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ÁCIDO SALICÍLICO EM SEMENTES DE SOJA E DESEMPENHO DE PLÂNTULAS SOB ESTRESSE HÍDRICO

Santa Maria, RS

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Tese apresentada ao Programa de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, da Universidade Federal de 1 Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Agronomia**

Orientador: Prof. Dr. Ubirajara Russi Nunes

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RESUMO

ÁCIDO SALICÍLICO EM SEMENTES DE SOJA E DESEMPENHO DE PLÂNTULAS SOB ESTRESSE HÍDRICO

AUTOR: Joner Silveira Dalcin
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Dentre os desafios enfrentados na produção da soja, o estresse por déficit hídrico é considerado um dos principais comprometedores da produtividade, estando associado a produção de radicais livres que causam diferentes danos a nível celular. Para contornar esta situação, um mecanismo de defesa é ativado, onde o ácido salicílico desempenha papel fundamental. Portanto, o objetivo deste estudo foi (1): avaliar a qualidade fisiológica de sementes comerciais de soja submetidas a diferentes concentrações de ácido salicílico (AS), diretamente no papel de germinação e em caixas gerbox por 24 horas; (2) avaliar os parâmetros fotossintéticos por condutância estomática e fisiológicos de plântulas de soja sob estresse hídrico após embebição de concentrações de ácido salicílico na germinação de sementes. No capítulo 1, cultivares NA 5909 RG e Tec Irga 6070 RR forma utilizadas, as quais foram embebidas em diferentes concentrações de ácido salicílico em papel de germinação e caixas gerbox por 24h, sendo posteriormente transferidos para papel de germinação neste último caso. Transcorridos 5 dias, determinamos o número de plântulas normais (primeira contagem), comprimento, massa fresca e seca de raiz e parte aérea de plântula. Concluiu-se que as concentrações de AS entre 250 e 750 µM podem ser utilizadas em sementes de soja, no entanto, acima de 1000 µM podem prejudicar os parâmetros da qualidade fisiológica. No capítulo 2, utilizou-se a cultivar Bayer® / Tec Irga 6070 RR, cujas sementes foram embebidas em 25 mL na solução de ácido salicílico (AS) durante 24 horas, sob as capacidades de retenção de 30, 50 e 70%. Em condição controlada foram adotadas as concentrações de zero, 250; 500; 750 e 1000 µM, avaliando-se as variáveis: comprimento, massa fresca e seca de raiz e parte aérea. Em casa de vegetação, utilizaram-se as concentrações de AS de zero, 500 e 1000 µM. Após 29 dias da semeadura, avaliaram-se a condutância estomática, comprimento, massa fresca e seca de raiz e parte aérea. As concentrações de 500 e 1000 µM de AS foram eficientes no déficit hídrico para as variáveis comprimento e comprimento das raízes, massa fresca e seca da parte aérea.

Palavras-chave: Déficit; Estômato; Qualidade fisiológica; Radical livre

ABSTRACT

SALICYLIC ACID IN SOYBEAN SEEDS AND SEEDLING PERFORMANCE UNDER WATER STRESS

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Among the challenges faced in soybean production, stress due to water deficit is considered one of the main productivity compromise, being associated with the production of free radicals that cause different damage at the cellular level. To circumvent this situation, a defense mechanism is activated, where salicylic acid plays a key role. Therefore, the objective of this study was (1): to evaluate the physiological quality of commercial soybean seeds submitted to different salicylic acid (SA) concentrations, directly in the germination paper and in gerbox boxes for 24 hours; (2) to evaluate the photosynthetic parameters by stomatal conductance and physiological of soybean seedlings under water stress after imbibition of salicylic acid concentrations in seed germination. In Chapter 1, cultivars NA 5909 RG and Tec Irga 6070 RR were used, which were soaked in different concentrations of salicylic acid in germinating paper and gerbox boxes for 24h and then transferred to germinating paper in the latter case. After 5 days, we determined the number of normal seedlings (first count), length, fresh and dry mass of root and shoots. It was concluded that SA concentrations between 250 and 750 µM can be used in soybean seeds, however, above 1000 µM can impair the physiological quality parameters. In Chapter 2, the Bayer® / Tec Irga 6070 RR cultivar was used, the seeds of which were soaked in 25 ml in salicylic acid (SA) solution for 24 hours under retention capacities of 30, 50 and 70%. In a controlled condition, concentrations of zero, 250; 500; 750 and 1000 µM were adopted, evaluating the variables: length, fresh and dry mass of root and shoot. In a greenhouse, SA concentrations of zero, 500 and 1000 µM were used. After 29 days of sowing, stomatal conductance, length, fresh and dry mass of root and shoot were evaluated. The concentrations of 500 and 1000 µM of AS were efficient in the water deficit for the variables length and root length, fresh and dry mass of the shoot.

Key words: Deficit; Stomata; Physiological quality; Free radical

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SUMÁRIO

1. INTRODUÇÃO

A cultura da soja apresenta um papel de destaque no cenário mundial de produção de grãos, sendo considerada atualmente uma *commodity* no Brasil e representando um dos principais produtos de exportação. Pertencente à família Fabaceae, a soja *Glycine max* (L.) Merrill, apresenta um grande potencial de mercado externo devido sua excelente adaptação às diferentes condições edafoclimáticas do Brasil, o que impulsiona seu cultivo em todas regiões do país.

A expansão da soja em nosso país se deu a partir da região Sul e sua introdução em novas áreas de plantio foi viabilizada graças à adoção de novas tecnologias, como o uso de sementes de melhor qualidade fisiológica, genética e sanitária, além de cultivares mais produtivas e adaptadas às diferentes condições de cultivo. Entretanto, as plantas estão sujeitas à diversas condições ambientais, muitas vezes drásticas, que podem causar estresses, afetando o desenvolvimento da cultura e diminuindo o potencial produtivo.

O déficit hídrico é considerado um dos principais desafios à boa produtividade desta cultura, tendo reflexo direto na produtividade. A disponibilidade de água é de extrema importância, principalmente nos estádios de germinação-emergência e floração-enchimento de grãos. Assim, o déficit hídrico, principalmente na fase de germinação-emergência, interfere no estabelecimento da cultura no campo, comprometendo o estande adequado de plantas.

O estresse causado pelo déficit hídrico promove alterações morfológicas, fisiológicas e bioquímicas, na germinação da semente até estádios reprodutivos da planta. Com isso, a campo observa-se o estabelecimento desuniforme das plantas, com desenvolvimento e crescimento retardado de algumas destas, o que certamente acarretará em perdas de desempenho da cultura como um todo.

Diante deste cenário, as células sofrem estresse oxidativo e são formados radicais livres, conhecidos como espécies reativas de oxigênio (EROs). Para contornar tais alterações, um complexo mecanismo de defesa é ativado, onde antioxidantes enzimáticos e não enzimáticos atuam controlando os níveis desses radicais livres impedindo que danos sejam causados nas células vegetais. Entretanto, em condições de elevado estresse, os danos podem ser irreversíveis.

O ácido salicílico (AS), o qual é considerado um fitormônio, atua como sinalizador do estresse, além de regular diversos processos fisiológicos e bioquímicos nas plantas.

Dependendo da dimensão do desafio, o AS atua aumentando e diminuindo suas concentrações, podendo, ainda, induzir a síntese de novas proteínas e auxiliar nos processos fotossintéticos, como abertura e fechamento estomático, relacionados com o estresse por déficit hídrico. Neste contexto, foram geradas as hipóteses que a aplicação exógena de ácido salicílico em sementes de soja pode influenciar a germinação dependendo da forma, tempo e concentrações de AS durante a embebição. Além disso, em condições de estresse hídrico, especialmente déficit, o aporte de AS durante a embebição na germinação das sementes pode aumentar a tolerância na fase inicial de plântula.

Desta forma, esta tese está composta por uma revisão de literatura sobre a cultura da soja, estresse hídrico nesta cultura e ácido salicílico, abordando os principais aspectos relacionados a função deste fitormônio. Na sequência são apresentados dois artigos científicos: i. Qualidade fisiológica de sementes de soja submetidas a diferentes concentrações de ácido salicílico, o qual teve por objetivo avaliar a qualidade fisiológica de sementes comerciais de soja submetidas a diferentes concentrações de AS, diretamente no papel de germinação e em caixas gerbox por 24 horas; ii. Ácido salicílico e seu efeito nos parâmetros fisiológicos e fotossintéticos em plântulas de soja sob déficit hídrico, o qual objetivou avaliar os parâmetros fotossintéticos por condutância estomática e fisiológicos de plântulas de soja sob estresse hídrico após embebição de concentrações de ácido salicílico na germinação de sementes.

2. REVISÃO DE LITERATURA

2.1 CULTURA DA SOJA

A soja [*Glycine max* (L.) Merrill] é uma planta anual e herbácea, da família Fabaceae. Esta cultura teve sua expansão ao redor do mundo com seu cultivo aumentando significativamente ao longo dos anos, em função de seu valor comercial e a necessidade de seu consumo em importantes países importadores (SEDIYAMA; SILVA; BORÉM, 2015).

