci* appeared to be due to an unnecessary expense of energy that plants invested when interacting with a metabolite useless for the cell defense apparatus. We found that at 100 µM of pyruvate, the values of gas exchanges did not differ from the stressed treatment, indicating absence of use of product at cell level; at the highest concentration (50,000 µM), we verify that exogenous application of pyruvate was harmful to the cells, based on reduction of gas exchange traits in stressed plants (Figure 3). Although gas exchange data using BR 1 is limited in literature, the reduction of *Ci* found here in stressed plants (22%) is in agreement with that found by Dutra et al, (2018) (28%), with the same cultivar submitted to 15 d- water stress at the beginning of the reproductive phase.

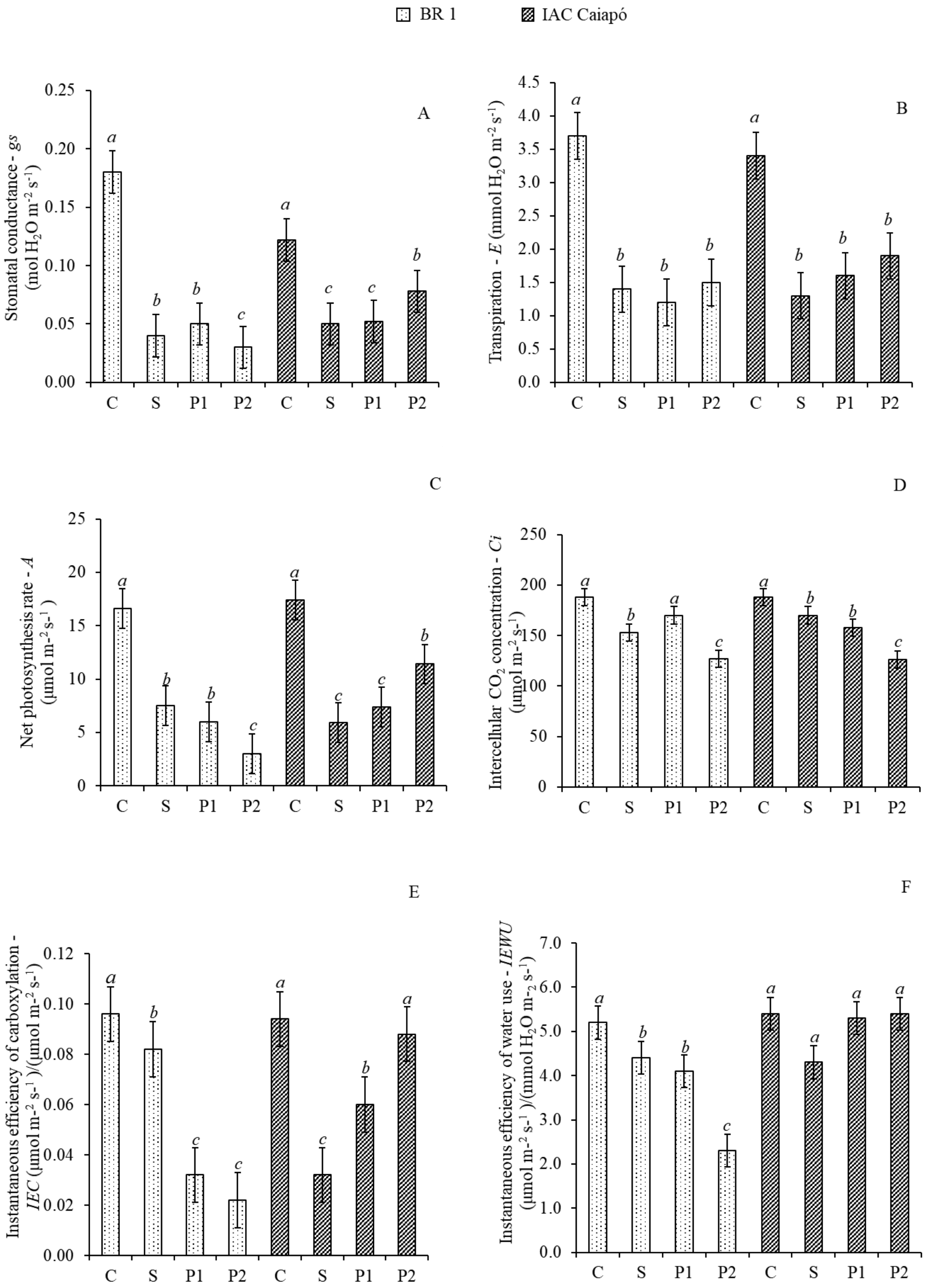

**Figure 3.** Gas exchanges in peanut cultivars submitted to drought and treated with exogenous pyruvate. A- Stomatal conductance (*gs*), B- Transpiration (*E*), C- Net photosynthesis rate (*A*), D- intercellular $CO_2$ concentration (*Ci*), E- instantaneous efficiency of carboxylation (*IEC*), F- instantaneous efficiency of water use (*IEWU*). C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

The two most expressive physiological effects resulting from exogenous application of pyruvate in IAC Caiapó plants were the full relief of plants to *IEC* and *IEWU* in P2 treatment (Figure 3E, F), whose values were similar to mean of control plants. In stressed treatment, the reductions of both traits were 66% and 20%, respectively. These results open perspectives for further adoption or improvement of IAC Caiapó to environments prone to drought, taking in account that water use efficiency is a parameter of crop quality and performance of plants under water deficit (Osakabe et al, 2014). IAC Caiapo is a commercial cultivar released by IAC for tropical environment, and a valuable germplasm for oil production, as well multiple resistance to several leaf fungi (Godoy et al, 1999; Santos et 2012).

Based on a general analysis of gas exchanges of peanut cultivars showed in Figure 3, we concluded that application of exogenous pyruvate at 50,000 µM enabled favorable results to sensible IAC Caiapó, minimizing the negative effects of water stress on physiology of the plants.

### 5.3.2. Relative water content in peanut stressed and treated with pyruvate

The RWC is the most adequate measure of plant water status, since it estimates the consequence of water deficit in cells, based on effects of leaf water potential and osmotic adjustment in stressed plants. Here, we found that the application of exogenous pyruvate did not alter the water status in stressed plants of tolerant BR 1, whose results are consistent with those found in *IEWU* (Figure 3F) and reinforces the natural autonomy of this cultivar to use its physiological resources to face situations of water stress (Santos et al, 1999; Pereira et al, 2016; Dutra et al, 2018). In cultivar IAC Caiapó, however, the cells recovered the water status with application of pyruvate in both treatments, whereas in stressed treatment the water content increased by 26% and 76%, respectively. This result possibly indicates that application of pyruvate has resulted in saving of water in peanut leaves, in order to facilitate the flow of other physiological processes.

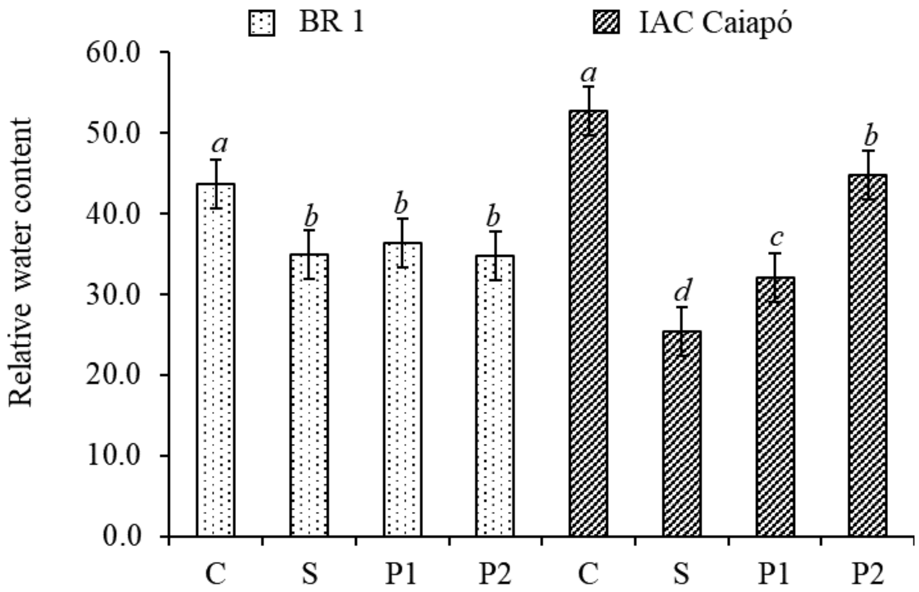

**Figure 4.** Relative water content (*RWC*) in peanut cultivars submitted to water stress and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

According to Levitt (1972), plants react to drought conditions through two manners, preventing and tolerating dehydration. Plants that prevent dehydration feature functions to perform the water adjustment of cells during drought, and tolerant genotypes have tissues that support longest period of dehydration, corresponding to a low RWC. As seen in Figure 4, the reduction of RWC in stressed BR 1 was only 20%, while in IAC Caiapó was 52%, attesting the tolerance of BR 1 to the condition of water deficiency (Gomes et al, 2007; Dutra et al, 2018).