Características relevantes no grão, como óleo, alto teor de proteína, baixo nível de água, valor nutritivo e variedade de produtos para consumo humano e animal, foram os impulsionadores de sua aceitação no mercado. Ainda, o valor comercial oferece rentabilidade para os produtores e fizeram com que esta fosse amplamente difundida. Da mesma forma, eventos biotecnológicos, cultivares adaptadas aos diferentes cenários, qualidade de sementes e investimento em pesquisa proporcionaram aumento da produtividade por área (FIOREZE et al., 2011; HAEGELE; BELOW, 2013; MASUDA; GOLDSMITH, 2009; MORANDO et al., 2014).

Durante as últimas safras, conforme dados da Companhia Nacional de Abastecimento – CONAB, o Brasil é o segundo maior produtor mundial do grão, com uma produção, na safra 2017/18 de 119.281,7 milhões de toneladas. A área plantada de soja no Brasil foi de 35.149,2 milhões de hectares e produtividade de 3.394 kg ha^{-1}. Na safra 2018/19 houve um leve aumento na área plantada, porém a produtividade foi menor, em função de estresses hídricos ocorridos em alguns locais do país, no entanto não ocorreram perdas que justificassem quebra de safra no país (CONAB, 2019).

2.2 ESTRESSE ABIÓTICO

Existem diferentes formas da planta processar a sinalização de um estresse e transformar esse sinal em proteção. Independentemente do tipo de estresse, seja biótico ou abiótico, o sistema de defesa da planta será ativado, podendo ser causado por temperaturas drásticas, salinidade, seca pragas, doenças ou outros tipos, tudo em função da integridade dos órgãos e sobrevivência (MORANDO, et al. 2014). Tal capacidade ocorre a nível celular, mediada por diversas enzimas, complexos antioxidantes e outros compostos que minimizam os possíveis danos (MIURA; TADA, 2014).

Particularmente, o estresse abiótico está relacionado às diferentes condições adversas de ambiente como estresse hídrico (excesso e déficit), concentrações de sais presentes no solo, baixas temperaturas, entre outros, podendo ter diferentes impactos nas plantas cultivadas. Tais condições adversas podem interferir e prejudicar o crescimento e desenvolvimento das culturas, impactando negativamente na produtividade (MOHAMMADI et al., 2012).

A água é um constituinte fundamental da célula e possui função vital em diferentes processos. Também representa grande parte do peso de uma planta de soja, sendo imprescindível a todos os processos bioquímicos e fisiológicos (TAIZ; ZEIGER, 2009). Logo, do ponto de vista econômico, o déficit hídrico pode ser considerado o estresse abiótico mais importante, pois afeta de modo direto a cultura independente do seu estádio de desenvolvimento (GAVA et al., 2015).

2.2.1 Déficit hídrico e seu efeito na germinação e estabelecimento inicial de plântulas

No sul do Brasil não são incomuns períodos de estiagens, os quais trazem grandes prejuízos à agricultura, uma vez que a grande parcela das lavouras ainda não possui sistemas de irrigação e a chuva constitui a principal fonte de água para as mesmas. Particularmente no Rio Grande do Sul, a disponibilidade hídrica é uma variável diretamente relacionada a expressão do potencial de rendimento da cultura de soja, independente da época de semeadura, do ciclo, cultivar e região do estado (CUNHA et al. 1998; FIOREZE et al., 2011).

Das etapas de desenvolvimento das plantas, a germinação se constitui em um estádio crítico, o qual tem consequências nas etapas subsequentes do ciclo de vida das mesmas (LEE; PARK, 2010). Esta fase de desenvolvimento inicia-se com a absorção de água e se encerra com o alongamento do eixo embrionário e protusão da raiz primária (MARCOS FILHO, 2015). Outros autores como Bewley e Black (1994) sugerem que este processo é dividido em três principais etapas: embebição, processo bioquímico preparatório e emergência propriamente dita (crescimento).

Particularmente, a fase de embebição, é de grande importância uma vez que a captação da quantidade adequada de água é imprescindível para o reinício da atividade metabólica após a maturidade da semente. Além disso, a água é fundamental para o metabolismo celular durante a germinação, garantindo a atividade enzimática, a solubilização e transporte de reservas e digestão hidrolítica de substâncias de reserva armazenadas. Esta é absorvida em três fases, iniciando pela rápida absorção de água por diferença de potencial, redução na velocidade de hidratação e respiração, e crescimento celular (embrião) (BEWLEY; BLACK, 1978; MARCOS FILHO, 2015).

Durante ou logo após o processo de germinação, o qual necessita primordialmente de água, a falta desse elemento pode ser o limitante para o crescimento e desenvolvimento da plântula ou para a própria produtividade final (MARCOS FILHO, 2015). Pode ser comum no período inicial de desenvolvimento da planta de soja ocorrer déficit hídrico, muitas vezes não drástico suficiente para a falência da planta, porém, o bastante pra afetar os processos fisiológicos e bioquímicos durante esse período (MORANDO, et al. 2014). Esse evento está relacionado à capacidade do material em tolerar essas condições, duração e intensidade do estresse. Na prática, solos que estão nessa situação, demandam em excesso que a planta busque água disponível com o solo em um potencial hídrico negativo, dificultando a absorção (FAROOQ, et al. 2009). Todavia, nesse estádio ainda existe um número reduzido de raízes, fato este determinante para a absorção de água em locais de maior profundidade (SILVA, et al. 2019).

Para superar esses momentos de estresse, desde que sejam momentâneos, a planta utiliza de mecanismos de defesa auxiliares, comandados por enzimas e complexos não enzimáticos, além de compostos fenólicos como o ácido salicílico (REZA; RIGI, 2014). Entende-se que o AS tem papel fundamental na defesa da planta, atuando ao lado de enzimas do complexo antioxidante na diminuição dos danos de radicais livres e até mesmo fisiologicamente no fechamento estomático (NOOREN, et al. 2009). Dessa forma, sua interação ou absorção durante o processo germinativo, plântula e na planta, poderá fornecer informações relevantes e principalmente atuar de forma positiva na redução dos danos causados pelo déficit hídrico.

2.3 ÁCIDO SALICÍLICO E SEU PAPEL NA TOLERÂNCIA À ESTRESSES ABIÓTICOS

Da mesma forma que os demais seres vivos, as plantas sofrem frequentemente ataques causados por agentes bióticos e abióticos. Como consequência destes, ocorrem adaptações e alterações profundas no metabolismo celular, entre a síntese de proteínas de defesa, expressas por genes específicos, os quais são ativados por metabolismos complexos (NÜRNBERGER et al. 2004; WU; BALDWIN, 2010). Estas proteínas exercem diferentes papéis na resistência e sobrevivência da planta, tanto de forma direta (combate ao agente agressor) como indireta (manutenção da estrutura e funções celulares) (PINHEIRO et al., 1999; CARVALHO et al., 2007).

Basicamente, o sistema de defesa atua de três formas: 1. Resistência constitutiva, inespecífica ou estática (recebida por herança genética, tornando as plantas imunes à determinados patógenos); 2. Resistência localizada (ativada no ponto onde há a agressão); 3.

Resistência sistêmica adquirida (*SAR- systemic acquired resistance*) que protege a planta de ataques subsequentes (GOTO, 1990; STICHER et al.,1997; CARVALHO et al., 2007).

Sob condições de estresse oxidativo ou devido às reações normais da cadeia de transporte de elétrons, são formados os radicais livres (RLs), como as espécies reativas de oxigênio (EROs), sendo prejudiciais ao sistema celular dependendo do nível de formação desses compostos. As reações que levam a formação destes são altamente reguladas, entretanto, pode ser perdida se o estresse for muito severo, aumentando consideravelmente a produção de RLs, os quais podem levar a uma cascata de eventos podendo começar com a peroxidação de lipídios, avançando para degradação de membranas e morte celular (CHAOUI et al., 1997; MAZHOUDI et al., 1997; GREGGAINS et al., 2000).

Em face ao aumento das espécies reativas de oxigênio (EROs) nas células, o sistema vegetal é protegido por diferentes eliminadores radicais, advindos do sistema enzimático e não-enzimático (MITTLER, 2002; GUPTA et al., 2013; KUMAR et al., 2013). Os componentes enzimáticos compreendem várias enzimas antioxidantes, tais como superóxido dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX) e enzimas do ciclo ascorbato-glutationa, tais como ascorbato peroxidase (APX) e glutationa redutase (GR) (ASADA, 1999; MITTLER, 2002; 2004). Os componentes não enzimáticos incluem os principais tampões redox celulares ascorbato (AsA) e glutationa (GSH), bem como tocoferol, carotenóides e compostos fenólicos (MITTLER et al., 2004, GRATÃO et al., 2005, SCANDALIOS, 2005).