In experimental conditions adopted in this work, we found that pyruvate at 50,000 µM demonstrated action on stomatal efficiency, with reflexes on photosynthesis and water saving of IAC Caiapó. The exogenous application had a stress-relieving effect, as the stress occurred next to flowering, when plants are more vulnerable to drought (Santos et al, 2012). The brief recovery seen in *gs*, whose opening controls the balance of water loss, suggests that exogenous pyruvate contributed to better use of water available in tissues, while facing the water stress. The opposite was found in absence of pyruvate, where stressed plants were more vulnerable.

In *Arabidopsis*, Shen et al. (2017) identified novel physiological roles of pyruvate and MPC1, in the regulation of stomatal closure, in plants submitted to drought (2-3 weeks) and treated with pyruvate at 10, 100, and 1000 µM. According to authors, plants exposed to drought stress responded by elevation of ABA, which induced elevation of the cellular pyruvate content and ROS accumulation to activate slow-type anion channels, finally inducing stomatal closure. With pyruvate treatment, the stomatal aperture decreased in size under higher concentrations of pyruvate until reaching 100 μM.

It is worth pointing out here that the application of pyruvate was made only on a tetrafoliolated leaf of each plant, taking into account the direction of the translocation (source-drain) (Tanaka and Fugita, 1979). Then, the pyruvate was absorbed by the leaf cuticle and translocated to the other organs of the plant. Based on results found with IAC Caiapó, it is possible that this cultivar could benefit from the relief of cell damage caused by water stress using minor concentrations of exogenous pyruvate, although other physiological traits might not have the same tendency. This proposition could be further deepened.

### 5.3.3. Antioxidative activity and proline in plants submitted to water stress and treated with exogenous pyruvate

Mitochondria are the major intracellular source of ROS and pyruvate has a relevant role in oxidative metabolism (Li et al, 2014; Shen et al, 2017). Pyruvate regulates stomatal closure indirectly by inducing ROS production, which are cytotoxic and highly detrimental to the cellular lipids, nucleic acids and proteins (Timon-Gomez, 2013; Shen et al, 2017). Genotypes able to maintain a low steady state of ROS are better adapted to tolerate stress conditions. Plants generally scavenge and dispose of these reactive substances by the use of antioxidant defense enzymes, such as SOD, CAT, POX, APX, GR, and other associated enzymes involved in the cellular detoxification mechanism. Here, we investigate the activity of SOD, CAT and APX in BR 1 and IAC Caiapó submitted to drought and treated with pyruvate exogenous.

As seen in Figure 5A, the profile of the antioxidant enzymes in BR 1 matches fully what is expected of a drought-tolerant germplasm. An input from SOD (45%), which dismutes the superoxide radicals, was displayed right at beginning of the antioxidative process, followed by degradation of the peroxides promoted by high activity of CAT (129%) and finished by APX action (increasing of 60%) in $H_2O_2$ degradation. This machinery operates integrally, aiming to minimize the effect of ROS generated from water stress in cells. These inputs found in leaves of stressed plants of BR 1were reported by Pereira et al (2012; 2015), that evaluated upright and runner genotypes submitted to drought during 7 and 10 days, respectively. The differential in this study was the mitigating action of pyruvate at 50,000 μM that totally restored the stressed plants to the normal condition, as seen in control treatment. It is possible that this action contributed to regulation of stomatal movements and reprogramming of key genes in the cell signaling network, responsible for regulating the activity of anionic channels of the guard cells.

As to IAC Caiapó (Figure 5B), the treatment of exogenous pyruvate did not contribute to mitigate the oxidative stress in plants, whose activity of the enzymes was milder, indicating slow response, often seen in sensitive genotypes (Nogueira et al, 1998).

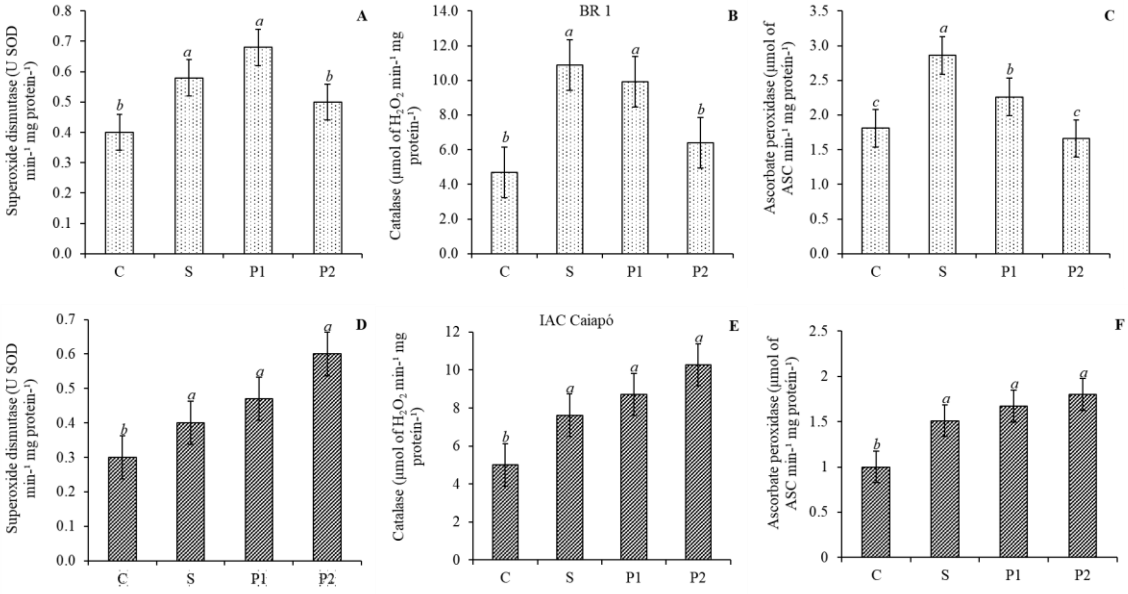

**Figure 5.** Antioxidative activity of SOD (A), CAT (B) and APX (C) in BR 1 and IAC Caiapó cultivars submitted to water stress and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 μM, respectively. Means with equal letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

Proline is widely cited in literature due to its influence on osmotic adjustment of plants under abiotic stress (Sivakumar et al, 2000; Alves et al, 2016). The Figure 6 displays the profile of free proline in both sensible and tolerant peanut cultivars. A significant increase in free proline was found in stressed cultivars, on average of 317%, confirming its relevant role on regulating of the osmotic potential in cells.

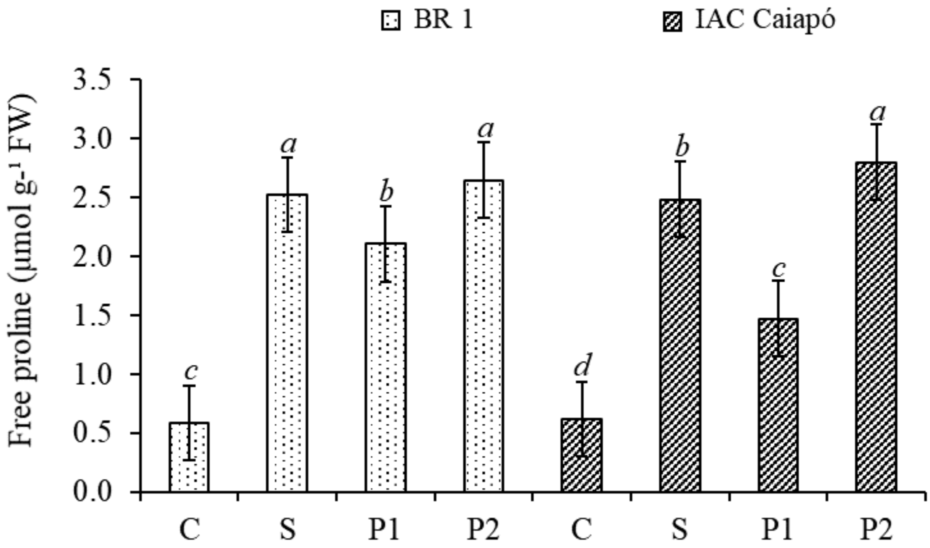

**Figure 6.** Free proline in peanut cultivars submitted to water deficit and treated with exogenous pyruvate. C- Control, S- Stressed, P1 and P2- Stressed + pyruvate at 100 and 50,000 µM, respectively. Means with equal letters do not differ statistically (Scott Knott test, $p \leq 0.05$).

In treatments with pyruvate, however, two effects were visibly found in both cultivars: a) mild and moderate recovery of osmotic adjustment in plants at 100 µM, in the order of 16% and 40% for BR 1 and IAC Caiapó, respectively. This attenuation may be indicative of an input provided by exogenous pyruvate, generating additional energy for the Krebs Cycle machinery and electron transport chain. Additionally, the action of proline could benefits in regulation of cytosolic acidity (Sivakumar et al, 2000), and b) negative interaction between the osmolite and the endogenous pyruvate, at 50,000 µM, indicating a possible disturbance on osmotic adjustment due to excess of pyruvate in cell environment.

In drought-tolerant plants, the use of proline in cellular defense machinery is more agile, allowing a more balanced response in coping with environmental stresses (Bhatnagar-Mathur et al, 2009; Alves et al, 2016). Proline protects plants against stress by stabilizing the mitochondrial electron transport complex II and scavenging the ROS and other free radicals (Hayat et al, 2012). In the literature, several studies have demonstrated the relationship between proline and the drought tolerance response in BR 1. The proline input in leaves of plants stressed for up to 15 d ranges from 50% to 450% before blooming, indicating wide plasticity depending on the duration of stress (Pereira et al, 2015; Alves et al, 2016). Based on this behavior, breeders have considered this germplasm as a robust parent in breeding works focused on the semi-arid environment (Santos et al, 1999; Gomes et al, 2007; Dutra et al, 2018). In Brazil, a team of peanut breeders reported top lines derived from crossing between BR 1 and allotetraploids (*A. batizocoi* K9484 x *A.duranensis* SeSn 2848)$_{4x}$ showing high tolerance to drought, endorsed by the high content of proline, after submission of water stress (Dutra et al, 2018).

This work revealed interesting information regarding to relief of the deleterious effects of water stress on sensitive plants of peanut, and better adjustment in the antioxidant and osmotic system of the tolerant cultivar, based on exogenous application of pyruvate. The range of concentrations adopted here were very wide because we based on the few works available in literature with exogenous pyruvate. Taking in account that a forecast of physiological and biochemical effects may occur in different species, we encourage further studies using intermediate concentrations assuming that may be possible to achieve noticeable responses in concentrations below 1000 μM.

## 5.4. ACKNOWLEDGMENTS

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## 5.5. CONFLICT OF INTERESTS

The authors hereby declare no conflict of interest.

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# CAPÍTULO II

## EXOGENOUS SOD MITIGATES CELL DAMAGE IN DROUGHT-SENSITIVE PEANUT

Artigo submetido para publicação na revista Journal of Experimental Botany

## 6. Exogenous SOD mitigates cell damage in drought-sensitive peanut

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**Abstract:** The superoxide dismutase SOD enzyme initiates the process of neutralization of cytotoxic effects caused by ROS. In drought-tolerant plants, SOD input is rapid due to their ability to adjust when they notice water unavailability, unlike in drought-sensitive plants. In order to investigate whether the exogenous application of SOD benefits drought-sensitive plants by ameliorating water stress, the present research used contrasting genotypes submitted to 11 days of water suppression treated with SOD concentrations (2.5, 5.0, and 7.5 U) on the surface of leaflets. The plants were assessed for growth, gas exchange, and activity of antioxidative enzymes. Overall, the contribution of exogenous SOD in mitigating the effects of water stress was more significative in stressed plants of drought-sensitive cultivars. Gas exchange recovery was observed in the plants as early as the initial SOD concentration (2.5 U), whereas higher exogenous SOD suply was required to prevent oxidative damage. The results are promising and drive more in-depth studies of the enzyme regarding the benefits on the phenology of runner genotypes, which are very productive but widely sensitive to environments with water restriction.

***Keywords:*** Antioxidative enzymes, metabolism, detoxifying enzymatic systems, tolerant plants, water deficit

***Abbreviations:*** ANOVA- analysis of variance, APX- ascorbate peroxidase, CAT- catalase, $H_2O_2$- hydrogen peroxide, $H_2O$- water, IRGA- Infrared Gas Analyzer, $O_2$- oxygen, ROS- reactive oxygen species, RWC- relative water content, SOD- superoxide dismutase, U- enzyme unit.

### 6.1. Introduction

Water deficit is one or the main limiting factors in agricultural production and may drastically compromise crops depending on its duration and intensity. Climate change has been occurring at a fast pace and does not allow cultures to adapt at the same rate. Genetic improvement programs have been proactive in identifying and selecting tolerant germoplasm, whose genotypes are often used in hybridization works to enhance the genetic basis of commercial cultivars (Dutra *et al.*, 2018).

Plant weakness caused by water shortage is observed phenotypically due to alterations in metabolism, impacting growth and $CO_2$ absorption, as consequences of changes in cellular water potential and in photosynthesis. Consequently, several ROS are generated, represented by free radicals that impact cellular defense. Under normal conditions, ROS may act as beneficial molecules that balance the cellular medium. However, under cellular stress conditions, they act as markers of oxidative stress, besides compromising lipids, proteins, and nucleic acids and causing cell death (Sharma et al., 2019).

Naturally, plants have evolved defense mechanisms over time to mitigate environmental adversities so as to maintain homeostasis. Stomatal closure is one of the signs of reaction, as is cellular adjustment based on the storage of osmoregulatory substances that prevent water imbalance in the cell and water loss to the soil or atmosphere (Farooq *et al.*, 2019). Plants react by increasing the production and storage of several osmolytes and by activating detoxifying enzymatic systems able to balance the cytotoxicity of ROS (Ashaf and Foolad, 2007; Gill and Tuteja, 2010; Choudhury *et al.*, 2017).

Antioxidative enzymes play a key role in the defense against ROS. SOD is a key enzyme in such process as it is the most effective antioxidant barrier present in the subcellular compartments (Gill and Tuteja, 2010). First reported in 1969 in bovine erythrocytes, SOD is considered a landmark in studies on the action of free radicals from oxygen metabolism, being found in most procaryote and eucaryote organisms, as well as the production in superoxide biological systems (Del Rio *et al.*, 2018).

In cells, SODs remove $O_2$•-, catalyzing dismutation, where one $O_2$•- is reduced to $H_2O_2$ and another is oxidated to $O_2$. With the removal of $O_2$•-, the risk of OH• formation inside the cell decreases. In tissues under environmental stress, the increase in SOD activity is directly related to tolerance in plants, which speed up the defense process against the imminent oxidative damage as a response to excess ROS generated in the cells (Pereira *et al.*, 2016; Dutra *et al.*, 2018). Under water stress conditions, tolerant plants speed up stomatal closure to reduce water loss (Hao *et al.*, 2013; Dutra *et al.*, 2018; Vison *et al.*, 2018). Consequently, ROS production in chloroplasts increases and is fought by two isoforms of SOD, namely, *Cu/Zn-SOD* and *Fe-SOD* (Huseynova *et al.*, 2014; Pilon *et al.*, 2011).

Abid *et al*. (2017) submitted 11 contrasting genotypes of beans (*Vicia faba* L.) to 30 days of water stress and observed increases in SOD between 99% and 289% among the tolerant genotypes and from 27% to 30% among the sensitive ones. Shafiq *et al*. (2019) found increases in SOD in corn between 31% and 37% among tolerant plants and from 4% to 21% among sensitive ones after 21 days of water suppression. The literature features several other papers in which drought tolerance can be related with increases in SOD in rice (Wang *et al*., 2019), soybean (Iqbal *et al*., 2019), and cotton (Prajapat *et al*., 2018), among others.

Peanut (*Arachis hypogaea* L.) is an oilseed plant of high dietary value that is grown in several regions of temperate and tropical climate worldwide. In semiarid environments, the occurrence of intermittent drought (also called *Indian summer* or *veranico*) during the rainy season disturbs the phenology of the crop, despite the tolerance against drought found in some cultivars (Pereira *et al*., 2016; Dutra *et al*., 2018).

Botanically, peanut is differentiated into two subspecies, namely, fastigiata and hypogaea, representing the upright and runner types, respectively. Cultivars of the fastigiata type have a shorter cycle and greater genetic variation for drought tolerance, whereas runners have longer cycles and are often more sensitive to water-restricted environments (Santos *et al*., 2012; Pereira *et al*., 2016; Dutra *et al*., 2018). Several works in the literature show the ability of peanut to perform osmotic adjustment to prevent oxidative damage when under drought conditions. Runner cultivars usually respond more slowly, which impacts gas exchanges and, consequently, the balance of antioxidative enzymes in the cellular defense process, especially SOD, which is responsible for initiating the detoxification steps (Pereira *et al*., 2015; Shinde *et al*., 2018; Vison *et al*., 2018).