Existem duas rotas metabólicas envolvidas na síntese de compostos fenólicos: a rota do ácido chiquímico e a rota do ácido malônico, sendo a primeira rota mais comum. Dentre os compostos de natureza fenólica, destaca-se o ácido salicílico que é composto de um anel aromático ligado a um grupo hidroxil (COLLI, 2012). O AS é produzido naturalmente pelas plantas por duas vias enzimáticas diferentes, partindo de ácido corísmico e sintetizado através das enzimas isocorismato sintase (ICS) ou fenilalanina amônia-liase (PAL) (MIURA; TADA, 2014; SEYFFERTH; TSUDA, 2014).

A enzima ICS converte corismato a isocorismato, que pela ação da enzima isocorismato piruvato liase, é convertido em ácido salicílico (AS). Por outro lado, se a via de formação ocorrer pela enzima PAL, o ácido benzóico é convertido em AS pela enzima ácido benzóico-2-hidroxilase. Após sua produção, o AS pode ser convertido em formas metiladas ou glicosadas, que também são translocadas via floema (KAWANO; BOUTEAU, 2013). A síntese do AS pode ocorrer tanto nos cloroplastos como nos peroxissomos das células, contudo, o exato local pode ser dependente dos compostos gerados e do tipo de estresse (TAIZ; ZEIGER, 2004; HERRERA-VÁSQUEZ; SALINAS; HOLUIGUE, 2015).

Vários efeitos fisiológicos e bioquímicos do AS nos sistemas das plantas têm sido documentados, incluindo efeitos na permeabilidade das membranas, respiração, além da modulação da resposta das plantas à estresses (BARKOSKY; EINHELLIG, 1993; SANERATNA et al., 2000). Ainda, destaca-se que o AS está envolvido nas respostas ao estresse oxidativo como um dos principais protagonistas, incluindo infecção por patógenos, a irradiação UV, déficit hídrico, dentre outros (RASKIN, 1992; STICHER; MAUCH-MANI; MÉTRAUX, 1998; SUNDAR et al., 2009; HAYAT el al., 2012; ROJAS et al., 2014).

Segundo Wrzaczek, Brosche e Kangasjarvi (2013), a ativação do ácido salicílico em plantas estressadas é precedido de oxidações originadas em diferentes compartimentos celulares, aumentando seus níveis pela formação de H_2O_2 mediada por oxidases e peroxidases extracelulares (JOO et al., 2005; TSUDA et al., 2008; KATAGIRI; TSUDA, 2010; MAMMARELLA et al., 2015). Notou-se isso principalmente quando há o ataque de patógenos, mas o mesmo processo ocorre de forma muito semelhante quando a planta está estressada por fatores abióticos, como exposição ao ozônio e UV-B, precedendo da sinalização via AS (GRANT; LOAKE, 2000; TORRES et al., 2002; JOO et al., 2005; OGAWA et al., 2007; GARCION et al., 2008; O'BRIEN et al., 2012).

Conforme comentado anteriormente, o AS também desempenha importante função frente aos diferentes tipos de estresses abióticos, tais como a exposição a luz, salinidade, déficit hídrico e temperatura (MATEO et al., 2006; LEE; KIM; PARK, 2010; WAN et al., 2012; MIURA; TADA, 2014). Em contraste aos estresses mencionados, estas condições geram o acúmulo de EROs nos cloroplastos e peroxissomos (APEL; HIRT, 2004; HOLUIGUE et al., 2007).

Maiores níveis de AS foram detectados em plantas com produção de EROs nos peroxissomos (via catalase2; cat2) e nos cloroplastos (via silenciamento do gene PRX do ascorbato tilacoidal; *taPX RNAi*). As evidências obtidas usando estes modelos indicam que o H_2O_2 originado nos cloroplastos e peroxissomos estimulam a biossíntese de AS, que é essencial para os principais desfechos do sistema de defesa: morte celular, reprogramação transcripcional e fechamento estomático (CHAOUCH et al., 2010; MARUTA et al., 2012; NOSHI et al., 2012; WAN; BOL; LINTHORST, 2012; SÁNCHEZ-MARTÍN et al., 2015).

Estudos complexos revelam resultados a níveis intracelulares, codificando genes específicos na biossíntese de AS, todavia, ainda permanecem desconhecidos os mecanismos pelos quais o H_2O_2 gerado nos cloroplastos, apoplastos e peroxissomos desencadeia a biossíntese de AS (NOSHI et al., 2012). Segundo GARCION et al. (2008) os genes ICS1 e ICS2 codificam para síntese do isocorismato, o qual é uma enzima chave no controle da biossíntese do AS em *Arabidopsis*. A regulação positiva do ICS1 foi detectada nas respostas ETI a patógenos, UV-B, ozônio e estresse hídrico, bem como em *cat2* (WILDERMUTH et al., 2001; ZHANG et al., 2010; WAN et al., 2012). No entanto, foram reconhecidos fatores que regulam a expressão de ICS1 e estão envolvidos na síntese de AS mediada por EROs (DU et al., 2009; ZHANG et al.,2010; VAN VERK; BOL; LINTHORST, 2011; GAO et al., 2013; SHI et al., 2014).

Além dos aspectos comentados acima, tem-se proposto que existe a sinalização e regulação de AS via presença de íons cálcio (Ca^{+2}), baseado na evidência da atividade de fatores expressos por ICS1 (DU et al., 2009; GAO et al., 2013; TRUMAN et al., 2013; SEYFFERTH; TSUDA, 2014). Alguns autores como Price et al. (1994), Pei et al. (2000) e Wrzaczek et al. (2013) propõe que o Ca^{+2} pode igualmente agir diminuindo a sinalização das EROs. Desta forma, a possibilidade de que a sinalização Ca^{+2} possa ativar a produção de AS via EROs, sugere que provavelmente um correto suprimento de Ca^{+2} na planta, possa interferir de forma benéfica na sinalização de um estresse em conjunto com o AS (DUBIELLA et al., 2013; GAO et al., 2013).

Curiosamente, alguns autores como Mou et al. (2003), Mateo et al. (2006) e Miura e Tada (2014) demonstraram que o AS apresenta um efeito ambivalente no sentido de tanto promover o acúmulo de EROs (pro-oxidante), como eliminação de EROs (antioxidante) em diferentes modelos de estresse, os quais incluem resposta induzidas à patógenos e respostas a outros tipos de estresse, como alta luminosidade, déficit hídrico, salinidade e estresse por frio.

Se por um lado o AS pode promover a produção de EROs durante eventos iniciais de sinalização, sendo essenciais para respostas de defesa, por outro essa produção pode ser maléfica em altos níveis (GARRETON et al., 2002; LEE; KIM; PARK, 2010; KHOKON et al., 2011). Portanto, a presença de EROs, se torna dependente dos níveis no sistema celular, sendo fundamental para a sinalização do aumento da concentração de compostos como o AS e enzimas que atuam para minimizar os efeitos drásticos de estresses. Ainda neste sentido, Lee, Kim e Park (2010) e Miura e Tada (2014), relatam que altas concentrações de AS promovem a produção de EROs, induzindo o estresse oxidativo e a redução da tolerância à seca e salinidade. Visto que isso dependerá basicamente dos níveis de produção de EROs, o excesso irá promover efeito negativo, já em níveis controlados fornece a sinalização básica para a formação de compostos importantes na tolerância aos estresses.

Relatos iniciais demonstraram a inibição, mediada pelo AS, da catalase e ascorbato citosólico (PRX), duas enzimas importantes na detoxificação de H_2O_2 (CHEN; SILVA; ANDKLESSIG, 1993; DURNER; KLESSIG, 1995). Neste sentido, observou-se que o AS, durante o fechamento estomático, impulsionou a produção de EROs por PRXs extracelulares, sendo talvez o efeito do déficit hídrico drástico a ponto de produzir em excesso EROs prejudicando o sistema celular de defesa da planta (KHOKON et al., 2011; MIURA et al., 2013).

De maneira oposta, demais evidências disponíveis apontam que o AS promove a remoção de EROs, sendo essencial a resposta antioxidante que restringe aumento súbito de EROs em resposta a bactérias avirulentas, alta luminosidade, ozônio e salinidade (GRANT; LOAKE, 2000; MATEO et al., 2006; YOSHIDA et al., 2009; LEE; KIM; PARK, 2010; CHAOUCH et al., 2010).

Desta forma, é claro que existe uma forte relação entre o AS e o complexo antioxidante enzimático e não enzimático, principalmente quanto à capacidade de intervir no estresse oxidativo, o qual é inevitável em células (SINGH et al., 2019). Ao longo da evolução houveram diferentes avanços dos processos metabólicos aeróbicos como a respiração e a fotossíntese, sendo estes motivos suficientes para que ocorra em algum momento a formação de EROs, tendo como principais locais de formação os peroxissomos, cloroplastos e mitocôndrias. São nessas organelas que muitas das funções bioquímicas e fisiológicas são sintetizadas, inclusive a formação desses compostos que atuam para minimizar os efeitos dos RLs (TAIZ; ZEIGER, 2004).