In face of such tendency, it is unknown whether exogenous SOD application would contribute to ameliorating the oxidative effects of water stress in the plants, particularly runner ones. Thus, the present study aimed to assess the behavior of two contrasting cultivars of peanut submitted to water stress and treated with exogenous SOD at different concentrations. The plants were assessed based on descriptors of growth, gas exchange, and antioxidative enzymes.

## 6.2. Material and Methods

### 6.2.1. Conducting the Experiment

The experiment was conducted in a greenhouse in the city of Campina Grande, PB, Brazil (07°13’ S; 53°31’ W) during the dry season. Seeds of both contrasting peanut cultivars

– BR 1 (subsp. fastigiata, upright and tolerant to drought) and IAC Caiapó (subsp. hypogaea, runner and sensitive to drought) were sowed in vases (15.6 tall x 10 cm diameter, capacity of 750 mL) containing plant substrate (Basaplant Base Agro, Brazil) and watered daily so as to maintain the substrate moisture at the level corresponding to that of field capacity. The assays were carried out separately as a function of the difference in the cycle of the two cultivars. When the genotypes were in phase V1 (Boote, 1982), corresponding to 11 days for BR 1 and 17 days for IAC Caiapó, the treatments were differentiated into five situations: Control (NS- No water stress), Stress due to water deficiency (WS- water stress during nine days), and Stress due to water deficiency + foliar application of SOD at 2.5 U (2.5WS), 5.0 U (5.0WS), and 7.5 U (7.5WS). A completely randomized experimental design with five repetitions was employed.

SOD applications started on the third day of water stress, when the stressed plants had stomatal closure estimated at 19.05% ($g_s$ of the stressed plants: 0.34 mol $H_2O$ $m^{-2}s^{-1}$; $g_s$ of the control plants: 0.42 mol $H_2O$ $m^{-2}s^{-1}$). In that occasion, the volume of water held in the substrate corresponded to 35%, representing 65% of the deficit to reach the field capacity. The product was applied for six days on the same leaflet using a flexible rod in the direction from the peduncle to the apex.

The plants were watered daily, maintaining the substrate moisture at the level corresponding to the field capacity, equivalent to 240 mL of water retention. At the end of the assay, the water content in the substrate was estimated at 86.4% and 28.8% for the control and stress treatments, respectively, based on the methodology described by Uhland *et al*. (1951). Soil moisture was controlled via daily application of 20 mL of water. That value was based on the differences between the weight of the vase at field capacity and after water loss during the day. Maximum and minimum temperature and relative air humidity measured during the assay were 21.5 °C and 43 °C, and 40% and 86%, respectively.

### 6.2.2. Growth Analysis

Plant growth was measured at the end of the assay based on the total number of leaves (NL), main stem height (MSH), dry mass of canopy (DMC), and dry mass of roots (DMR). The last two were obtained after drying in a forced air oven at 65 °C for 72 h.

### 6.2.3. Gas Exchanges and Relative Water Content

Gas exchanges were analyzed between 9:00 and 11:00 a.m. using an IRGA (model LCpro-SD) with no artificial carbon source and using an artificial light source of 1,200 µmol

$m^{-2}m^{-1}$. The traits analyzed were: Rate of liquid photosynthesis (*A*), intercellular $CO_2$ concentration (*Ci*), stomatal conductance ($g_s$), and transpiration (*E*). Such data were used to estimate the instantaneous carboxylation efficiency (*IEC=A/Ci*) and the instantaneous water-use efficiency (*IEWU=A/E*) (Dutra *et al*., 2018).

Relative water content (*RWC*) was estimated from the mass of six foliar discs collected from mature leaves located in the upper part of the plant according to the methodology by Weatherley (1950): RWC=[(FM-DM)/(TM-DM)]x100, where FM- fresh matter (moment of collection), TM- turgid matter (discs immersed in water for 12 h), and DM- dry matter (discs dried in an oven at 60 °C until constant weight).

### 6.2.4. Antioxidant Enzyme

At the end of the assay, 200 mg of leaves were collected and stored at -80 °C for extraction of total proteins (Pereira *et al*., 2012). Proteins were quantified using the method by Bradford (1976) in a spectrophotometer at 595 nm. Antioxidant activity was estimated in a spectrophotometer using SOD (Bulbovas *et al*., 2005), CAT (Azevedo *et al.*, 1998), and APX (Nakano and Asada, 1981) enzymes at wavelengths of 560 nm, 240 nm, and 290 nm.

### 6.2.5. Statistical Analysis

The data were first submitted to Shapiro-Wilk normality test (Shapiro and Wilk, 1965) with transformation into Log X at base 10 of those failing to meet the criteria for normalization of the distribution of deviations (Pimentel-Gomes, 2000). With the assumptions of normality met, analysis of variance using F-test ($p<0.05$) was performed based on orthogonal contrasts of estimates of means of the treatments and comparisons by Student's *t*-test at 5% confidence (Barbosa and Maldonado Júnior, 2015). The following contrasts were compared:

$\hat{Y}_1$=4*0NS-0WS-2.5WS-5.0WS-7.5WS
$\hat{Y}_2$=3*0WS-2.5WS-5.0WS-7.5WS
$\hat{Y}_3$=0WS-2.5WS
$\hat{Y}_4$=0WS-5.0WS
$\hat{Y}_5$=0WS-7.5WS

In the $\hat{Y}_1$ contrast, the mean of the control treatment is compared with the group of means of the stress treatments and SOD concentrations; for $\hat{Y}_2$, the mean of the stress treatment is compared with the SOD concentrations; $\hat{Y}_3$, $\hat{Y}_4$, and $\hat{Y}_5$, compare the mean of the stress treatment with the mean of the stress treatment with the plots treated with 2.5, 5.0, and 7.5 U

SOD $\mu L^{-1}$, respectively. The analyses were carried out using the computational system for statistical analyses Sisvar 5.6 (Ferreira, 2019).

## 6.3. Results and Discussion

In this study, exogenous SOD use was tested aiming to investigate its mitigating effect in peanut plants submitted to water stress. To support such hypothesis, five orthogonal contrasts were established so as to lay basis for the experimental design proposed, comprising two controls, namely: overall control of the entire experiment (plants that did not face water stress) and one control comparing the mitigating effect of SOD at three concentrations (plants that faced water stress). Table 1 presents a synthesis of the ANOVA with the unfolding of the orthogonal contrasts. We found that, for all 14 traits analyzed, contrast $\hat{Y}1$ was the most adequate as it enabled discriminating most responses relevant to the questions of the assay, with a high percentage of significance ($p \leq 0.01$). Hence, the discussion of the results will be based on that contrast.

### 6.3.1. Growth of the Plants Treated with Exogenous SOD

The two peanut cultivars were affected differently during the short water stress period. However, for early-maturing BR 1, exogenous SOD application did not have a differential effect on the traits analyzed, although a difference among treatments was found for MSH, DMC, and DMR (Table 1, Fig. 1). For IAC Caiapó, statistically significant differences were observed for MSH and DMR (Table 1). However, the benefit of SOD was seen only for MSH (Fig. 2), whose mean height at the three SOD concentrations was 67% higher than the stressed treatment, which suggests a relieving effect for the stressed plants at the beginning of growth.

**Table 1**. Summary of analysis of variance for growth traits in contrasting peanut cultivars submitted to water suppression and treated with exogenous SOD.

| **SV** | **DF** | **Mean square BR 1** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **NL** | **MSH** | **DMC** | **DMR** | $g_s$ | ***E*** | ***A*** | ***Ci*** | ***IEC*** | ***IEWU*** | ***RCW*** | **SOD** | **CAT** | **APX** |
| *T* | 4 | $0.02^{ns}$ | $46.1^{**}$ | $0.50^{**}$ | $10x10^{-5**}$ | $0.01^{**}$ | $6.0^{**}$ | $128.6^{**}$ | $2,714.6^{**}$ | $5x10^{-3**}$ | $0.6^{ns}$ | $73.5^{**}$ | $0.1^{**}$ | $19.3^{**}$ | $0.2^{**}$ |
| Error | 20 | $4x10^{-3}$ | 2.0 | 0.01 | $5x10^{-6}$ | $1.1x10^{-4}$ | 0.2 | 1.6 | 274.6 | $8x10^{-5}$ | 0.4 | 4.3 | $3x10^{-3}$ | 0.4 | $4x10^{-3}$ |
| $\hat{Y}_1$ | 1 | $0.07^{**}$ | $175.5^{**}$ | $1.7^{**}$ | $3x10^{-4**}$ | $0.07^{**}$ | $22.9^{**}$ | $462.2^{**}$ | $5,461.2^{**}$ | $0.01^{**}$ | $0.8^{ns}$ | $128.9^{**}$ | $0.3^{**}$ | $16.2^{**}$ | $0.5^{**}$ |
| $\hat{Y}_2$ | 1 | $0.03^{ns}$ | $0.4^{ns}$ | $0.08^{ns}$ | $5x10^{-5ns}$ | $4x10^{-5ns}$ | $0.2^{ns}$ | $10.4^{ns}$ | $20.4^{ns}$ | $9x10^{-3**}$ | $0.4^{ns}$ | $62.8^{**}$ | $0.02^{ns}$ | $56.7^{**}$ | $0.3^{**}$ |
| $\hat{Y}_3$ | 1 | $0.02^{ns}$ | $0.5^{ns}$ | $4x10^{-3ns}$ | $2x10^{-5ns}$ | $1x10^{-3ns}$ | $0.5^{ns}$ | $17.2^{*}$ | $960.4^{ns}$ | $7x10^{-3**}$ | $8x10^{-4ns}$ | $151.4^{**}$ | $5x10^{-2ns}$ | $24.8^{**}$ | $0.2^{**}$ |
| $\hat{Y}_4$ | 1 | $7x10^{-3ns}$ | $0.0^{ns}$ | $0.03^{ns}$ | $4x10^{-5ns}$ | $1x10^{0ns}$ | $0.4^{ns}$ | $23.4^{**}$ | $1,768.9^{ns}$ | $6x10^{-3**}$ | $0.17^{ns}$ | $14.5^{ns}$ | $3x10^{-3ns}$ | $44.3^{**}$ | $0.2^{**}$ |
| $\hat{Y}_5$ | 1 | $0.03^{ns}$ | $4.4^{ns}$ | $0.2^{*}$ | $6x10^{-5*}$ | $3x10^{-4ns}$ | $0.04^{ns}$ | $1.1^{ns}$ | $0x10^{0ns}$ | $5x10^{-3**}$ | $1.2^{ns}$ | $10.9^{ns}$ | $0.1^{**}$ | $46.5^{**}$ | $0.2^{**}$ |
| Mean | | 1.0 | 10.5 | 0.8 | 0.0 | 158.6 | 1.8 | 8.0 | 0.07 | 0.05 | 4.2 | 39.1 | 0.7 | 6.6 | 0.4 |
| CV (%) | | 6.1 | 13.5 | 15.0 | 5.6 | 14.8 | 22.5 | 15.7 | 10.4 | 16.7 | 15.9 | 5.3 | 7.3 | 9.3 | 13.8 |
| | | **Mean square IAC Caiapó** | | | | | | | | | | | | | |
| *T* | 4 | $9x10^{-3ns}$ | $12.5^{**}$ | $0.03^{ns}$ | $1x10^{-4*}$ | $6x10^{-3**}$ | $4.4^{**}$ | $129.0^{**}$ | $367.4^{ns}$ | $4x10^{-3**}$ | $3.2^{**}$ | $601.9^{**}$ | $0.2^{**}$ | $11.3^{**}$ | $3.1^{**}$ |
| Error | 20 | $2x10^{-3}$ | 0.3 | $6x10^{-3}$ | $2x10^{-5}$ | $1x10^{-4}$ | 0.2 | 1.1 | 349.7 | $7x10^{-5}$ | 0.2 | 4.2 | $3x10^{-3}$ | 0.7 | 0.01 |
| $\hat{Y}_1$ | 1 | $3x10^{-2**}$ | $14.6^{**}$ | $0.03^{ns}$ | $3x10^{-4**}$ | $0.02^{**}$ | $16.6^{**}$ | $500.3^{**}$ | $812.2^{ns}$ | $1x10^{-2**}$ | $9.8^{**}$ | $2214^{**}$ | $0.4^{**}$ | $0.2^{ns}$ | $10.3^{**}$ |
| $\hat{Y}_2$ | 1 | $0x10^{0ns}$ | $34.3^{**}$ | $0.07^{ns}$ | $1x10^{-4ns}$ | $4x10^{-5ns}$ | $7x10^{-4ns}$ | $0.6^{ns}$ | $120.4^{ns}$ | $10x10^{-4*}$ | $2.2^{ns}$ | $97.3^{**}$ | $0.1^{**}$ | $0.1^{ns}$ | $0.2^{*}$ |
| $\hat{Y}_3$ | 1 | $2x10^{-4ns}$ | $27.9^{**}$ | $0.08^{**}$ | $2x10^{-4*}$ | $2x10^{-4ns}$ | $0.28^{ns}$ | $6.8^{ns}$ | $396.9^{ns}$ | $5x10^{-3**}$ | $1.3^{ns}$ | $173.7^{**}$ | $0.06^{**}$ | $1.9^{ns}$ | $0.8^{**}$ |
| $\hat{Y}_4$ | 1 | $2x10^{-4ns}$ | $17.9^{**}$ | $0.03^{ns}$ | $9x10^{-5ns}$ | $2x10^{-4ns}$ | $0.05^{ns}$ | $0.4^{ns}$ | $102.4^{ns}$ | $9x10^{-5ns}$ | $2.7^{*}$ | $57.5^{**}$ | 0.3** | $0.6^{ns}$ | $0.4^{**}$ |
| $\hat{Y}_5$ | 1 | $9x10^{-4ns}$ | $23.4^{**}$ | $0.03^{ns}$ | $6x10^{-6ns}$ | $3x10^{-4ns}$ | $0.13^{ns}$ | $1.7^{ns}$ | $10.0^{ns}$ | $4x10^{-5ns}$ | $0.7^{ns}$ | $11.5^{ns}$ | $7x10^{-3ns}$ | $0.07^{ns}$ | 0.2* |
| Mean | | 0.9 | 7.9 | 0.61 | 0.03 | 176.80 | 1.76 | 8.5 | 0.06 | 0.05 | 5.0 | 33.9 | 0.66 | 10.57 | 1.96 |
| CV (%) | | 4.2 | 7.2 | 12.30 | 10.53 | 18.41 | 23.37 | 12.55 | 10.58 | 15.71 | 9.38 | 6.07 | 7.97 | 10.57 | 6.11 |

SV- Source of variance, DF- Degree of freedom, NL- number of leaves, MSH- Main stem height, DMC- Dry mass of canopy, DMR- Dry mass of roots, $g_s$- Stomatal conductance, *T*- Transpiration, *A*- Photosynthesis, *Ci*- Intercellular $CO_2$ concentration, *IEC*- Instantaneous efficiency of carboxylation, *IEWU*- Instantaneous efficiency of water use, *RWC*- Relative water content. Ŷ- Orthogonal contrasts for comparison of means among treatments: $\hat{Y}_1$=4*0NS-0WS-2.5WS-5.0WS-7.5WS, $\hat{Y}_2$=3*0WS-2.5WS-5.0WS-7.5WS, $\hat{Y}_3$=0WS-2.5WS, $\hat{Y}_4$=0WS-5.0WS, $\hat{Y}_5$=0WS-7.5WS. CV- coefficient of variation. $^{ns-}$ Not significant; $^{**,}$ *- Significant, F-test ($p \leq 0.01$ and $p \leq 0.05$, respectively).

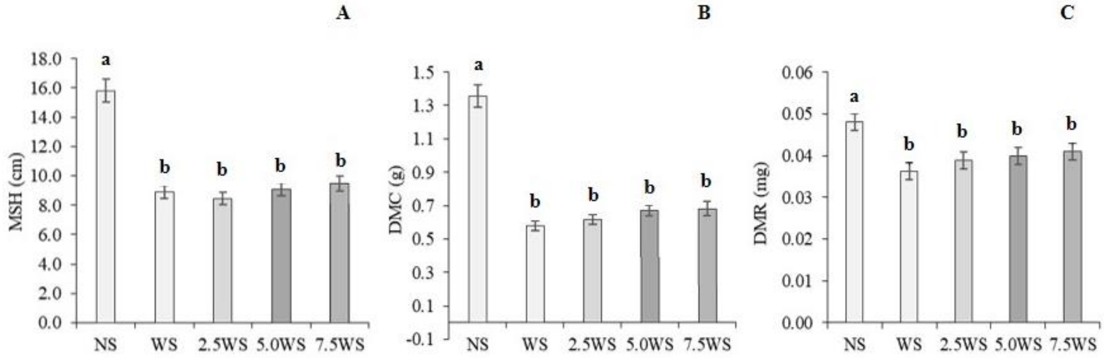


**Figure 1.** Vegetative growth of cultivar BR 1 submitted to drought and treated with exogenous SOD. A- MSH- Main stem height, B- DMC- Dry mass of canopy, C- DMR- Dry mass of roots. NS- No water stress, WS- water stress, 2.5WS, 5.0WS, and 7.5WS- water stress treated with 2.5, 5.0, and 7.5 U of SOD.