Esse processo de sinalização e intervenção na defesa contra o estresse hídrico, está associado ao nível de EROs e a atividade das enzimas mediadas por compostos de origem diferente, mas que possuem relação muito específica (JANDA, et al. 2014). Todavia, pelo o exposto, os processos que ocorrem nas células não são ordenados. Existem muitos fatores que contribuem para variações nos resultados, como a espécie, cultivar, manejo, condições ambientais e principalmente o nível de estresse (HAYAT, et al. 2010).

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3. CAPÍTULO I – Artigo Científico

Este capítulo originou um artigo científico aceito para publicação na revista Journal of Agricultural Science

Salicylic Acid Concentrations and Its Effects on the Physiological Quality of Soybean Seeds

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Abstract

The objective of this study was to evaluate the physiological quality of commercial soybean seeds submitted to different concentrations of salicylic acid (SA), directly on germination paper and gerboxes for 24 hours. Seeds of cultivars NA 5909 RG and Tec Irga 6070 RR were soaked in salicylic acid solutions with concentrations of zero, 250, 500, 750, 1000, 1500, 2000, 3000, 4000 and 5000 µM. Seed imbibition occurred in two ways: (1) germination paper moistened with salicylic acid solutions; (2) imbibition of the seeds in salicylic acid solutions in gerbox boxes for 24 hours and subsequent sowing on germinated paper moistened with distilled water. On the fifth day after sowing, the number of normal seedlings (first count), length, fresh and dry mass of root and shoot were determined. It was concluded that the concentrations of SA between 250 and 750 µM can be used in soybean seeds, however, above 1000 µM may impair the parameters of physiological quality. The gerbox method for 24 hours provided the best results without the drastic reduction of the parameters in the lowest concentrations of SA.

Keywords: vigor, germination, imbibition

1. Introduction

Soybean cultivation has a global importance in agribusiness because it is used for different objectives, both for animals and humans (Fioreze et al., 2011). The demand for higher productivity is achieved with the use of techniques and management, and the beginning of a crop will only have uniformity with the use of seeds with high physiological potential (Marcos Filho, 2015a). However, biotic and abiotic stresses may decrease yield throughout the growing season in the field, from germination to harvesting (Danquah et al., 2014; Jaleel et al., 2009; Morando et al., 2014).

To maintain its capacity to tolerate stresses, the plant uses its complex defense system, which is composed of different mechanisms and specific compounds that aid in this process (Yang & Dong, 2014). Among these, salicylic acid (SA) is considered a plant hormone present in numerous plant species and acts in important biochemical and physiological processes, such as growth and development, stomatal closure, nutrient absorption, chlorophyll and protein synthesis, foliar abscission and seed germination (Hayat, 2010; Miura & Tada, 2014; Vazirimehr & Rigi, 2014). In addition, it has an influence on the response to different types of stress, through chemical signaling in plant cells together with antioxidant complex enzymes (Janda et al., 2014; Parmoon et al., 2017). Also, other enzymes such as superoxide dismutase, peroxidases, catalases, among others, have the function of reducing the damage caused by free radicals, such as reactive oxygen species (ROS), which are formed mainly in moments of stress (Hayat et al., 2012; Lee, Kim, & Park, 2010).

Studies that perform the exogenous application of SA do not have consensus about the concentration to be used, the time of contact or route for absorption. There is concrete information that low concentrations of SA aid in antioxidant capacity; already, high concentrations may lead to oxidative stress or susceptibility to abiotic stress (Miura & Tada, 2014). However, each situation may be influenced by the study conditions, which consequently increase the variability of the results. As an example, Kang et al. (2012) observed that high concentrations decreased important parameters in the wheat crop, such as lower seedling growth, increased activity of antioxidant enzymes, decreased photosynthesis, thus increasing plant stress levels.

Tang et al. (2017) point out that the concentration of 0.5 mM and osmotic potential of -1.03 MPa in the germination of soybean seeds reduced the effect of high water stress. Similarly, Al-Hakimi (2006) suggested that the concentration of 0.6 mM SA promoted a water deficit inhibitory effect on several parameters evaluated in leaves and roots. However, the role of SA and its various functions in the plant defense system is not yet known, so studies that specifically elucidate its performance are needed (Janda & Ruelland, 2015).

It is noticed that although there are several studies using SA, a pattern of concentrations and mode of imbibition of the compound after seed germination has not yet been established. Therefore, the objective of the study was to verify the effect of different concentrations of SA on some parameters of physiological quality in soybean commercial seeds.

2. Material and Methods

The study was conducted at the Laboratory of Seed Research and Development (LDPS) of the Department of Plant Science at the Federal University of Santa Maria, in the Rio Grande do Sul state. Commercial soybean seeds of Nidera® NA 5909 RG and Bayer®/Tec Irga 6070 RR cultivars produced in the 2015/2016 crop were used, both of which are recommended for cultivation in the state.

Initial evaluation of seeds: samples from both cultivars were evaluated separately for physical and physiological characteristics by performing the following tests: thousand seed weight, moisture content, first count of germination, germination test, root and shoot length, dry mass of seedlings, mass electrical conductivity and emergence of seedlings on field (Krzyzanowski; Vieira, & França Neto, 1999; Nakagawa, 1999; Brasil, 2009).

Physiological quality of soybean seeds submitted to SA concentrations: after the initial characterization, the seeds were submitted to germination under different concentrations of SA (Sigma-Aldrich®): zero; 250; 500; 750; 1000; 1500; 2000; 3000; 4000; 5000 μM. In the literature, there are different studies including soybean, but there is no consensus among researchers of the most adequated concentration (Al-Hakimi, 2006; Tang et al., 2017).

The SA was supplied to the seeds in two forms: (1) in the germinated paper moistened with the SA solutions; (2) imbibition of the seeds in SA solutions in gerbox for 24 hours. For the first group, four replications of 50 seeds were sown on rolls of germination paper with the SA solutions mentioned, in the proportion of 2.5 times the weight of the dry paper. The rolls were packed in plastic bags and kept in a BOD (Box Organism Development) germination chamber under constant light and temperature of 25 ºC.

In the second option, four replicates of 50 seeds were placed in gerbox boxes to soak on three sheets of germinating paper moistened with 25 mL of SA solution at the same concentrations. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 ºC. After this imbibition period, the seeds were placed to germinate on germinated paper moistened only with distilled water, in the proportion of 2.5 times the dry paper mass. The rolls were packed in plastic bags, kept in BOD, under continuous light and at 25 ºC (Brasil, 2009).

For both ways of SA treatment, on the fifth day after sowing, the percentage of normal seedlings was evaluated and, at the eighth day, the percentage of germination was estimated according to Brazil (2009). In addition to the germination test, on the fifth day after sowing, ten normal seedlings were removed in sequence from the upper part of the germinating roller, to compose the length, fresh and dry mass of root and shoot, being the cotyledons removed (Nakagawa, 1999).

Statistical analysis: the study was conducted in a completely randomized design with four replicates per treatment, each replicate being composed of 50 seeds. Initially, the assumptions of the mathematical model were verified by Action® software (Equipe Estatcamp, 2014). In case of non-compliance with the normality of the errors and homogeneity of the variances, the data were transformed by the methodology $\sqrt{x}$. The percent data were transformed by the equation: arcsin$\sqrt{\%/100}$.

Seeds imbibition forms with SA (germination paper and gerbox for 24 hours) and cultivars were analyzed separately. Data were submitted to analysis of variance by the F test ($p < 0.05$) and regression analysis ($p < 0.05$) using the Sisvar® software (Ferreira, 2011).

3. Results and Discussion

The physical and physiological characterization of the seeds of the two commercial cultivars is presented in Table 1. It is observed that both have adequate humidity and germination above 80%, however it is noticed a difference in the physiological quality between these materials. The data of the initial characterization were not submitted to statistical analysis, once the objective was only to characterize the cultivars regarding the physiological quality. According to Marcos Filho (2015b), the correct characterization of seed lot quality is extremely important in the use of vigor tests and to understand the results that can be found in the most diverse studies.

Table 1. Thousand seed weight (TSW), moisture (M), first count (FC), germination (G), radicle length (RL), shoot length (SL), radicle dry mass (RDM), shoot dry mass (SDM), electrical conductivity (EC), field emergence (FE) and emergence speed index (ESI) of soybean seeds Tec Irga 6070 RR and NA 5909 RG

Cultivar	TSW	Moisture	FC	G	RL	SL	RDM	SDM	EC	FE	ESI
	g	----------- % ----------- -			----- cm -----		------- mg ------		µS cm^{-1} g^{-1}	%	
Tec Irga 6070 RR	137	12,97	83	92	7,6	14,9	87	198	55,8	97	14,3
NA 5909 RG	163	9,85	67	93	9,0	4,4	74,5	166,2	70,9	84	7,5

The data on vigor and germination for both cultivars and imbibition forms are shown in Figure 1. There was no significant difference in the germination for the cultivar Tec Irga 6070 RR (Figures 1A and 1B). In general, it is noted that the vigor and germination are very similar for the two materials and imbibition method. Thus, at concentrations ranging from 250 and 1000 μM occurred the increase of the percentage values, however, from 1000 to 5000 μM these values decreased. In comparison with the control, concentrations of 500 and 750 μM demonstrated the highest values (Figures 1A-1D). Once there was no type of stress involved, these results were due to the concentrations of SA used.