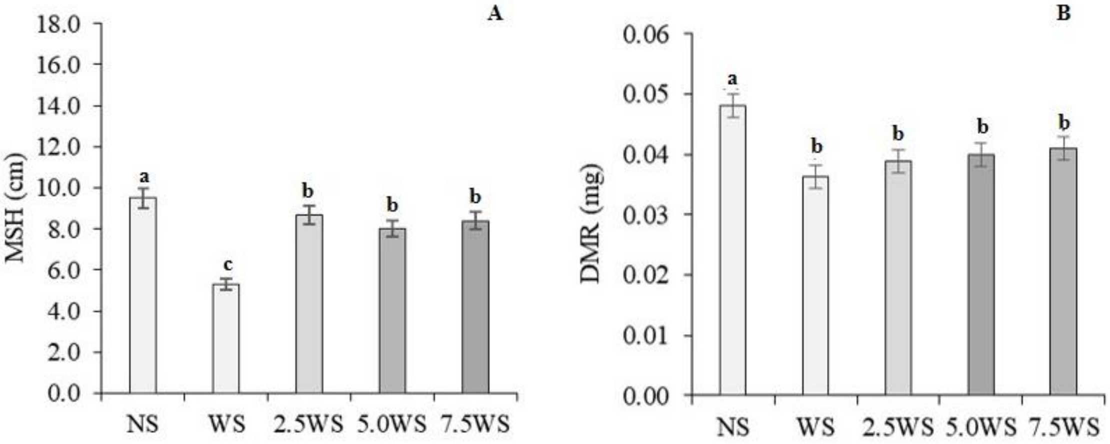


**Figure 2.** Vegetative growth of cultivar IAC Caiapó submitted to drought and treated with exogenous SOD. A- MSH- Main stem height, B- DMR- Dry mass of roots. NS- No water stress, WS- water stress, 2.5WS, 5.0WS, and 7.5WS- water stress treated with 2.5, 5.0, and 7.5 U of SOD.

The reduction in height is one of the main indicators of the behavior of peanut plants when facing a water suppression situation, even in the early phase. Such strategy aims at minimizing energy in the photosynthetic machinery during growth, even because plant height is negatively correlated with pod production in upright plants (Luz *et al*., 2010; Arruda *et al*., 2015). As stress advances, other growth traits are also impacted, leading to different disorders as a function of the tolerance of the genotype. The present work noticed that BR 1 height was more impacted (43%) than that of IAC Caiapó (29%), which is justified by the upright and runner phenotypes, respectively. Runner plants often have main stem between 8 and 12 cm, whereas upright ones range from 25 to 50 cm (Santos *et al.*, 2012). Therefore, the differences become more noticeable in fastigiata plants than in hypogaea ones. Since water suppression occurred early in the cycle, BR 1 did not need additional substrate to alleviate stress as it is tolerant to drought. The opposite took place with the runner cultivar, whose management greatly depends on water (Santos *et al.*, 2012; Duarte *et al.*, 2013). Hence, exogenous SOD application, irrespective of concentration, relieved the growth of the plants, which recovered 84% of their height when compared with the control specimens.

### 6.3.2. Gas Exchanges and Relative Water Content

Seven physiological traits were selected to evaluate stressed plants treated with exogenous SOD. Except for *IEWU* for BR 1, and *Ci* for IAC Caiapó, both cultivars exhibited significant differences ($p \leq 0.01$) for all traits assessed (Table 1). The evaluation of gas exchange of the early-maturing cultivar BR 1 showed a reduction in $g_s$ and $E$ by 72% and 80%, respectively, compared with the control plants (Fig. 3). Such drastic behavior is very characteristic of that cultivar, which properly adjusts the regulation of guard cells to promote stomatal closure, thereby favoring water conservation (Graciano *et al.*, 2016; Pereira *et al.*, 2016; Dutra *et al.*, 2018).

SOD application had no effect on those traits in stressed plants, however, at 7.5 U, an increase by 9% was observed in photosynthesis rate compared with the average of the stressed treatment. This caused an increase in the activity of the cell's antioxidant detoxification mechanism. That suggests the input of exogenous SOD provided to the plants under stress contributed to maintaining the activities of the photosynthesis apparatus, aiding in breaking down stored ROS, since SOD is present at higher concentration in ROS-producing cell organelles, particularly in chloroplasts (Huseynova *et al.*, 2014).

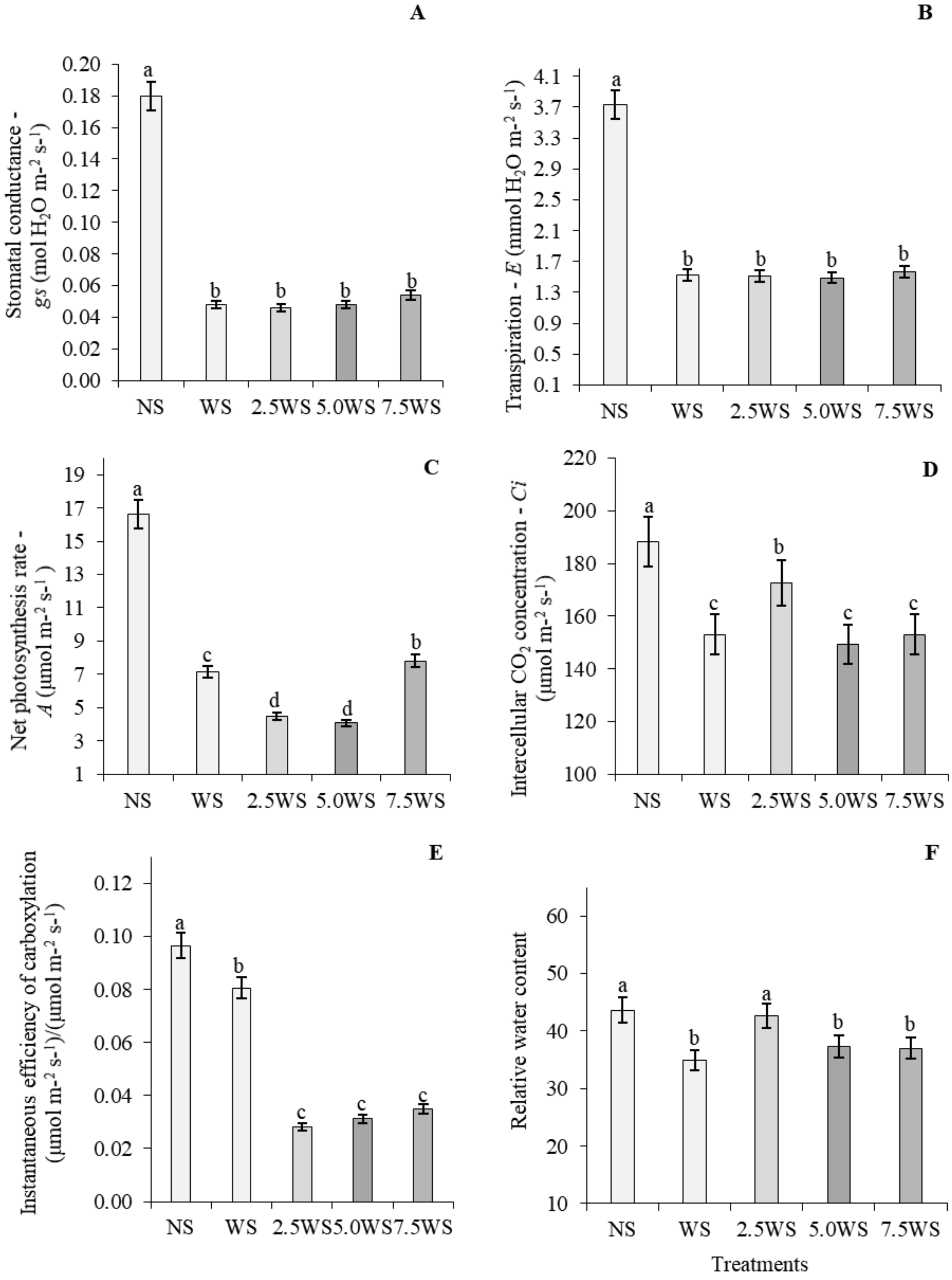


**Figure 3.** Gas exchange of cultivar BR 1 submitted to water stress and treated with exogenous SOD: A- Stomatal conductance, B- Transpiration, C- Photosynthesis rate, D- Internal carbon, E- Instantaneous efficiency of carboxylation, F- Relative water content. NS- No water stress, WS- water stress, 2.5WS, 5.0WS, and 7.5WS- water stress treated with 2.5, 5.0, and 7.5 U of SOD.

Naturally, SOD acts on the antioxidative metabolism by eliminating ROS during environmental stress conditions (Farooq *et al.*, 2019). The agility of such process depends on how much SOD the cell is able to expression to maintain the defense process, which is what differentiates tolerant and sensitive plants. The response observed in the present study shows that exogenous SOD helped re-establish cell equilibrium since, with the brief recovery of *A* in the 7.5W treatment, $CO_2$ concentration within substomatal chambers was increased by 13%, with the application of just 2.5 U of exogenous SOD, which seemed sufficient for the photosynthetic machinery, even with a great reduction in $g_s$ and *E*.