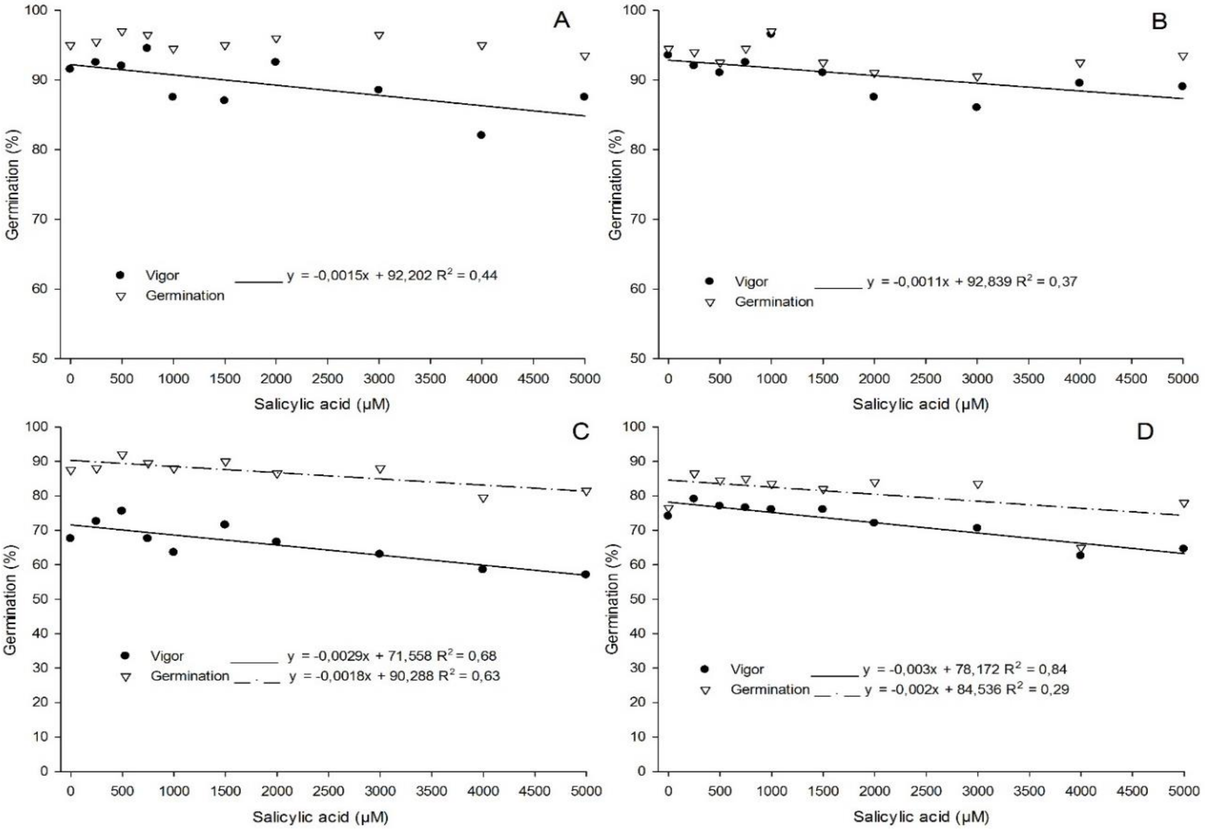

Figure 1. Percentage of vigor and germination for the plants Tec Irga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

Most studies report that positive results with SA are usually found in the presence of stress. For example, Al-Hakimi (2006) detected increases in the cell wall metabolism of leaves and roots of soybeans using the concentration of 600 μM with the presence of water deficit. In the same context, Lee, Kim, and Park (2010) showed that under normal conditions during the germination process the SA is not essential, only in the stress situation.

Therefore, the significance of high and low concentrations of SA may vary according to species, type of stress evaluated, the period of imbibition or contact of seeds, absorption medium and even the parameters and target of study. Parmoon et al. (2017) evaluated the treatment of seeds of *Sylibum marianum* with SA, and concluded that concentration of 1000 mg L^{-1} considerably improved germination and vigor of the aged seeds and seedling growth, in addition to the greater antioxidant activity.

Interestingly, Siddiqui et al. (2018) used 100 µM of SA in the treatment of wheat seeds, and there was an increase in germination and plant height due to the increase of proline and total soluble carbohydrates that suppressed the formation of ROS in the roots. It is observed that this concentration is lower than that used in this study, therefore, depending on the species, the sensitivity and response to SA may be completely different.

Figure 2 shows the seedling lengths for each organ and total. Again, the behavior of both materials was similar, but on the germination paper, the values were lower for the three variables studied (Figures 2A and 2C). In gerbox there was an increase comparing the imbibition methods (Figures 2B and 2D). It is noteworthy that there was a different behavior for Tec Irga 6070 RR in the gerbox, which at the concentration of 5000 µM there was an increase in the values, the same did not occur in the paper and for the cultivar NA 5909 RG.

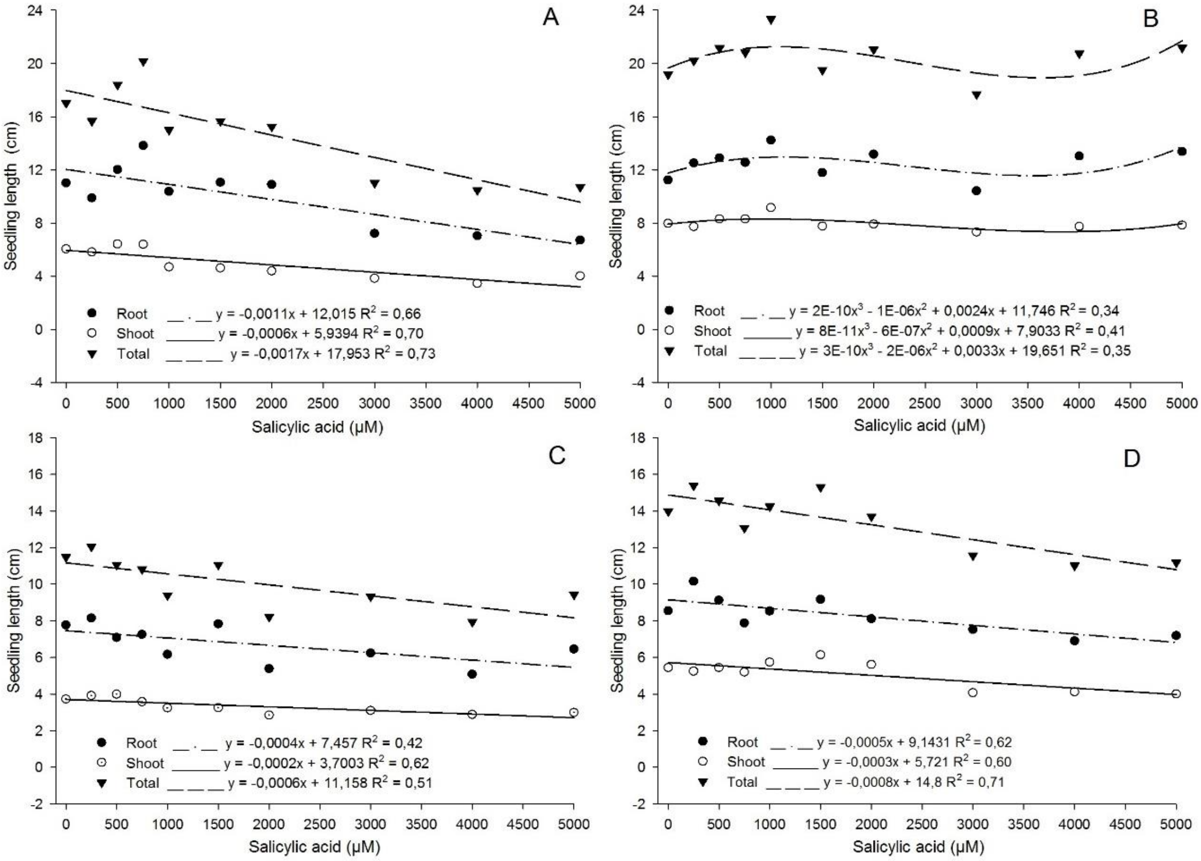

Figure 2. Root, shoot and total length for the cultivars Tec Irga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

The time of contact of the concentrations with the seeds in each method was different, and this factor may have contributed to the difference in SA absorption. Taking this into account, it is inferred that as a defense practice, at the highest concentrations, such as 4000 and 5000 µM, the seedling in an attempt to alleviate the possible stress caused, emitted root and shoot to overcome this moment, however, for NA 5909 RG this behavior was different. High concentrations of SA lead to oxidative stress and an increase in ROS that impair cellular function at low concentrations, such as 10 µM SA, used by Lan et al. (2016) resulted in less accumulation of aluminum in soybean roots and in the activation of antioxidant enzymes, which helps to reduce the stress caused by this element.

The fresh mass values for the two cultivars and imbibition methods are described in Figure 3. Similar to that exposed for the seedling lenght (Figure 2), the fresh mass behavior exhibited a trend of higher weights for the gerbox method and smaller in the paper. In addition, the range of concentrations between 250 and 1000 µM, but mainly at 500 and 750 µM, showed the highest fresh root weight, shoot and total weight for both cultivars. Likewise, in the majority of cases, concentrations above 1000 µM affected the parameters evaluated (Figure 3).

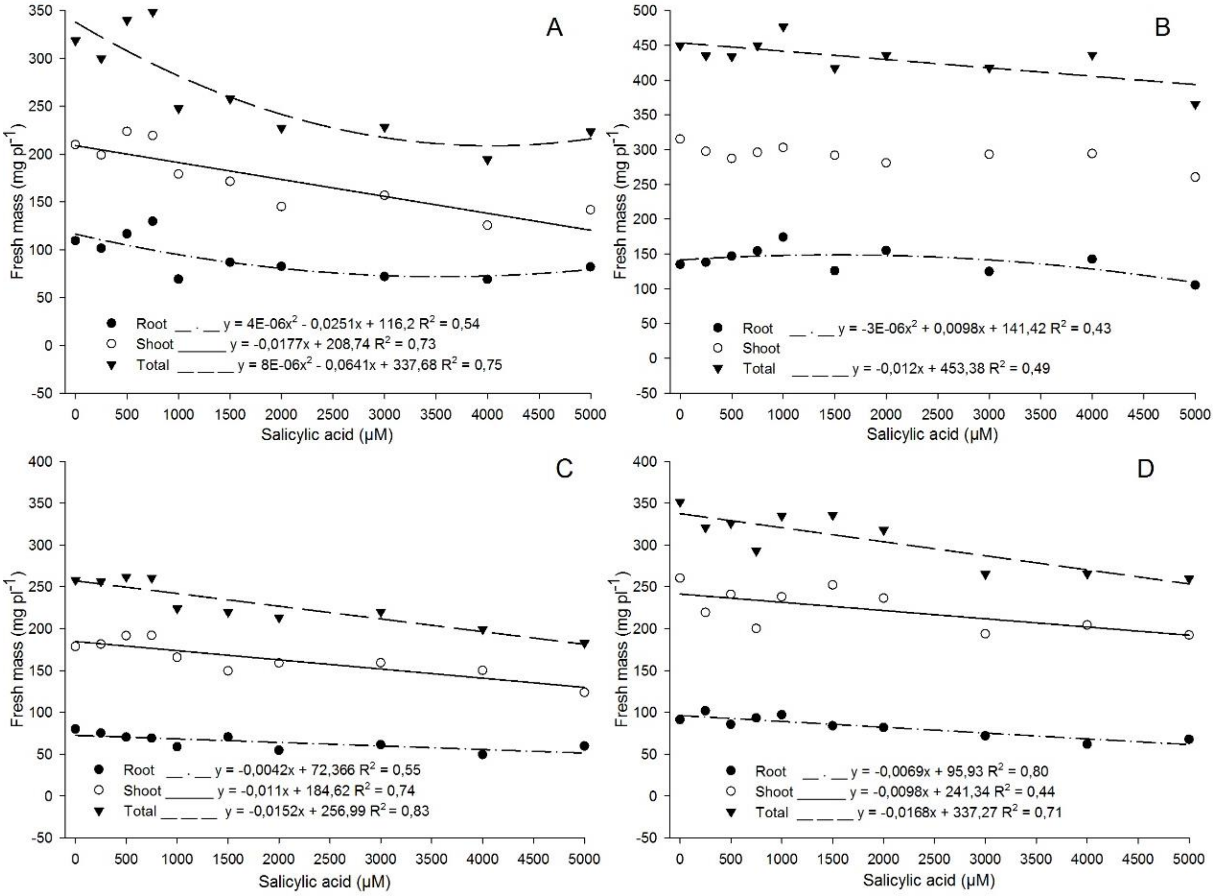

Figure 3. Fresh root, shoot and total mass for the cultivars Tec Irga 6070 RR (A) paper, (B) gerbox, and NA 5909 RG (C) paper and (D) gerbox

These results are similar to those obtained by Coronado, López, and Saavedra (1998), where the foliar application of SA with concentrations higher than 5000 µM in soybean crop increases leaf length and mainly root values. However, this was a field situation, where higher concentrations of SA may be required. In these situations, root growth may be potentiated after treatment with SA, giving the plant greater ability to absorb water and nutrients during times of water stress (Kadioglu et al., 2011).

Figure 4 shows the dry mass values for the two cultivars and imbibition methods. As expected, again the tendency that occurred in the previous variables was noticed for the dry mass, because there was a decrease in the fresh mass and length in concentrations higher than 1000 µM and mainly in 5000 µM.

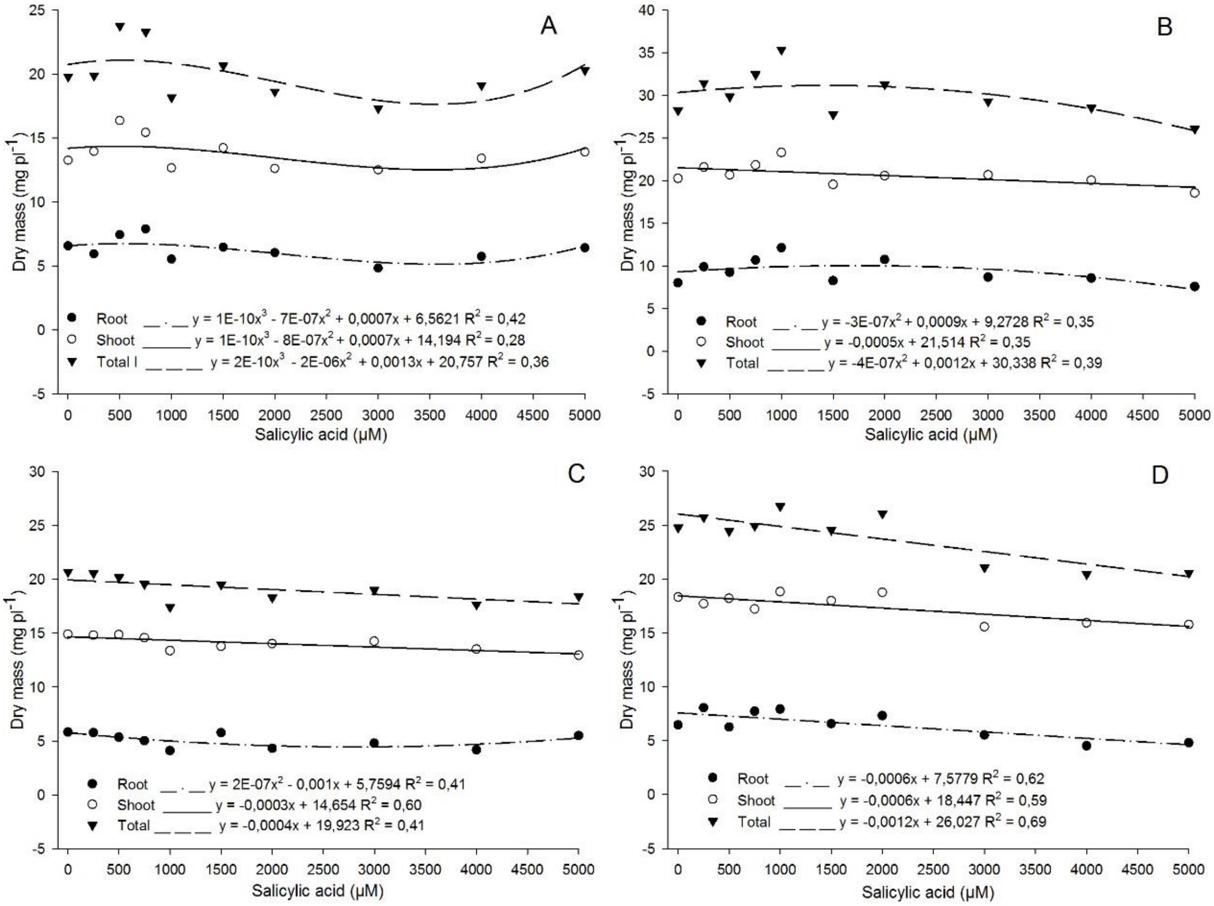

Figure 4. Dry root, shoot and total mass for the cultivars Tec Irga 6070 RR (A) paper, (B) gerbox and NA 5909 RG (C) paper and (D) gerbox

Therefore, for the dry mass in the two methods in both cultivars the result was similar, and the concentrations between 250 and 1000 µM showed the best results, being these values higher than the control group in many cases. Differently, Al Sahil (2016) did not detect a significant increase of fresh, dry mass and seedling length with the use of SA in the concentrations of 0.5 and 1 ppm, only in the association with gibberellin for the percentage of germination under saline stress in seeds of cucumber. Similarly, Mazraei1, Ganjalil, and Rad (2016) did not reach significance for the parameters of thousand seed weight and wheat yield using the concentrations 900, 1800 and 2700 µM.