The lack of *IEC* response in the SOD treatments suggests the carbon available in the stomatal chambers was not fully demanded since the input of 2.5 U SOD was sufficient to recover nearly all of the *Ci* at 89%, based on the average of the control treatment. All this gas balance benefited the *RWC* of BR 1 because, by preventing water loss by *E*, the plants possibly adjusted their water potential, leaving it more negative and allowing for greater water retention in the cell. That enabled full re-establishment of *RWC* with low SOD input, which, when absorbed by foliar cuticles, helped eliminate ROS, thus enabling the maintenance of physiological functions due to the reduction in cell damage.

As for the gas exchanges of the late-maturing IAC Caiapó cultivar, we found that, just like the early-maturing BR 1 cultivar, plants also benefited from additional SOD input. The difference is that the late-maturing cultivar benefited from SOD at the early signs of stress and at low concentration (2.5 U), which alleviated the $g_s$, *T*, and *A* of plants by 20%, 31%, and 33%, respectively, compared with the WS treatment (Fig. 4). Although it seems like modest gains, such recovery was sufficient to ensure adequate carbon flow in the cells, based on the expressive increase by 160% in carboxylation efficiency, represented by the *IEC*. Despite the lack of statistically significant difference in the *Ci* among the treatments (Table 1), $CO_2$ stores reduced further diffusive limitations, maintaining water availability in the cells and minimizing oxidative damage.

As seen in Fig. 4, the *IEWU*, which records the water status of cells during stress, increased by 19% compared with the average of the treatments with exogenous SOD. That contributes to the recovery of *RWC* by around 28% based on the averages of the 2.5 WS and 5 WS treatments compared to the stressed treatment (WS).

The role of SOD in the cell medium when faced with environmental stress shows the importance of the enzyme in setting off the antioxidative metabolism. By minimizing excess ROS promoted by cell stress, SOD sets off the dismutation steps to eliminate reactive species. In tolerant species, the amount of SOD acting on the biochemical metabolism must be reasonably produced to protect the cells from oxidative damage. To the extent of our knowledge, the literature has no report on the response to the application of exogenous SOD in plants under water stress. The results of this research, based on data on gas exchange, provide an exciting overview of the benefit of the enzyme in mitigating water stress, at least in C3 plants. Given the averages of the control treatment of the two cultivars studied, it can be seen that the results become more significant when the *RWC* of IAC Caiapó is shown to reduce by 54% in the stressed treatment (WS). However, with SOD input between 2.5 and 5 U, such loss decreased to 40% (Fig. 4). In the drought-tolerant cultivar, *RWC* reduction was lower at 24% compared to the stressed treatment, however, exogenous SOD application in those plants completely recovered the water condition in plant cells. We cannot state whether such response is predictable in tolerant specimens; further studies with other tolerant genotypes are needed to make such claim. However, it is well known that the lowed requirement of exogenous SOD by cultivar BR 1 is precisely due to its broad ability of adjustment when facing water restriction situations, accumulating large amounts of organic solutes, especially proline, a potent osmoregulatory of plants under stress (Nogueira and Santos, 2000; Alves *et al*., 2016; Pereira *et al*., 2016; Dutra *et al*., 2018).

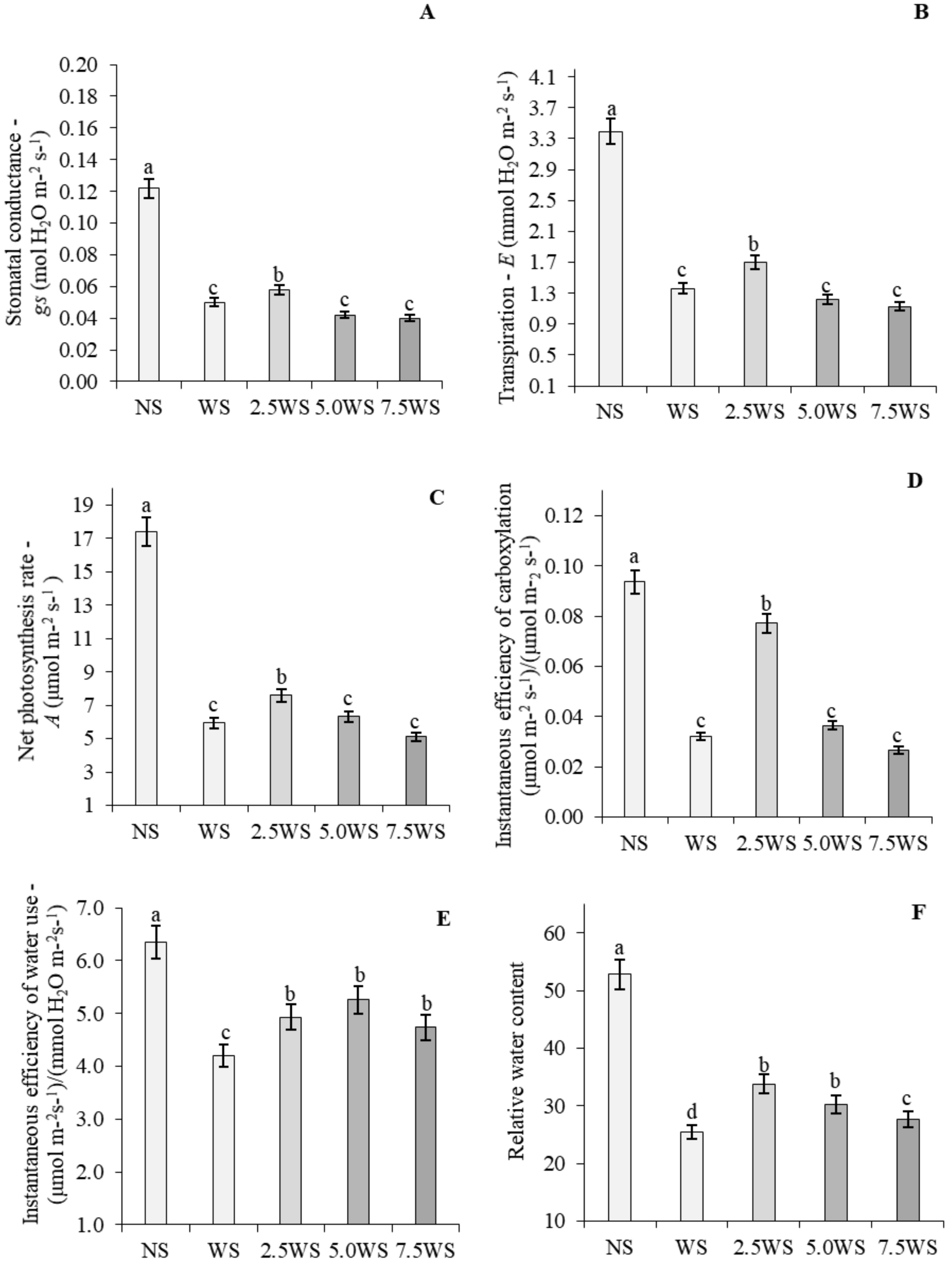


**Figure 4.** Gas exchange of cultivar IAC Caiapó submitted to water stress and treated with exogenous SOD: A- Stomatal conductance, B- Transpiration, C- Photosynthesis rate, D- Internal carbon, E Instantaneous efficiency of carboxylation, F- Relative water content. NS- No

water stress, WS- water stress, 2.5WS, 5.0WS, and 7.5WS- water stress treated with 2.5, 5.0, and 7.5 U of SOD.

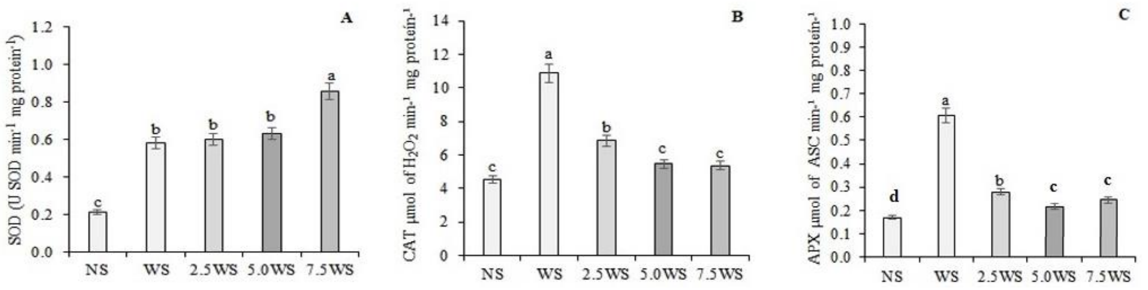
The main role of SOD involves antioxidant defense against ROS, particularly against superoxide radical (Akay *et al.*, 2010). The results obtained in this study show that SOD application played a key role in maintaining the main parameters investigated. The positive interaction between foliar application of SOD and the joint response of vegetative growth and preservation of gas exchange parameters and of RWC point to the protective action of SOD during drought stress, i.e., it minimizes damage.