Lisboa et al. (2017) observed that for different sweet sorghum cultivars, there was drastic reduction of parameters such as germination, germination speed index, number of leaves, length and dry mass of shoot and root in concentrations ranging from 5000 to 20000 µM. This study suggests that it is not necessary to use concentrations above 5000 µM and that the use of SA concentrations in the range between 250 and 1000 µM can be used in soybean aiming less damage in face of different kind of stresses. In addition, only 24 hours of imbibition in the gerbox method has not affected most of variables evaluated. For the paper method, there were also variables with increase in values, however, the values are smaller when compared to the time of 24 hours of imbibition of the seeds in gerbox. These results corroborate with Gupta, Meena and Datta, (2017) which used 500 µM of SA and observed the increase of germination, root length and aerial part, fresh and dry mass of soybean under heavy metal stress.

In this context, positive results were found by Kuchlan, Kuchlan, and Husain (2017), where SA foliar application provided higher productivity, sanity, germination, and vigor of soybean seeds during storage. Similarly, the same methods used by Fernandes et al. (2019) in the bean culture showed that 24 hours of imbibition, as opposed to the germination paper, up to 1000 μM provided positive results for germination and other variables analyzed. In fact, this concentration range proved to be the most adequate in the evaluated parameters, serving as the basis for the work and according to the literature.

Therefore, future work should be carried out in an attempt to elucidate the parameters of different species, including in the soybean crop. Many of these studies show that there is a concrete effect on the antioxidant activity against biotic and abiotic stresses, and extensive studies are already underway to determine role of this plant hormone by molecular technics (Kang, Li, & Guo, 2014).

Thus, it is suggested that concentrations above 1000 μM impair the evaluated parameters, and the range between 250 and 750 μM can be used in future studies with soybean seeds to evaluate the behavior under stress. In addition, the gerbox method for 24 hours provided the best results without drastic decrease of the parameters in lower concentrations of SA.

4. Conclusions

Concentrations of SA between 250 and 750 μM can be used in soybean seeds, however, above 1000 μM, the use of this compound may impair physiological quality parameters.

The gerbox method for 24 hours provided the best results without drastic reduction of the parameters in the lowest concentrations of SA.

The behavior in relation to the treatments used was similar for the two cultivars analyzed.

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4. CAPÍTULO II – Artigo Científico

Este capítulo originou um artigo científico aceito para publicação na Journal of Agricultural Science

Salicylic Acid and Its Effect on Physiological and Photosynthetic Parameters in Soybean Seedlings Under Water Deficit

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Abstract

The objective of this study was to evaluate the physiological parameters and photosynthetic by the stomatal conductance of soybean plants under water deficit after imbibition in different concentrations of salicylic acid during germination. The initial seed quality of the cultivar Bayer®/Tec Irga 6070 RR was evaluated. The soybean seeds were soaked in 25 ml in the salicylic acid solution (SA) for 24 hours and the retention capacities of 30, 50 and 70% were adopted. Under controlled conditions, concentrations of zero, 250, 500, 750 and 1000 µM, evaluating the variables length, fresh and dry mass of root and shoot. In the greenhouse, the concentrations of SA of zero, 500 and 1000 µM were used. After 29 days of the seedling, the stomatal conductance, length, fresh and dry mass of root and shoot were evaluated. The results showed that the uptake of SA in the germination aided seedlings under water deficit. The retention capacity of 30% simulated the water deficit, damaging the physiological parameters of soybean seedlings in both environments. The concentrations of 500 and 1000 µM of SA were efficient when there was stress due to water deficit in the retention capacity of 30% for the variables root length, and length, fresh and dry shoot mass.

Keywords: conductance, stomata, enzyme, drought

1. Introduction

Soybean production has global economic importance in agribusiness, and, in order to guarantee safety and production increase, it is necessary to use different techniques and management practices. In addition to these aspects, the beginning of a crop will have its uniformity determined by the use of seeds with high physiological potential, among other aspects (Marcos Filho, 2015b). However, biotic and abiotic stresses may decrease productivity throughout the growing season (Danquah et al., 2014; Jaleel et al., 2009; Morando et al., 2014).

Among the most common stresses, water deficit is considered one of the major causes of crop losses, especially in critical stages of development, such as germination and seedling establishment (El Sabagh et al., 2015). This stress promotes morphological, physiological and biochemical changes resulting in oxidative stress and formation of reactive oxygen species (ROS) (El Sabagh et al., 2019).

In order to aid in this process, the plant hormone salicylic acid (SA) plays an essential role in relation to biotic and abiotic stresses, an aspect explored by different researches. Still, SA is responsible for important biochemical and physiological processes, such as growth and development, stomatal closure and nutrient absorption (Hayat, 2010; Janda et al., 2014; Miura & Tada, 2014; Vazirimehr & Rigi, 2014; Parmoon et al., 2017).

The seed has become a vehicle where products and technologies are applied in order to increase soybean yield. The application of SA has already been used with promising results, however, it is evident that there are gaps to be filled, since the results are variable, according to the species, cultivar, and type of stress observed (Fahad et al., 2017). Therefore, the objective of this study was to evaluate the photosynthetic and physiological parameters of soybean plants under water deficit after imbibition of different doses of salicylic acid during the germination of seeds.

2. Material and Methods

The work was conducted at the Didactic and Seeds Research Laboratory (LDPS) of the Plant Science Department of the Federal University of Santa Maria, Santa Maria, RS. Commercial soybean seeds of cultivar Bayer®/Tec Irga 6070 RR produced in the 2015/2016 crop was used, which is recommended for cultivation in the state of Rio Grande do Sul.

Initial evaluation of seeds: samples from Tec Irga 6070 RR cultivar were evaluated for physical and physiological characteristics by performing the following tests: thousand seed weight, moisture content, first count of germination, germination test, root and shoot length, dry mass of seedlings, mass electrical conductivity and emergence of seedlings on field (Nakagawa, 1999; Brasil, 2009).

Evaluation of physiological parameters in soybean seedlings under water deficit on controlled conditions: the seeds were submitted to soaking in gerbox boxes under three germination papers moistened with 25 mL of salicylic acid solution (SA) (Sigma-Aldrich®). The concentrations of SA on the solutions were: zero, 250, 500, 750 and 1000 μM. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 ºC. After this time of imbibition, the seed samples were transferred to plastic cups containing 350 g of sand, where sand retention capacities were determined (30, 50 and 70%) to simulate the water stress, according to Brasil (2009).

Four replicates of 3 seeds were sown in each glass, which was kept in BOD, under continuous light, and at 25 ºC, and the amount of water required to maintain their retention capacity was supplied daily, according to Brasil (2009). On the eighth day after sowing the seedlings were removed from the cups with sand and washed for the evaluation of length, fresh and dry mass of root and shoot, without the cotyledons (Nakagawa, 1999).

Evaluation of physiological and photosynthetic parameters in soybean seedlings under water deficit in greenhouse: the seeds were submitted to imbibition in gerbox-type boxes under three germination paper moistened with 25 mL of salicylic acid solution (AS) (Sigma-Aldrich®). The concentrations of SA on the solutions were: zero, 500 and 1000 μM. The seeds remained soaked for 24 hours in BOD under constant light and temperature of 25 ºC. After this time of imbibition, the seeds were transferred to vessels containing 1800 g of substrate, where sand retention capacities were determined (30, 50 and 70%) to simulate the water deficit, according to Brasil (2009).

A series of six replicates of three seeds were carried out in each pot, being these in a greenhouse and random distributed, in order to avoid variations caused by temperature and sun position. Daily, water was supplied in order to maintain the retention capacity, according to Brasil (2009).

After the period of 29 days when the plants were in the phenological stage V2-3 (2 or 3 leaves-varying according to the treatment) an evaluation of the stomatal conductance of water vapors (Gs-mol H_2O m^{-2} s^{-1}) in the developed leaves of two plants (Portable System of Photosynthesis LCi-SD (Fehr & Caviness, 1977). After this evaluation, the plants were removed from the substrate and washed for evaluation the length, fresh and dry mass of the root and shoot (Nakagawa, 1999).

Statistical analysis: the sand experiment was conducted in a completely randomized design (RD), with four replications per treatment, each replicate being composed of three plants. In the greenhouse experiment also RD used, being composed of six replicates per treatment, each replicate being composed of three plants. Initially, the assumptions of the mathematical model were verified by the software Assistat (Silva & Azevedo, 2009). In case of non-compliance with the normality of the errors and homogeneity of the variances, the data were transformed by the methodology $\sqrt{x}$. For the two experiments the data were submitted to variance analysis by the F test ($p < 0.05$) and comparison of means ($p < 0.05$) through the software Sisvar® (Ferreira, 2011).