### 6.3.4. Antioxidant Enzyme

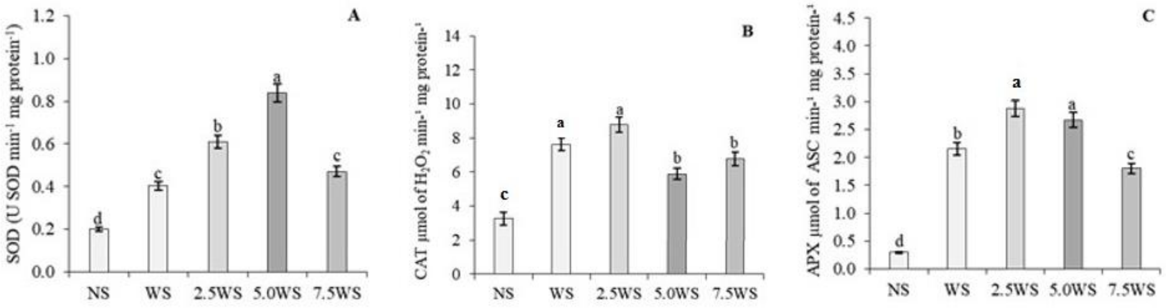
The activity of antioxidative enzymes in the treatments of this study showed a statistically significant difference between cultivars BR 1 and IAC Caiapó, indicating the application of exogenous SOD contributed to the reduction in cell damage in stressed plants (Table 1).

Figure 5A shows the profile of the enzyme activities in the different treatments applied to BR 1. It can be seen that SOD absorption at 7.5 U in the stressed plants resulted in a 50% increase in cell SOD availability when compared with the WS treatment. With the amount of SOD available in the cell, the processes of dismutation and consequent elimination of ROS were optimized with support of the activities of CAT and APX. CAT response shows that, at 2.5 U, the process of elimination of $H_2O_2$, generated from the dismutation of the superoxide radical, decreased by 36% when compared with the mean of WS. At higher concentrations (5 and 7.5 U), the reduction was by 55%, leading to greater activity of $O_2$ and $H_2O$ for the cells, with lower cellular wear, considering the trend of proximity to the plants of the control treatment.

Such condition was also observed in the activity of APX, which used a lower amount of substrate to reduce the $H_2O_2$ remaining from the process of neutralization into $H_2O$. As seen in Figure 5, adding 2.5 U SOD contributed to reducing the work of APX by 50% and, at higher concentrations, by more significant 67%. That shows the extra input of 7.5 U SOD for the stressed plants was quite adequate to help the cascade of events of those enzymes, minimizing the wear of the cellular machinery often caused by oxidative stress.



BR 1



IAC Caiapó

**Figure 5.** Antioxidative activity of BR 1 and IAC Caiapó submitted to water stress and treated with exogenous SOD. NS- No water stress, WS- water stress, 2.5WS, 5.0WS, and 7.5WS- water stress treated with 2.5, 5.0, and 7.5 U of SOD.

In cultivar IAC Caiapó, the triggering of the enzymes followed the same pace as in BR 1 in terms of use of exogenous SOD to minimize cell damage in plants under stress. The difference between them was based on the percentage of mitigation, which was slower in the runner cultivar precisely as it is more sensitive to drought. In addition, IAC Caiapó required a higher supply of SOD to respond to the cellular defense process. Thus, the input of 5 U of exogenous SOD enabled a significant increase by 200% of SOD available in the cells based on the average of the plants in the stressed treatment (Fig. 5). That is a very encouraging result since, as it initiates the antioxidative process, the increase in SOD allowed the steps of dismutation and neutralization of reactive species to be carried out unimpeded, without exhausting the cellular machinery. Despite this input, the agility in $H_2O_2$ reprogram metabolism by CAT and APX in stressed plants was modest when considering the reduction in activity of those enzymes in stressed plants. CAT (5 to 7.5 U) and APX (7.5 U) reduced activity by only 19% and 22%, when compared with WS.

These results show that, in both cultivars, exogenous SOD contributed to minimizing oxidative damage in plants water stress. The rate of response of BR 1, however, was more agile since it managed to scavenging the effects of ROS, reaching closer to the average of the control plants, as seen for APX, which scavenging system process.

In the chain of events of antioxidative enzymes, SOD, CAT, and APX act in a coordinated manner to eliminate ROS and prevent cell damage (Sharma *et al*., 2019), In resistant plants, an input is often seen in SOD of stressed plants to favor the steps of $H_2O_2$ neutralization and $O_2$ and $H_2O$ release to the cells by CAT and APX (Pereira *et al*., 2015; Farooq *et al*., 2019). The literature has no report of the application of exogenous SOD under stress, however, as it is involved in the cellular defense processes, it is expected that, an increase in enzyme activity, benefitting plants tend to draw closer to the normality condition. This study verified such ability in the drought-resistant cultivar BR 1. However, in the drought-sensitive cultivar, despite the slower response, the mitigating effect of exogenous SOD was more beneficial if the reductions in activity exhibited by the stressed plants when compared with the control treatment are taken into account. This is a prospective study and it lays basis for other genotypes to be studied, including to explore the possible practical benefits of the enzyme in peanut management.

## 6.4. Acknowledgments

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## 6.5. Conflict of interests

The authors hereby declare no conflict of interest.

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## 7. CONSIDERAÇÕES GERAIS

Durante o enfrentamento do estresse hídrico, as plantas reagem diferentemente de modo a minimizar os efeitos drásticos que a falta de água produz nos vários eventos celulares. Nas plantas tolerantes, ações integradas são agilizadas em nível celular, de modo a reduzir perda de água e favorecer o ajustamento osmótico, minimizando os oxidativos. Como resultado, a planta consegue finalizar o ciclo e gerar frutos, embora sinta o impacto da desidratação; nas plantas sensíveis, os processos de agilização de respostas de defesa são menos ágeis, motivo pelo qual os danos celulares são maiores, refletindo na fenologia de crescimento e produção. Várias pesquisas disponíveis na literatura têm demonstrado o papel da suplementação de compostos orgânicos na mitigação de estresses ambientais em plantas sensíveis a seca. Tais compostos, geralmente envolvidos em rotas de ajustamento osmótico ou defesa contra danos oxidativos, têm papel ativo nas plantas tolerantes, motivo pelo qual são menos responsivos, quando aplicados de forma exógena. Nas plantas sensíveis, contudo, a contribuição desses compostos no aliviamento do estresse hídrico tem demonstrado benefícios diretos no crescimento, o que abre perspectiva para uso suplementar em lavouras conduzidas em ambientes com irregularidade pluvial. O amendoim é uma oleaginosa alimentar de grande valor comercial. Atualmente o mercado brasileiro é dominado por cultivares rasteiras que possuem um elevado potencial para produção de grãos, porém são muito dependentes de ambiente com regularidade hídrica. Isso limita a adoção desses materiais em ambiente semiárido.

Nesse trabalho, os esforços da pesquisa foram focalizados no papel do piruvato e SOD exógenos aplicados em plantas de amendoim no início do crescimento, usando-se como referencial cultivares contrastantes quanto a tolerância e sensibilidade a seca. Baseados nos resultados obtidos, verificou-se que a aplicação de ambos compostos influenciaram positivamente na fisiologia e bioquímica da cultivar sensível a seca, aliviando os efeitos do estresse hídrico, refletidos nas respostas das trocas gasosas, fotossíntese, prolina e enzimas antioxidativas. A mitigação do estresse ficou devidamente demonstrada, abrindo perspectiva para posterior adoção de cultivares rasteiras de amendoim em ambientes com restrição hídrica. As concentrações adotadas para cada composto orgânico foram baseadas em evidencias reportadas em espécies de metabolismo C3.

A execução desse trabalho permitiu vislumbrar um leque de possibilidades de pesquisas que poderão agregar mais valor e conhecimento a respeito dos benefícios dos piruvato e SOD exógenos em plantas sob estresse hidrico. Para o avanço de pesquisas nesse segmento, recomenda-se a) aprofundar esse estudo com outras cultivares rasteiras, de modo a verificar possíveis respostas de genótipo-dependência, b) estimar concentrações que permitam respostas ajustadas de modo a favorecer a economicidade nas aplicações e c) validar esses resultados em condições de campo, com vista a posterior recomendação de uso, no manejo do amendoim em ambiente semiárido.