3. Results and Discussion

The physical and physiological characterization of the Bayer®/Tec Irga 6070 RR soybean seeds cultivar is presented in Table 1. It can be observed that there is adequate humidity and the germination rate was above 80%. The data of the initial characterization was not submitted to statistical analysis, once the objective of this evaluation was only to characterize the physiological quality of the cultivar (Marcos Filho, 2015a).

Table 1. Thousand seed weight (TSW), moisture (M), first count (FC), germination (G), radicle length (RL), shoot length (SL), radicle dry mass (RDM), shoot dry mass (SDM), electrical conductivity (EC), field emergence (FE) and emergence speed index (ESI) of Tec Irga 6070 RR

TSW	Moisture	FC	G	RL	SL	RDM	SDM	EC	FE	ESI
g	-------------- % -------------			------ cm -----		-------- mg --------		ìS cm^{-1} g^{-1}	%	
137	12.97	83	92	7.6	14.9	87	198	55.8	97	14.3

Table 2 presents the results concerning the variables of length, fresh and dry mass of root and dry mass of shoot in the experiment under controlled conditions. There was no interaction between the factors, therefore, they were analyzed separately. In the retention capacity of 70%, which simulated water excess, the variables length and root dry mass decreased considerably. In the same way, Wijewardana, Reddya, and Bellaloui (2019) verified that some physiological parameters, chemical and quality factors were affected by water excess.

Table 2. Mean root length (RL) (cm), fresh root mass (FRM), root dry mass (MDR) (g) and dry shoot mass (DSM) (g) as a function of the water retention capacity (%) and salicylic acid (SA) concentrations (µM) under controlled conditions

Retention Capacity	RL	FRM	MDR	DMS
30	8.03 a	0.33 ^{ns}	0.055 a	0.036 ^{ns}
50	8.36 a	0.37	0.051 a	0.050
70	6.39 b	0.35	0.019 b	0.036
Salicylic Acid	RL	FRM	MDR	DMS
0	6.78 b	0.27 b	0.025 b	0.026 ^{ns}
250	7.13 b	0.30 b	0.037 a	0.030
500	8.28 a	0.32 b	0.046 a	0.060
750	8.24 a	0.41 a	0.055 a	0.037
1000	8.27 a	0.43 a	0.046 a	0.045
CV (%)	10.94	10.76	22.74	32.03

Note. * Means followed by the same letter do not statistically differ between each other by the Scott-Knott test (P > 0.05).

In environments where there is moisture restriction or excess, the root is one of the first organs to signal its occurrence, after all, the roots provide nutrition and water for all processes. Other structures, as well as the aerial part and leaves are structures responsible for managing evapotranspiration, regulating the balance between soil, plant and atmosphere (Taiz & Zieger, 2004; Silva et al., 2019).

As for SA, it is noticed that between the concentrations of zero up to 1000 µM there was a tendency to increase the values of the variables evaluated in higher concentrations of this plant hormone. These differences were evident in the root variables, with the lowest values being in the control that did not receive SA. Thus, it is seen that the compound does not harm the plant, but rather assists in the processes, as reported by Al-Hakimi (2006), and Kabiri, Farahbakhsh, and Nasibi (2012), who obtained positive results using SA at concentrations of 600 µM and between 500 and 1000 µM, respectively.

Table 3 shows the results of the interaction between SA and retention capacity for the variables length and fresh mass of shoot. These results demonstrate that under controlled conditions the efficiency of SA in the different treatments was not clearly evident. For most of the evaluated variables, the different retention capacities, as well as the concentrations of SA, did not present statistically relevant results, since different studies demonstrate that the SA aided in stress conditions. As reported by Mahmood, Sajid, and Khilji (2018) in seeds and maize plants under salt stress, they obtained the best results in the concentration of 500 µM AS.

Table 3. Average shoot length (SL) (cm) and fresh shoot mass (FSM) (g) as a function of water retention capacity (%) and salicylic acid concentrations (µM) under controlled conditions

Salicylic Acid	Retention Capacity					
	SL			FSM		
	30	50	70	30	50	70
0	12.29 ^{ns} B	13.54 B	12.67 B	0.32 $^{ns\ ns}$	0.28 $^{ns\ ns}$	0.21 A
250	12.29 ^{ns} B	11.77 B	15.36 A	0.32 ^{ns}	0.32	0.22 A
500	14.25 ^{ns} B	16.35 A	12.03 B	0.30 a	0.34 a	0.06 bB
750	17.83 ^{ns} A	14.57 A	16.21 A	0.35 a	0.35 a	0.24 aA
1000	14.97 ^{ns} B	17.16 A	18.10 A	0.31 b	0.43 a	0.22 bA
CV (%)	8.71			13.95		

Note. * Means followed by the same uppercase letter in the column and lowercase case letter on the line do not statistically differ between each other by the Scott-Knott test (P < 0.05).

Table 4 represents the results found for the variables fresh and root dry mass and stomatal conductance in the greenhouse experiment. As there was no interaction for these variables, the factors were analyzed separately. The retention capacity of 50%, not interfered negatively in the values, which suggests that this would be the closest percentage to the field capacity conditions.

Table 4. Mean fresh root mass (FRM) (g), root dry mass (RDM) (g) and stomatal conductance (SC) as a function of water retention capacity (%) and salicylic acid concentrations (μM) at greenhouse

Retention Capacity	FRM	RDM	SC
30	2.50 b	0.19 b	0.06 b
50	3.28 a	0.26 a	0.08 a
70	1.24 b	0.10 c	0.06 b
Salicylic Acid	FRM	RDM	SC
0	2.03 ^{ns}	0.15 b	0.07 a
500	2.33	0.18 b	0.05 b
1000	2.66	0.22 a	0.08 a
CV (%)	28.57	26.56	28.62

Note. * Means followed by the same letter do not statistically differ between each other by the Scott-Knott test ($P > 0.05$).

When 1000 μM of SA was used, there was an increase in root dry mass and an increase in stomatal conductance (Gs), indicating its positive effect on these variables. According to Miura and Tada (2014), SA is important in the process of stomatal closure, which supports the results obtained.

This assessment may be influenced by many factors such as the solar radiation of the moment, time, or even leaf size. These factors may still be related to the initial vegetative stage of plants with young leaves. However, specific studies of the involvement of SA in these physiological and photosynthetic processes have shown that its effect is beneficial and may be important signaling of stress along with antioxidant enzymes and other compounds on soybean (Ardebili, Iranbakhsh, & Ardebili, 2019).

When the variables length, fresh and dry mass of shoot and root length were evaluated, it was observed that there was a decrease in the values in all treatments in the control without SA in the retention capacity of 30 and 70%, which were detrimental to the development of the seedlings (Tables 4 and 5). Still, it is noted that the concentrations of 500 and especially 1000 μM provided positive results when the plants were under stress. Similar results were found in the study conducted by Arivalagan and Somasundaram (2015) in sorghum, in which plants under water deficit had variables such as length, fresh and dry mass of root and shoot, and photosynthetic pigments improved with the application of 1000 μM SA.

Table 5. Average shoot length (SL) (cm), fresh shoot mass (FSM) (g), dry shoot mass (DSM) (g) and root length (RL) as a function of the retention capacity of water (%) and concentrations of salicylic acid (SA) (μM) in greenhouse

SA	Retention Capacity											
	SL			FSM			DSM			RL		
	30	50	70	30	50	70	30	50	70	30	50	70
0	23.79 bB	49.29 a ^{ns}	21.87b^{ns}	2.79 bB	10.32a^{ns}	1.67 b ^{ns}	0.65 bB	1.74 a ^{ns}	0.28 b ^{ns}	9.57 bB	29.19 a ^{ns}	7.60 b ^{ns}
500	28.76 bB	44.69 a	27.20 b	3.79 bB	7.76 a	2.34 b	0.44 bB	1.32 a	0.40 b	17.39bA	29.49 a	10.39 c
1000	45.55 aA	43.47 a	27.61 b	8.69 aA	7.04 a	2.86 b	1.46 aA	1.22 a	0.50 b	19.67bA	26.47 a	11.85 c
CV (%)	14.66			24.45			27.19			14.26		

Note. * Means followed by the same uppercase letter in the column and lowercase case letter on the line do not statistically differ between each other by the Scott-Knott test ($P < 0.05$).

This result is extremely important and clearly shows the positive effect of SA use on the occurrence of water deficit (La et al., 2019). It should be noted that the use of SA in this study was restricted to the process of imbibing the seeds for 24 hours and after it was performed the simulation of water deficit in the retention capacity of 30% of water. Similarly, results of Sharafizad et al. (2013), and Habibi and Abdoli (2013) corroborate the use of the same SA absorption method, with concentrations below 2000 μM demonstrating efficient results.

The compound was not added during the conduction of the assay, either in solution or foliar. Although, other studies evaluate the application of these compounds by supplying solutions with water or foliar application in times of stress (Damalas, 2019; Kareem, Rihan, & Fuller, 2019; Osama et al., 2019).

The use of SA in the seed as a carrier may be an easy and effective way of absorbing the compound in the early stage of seedling growth. The most common means for this is seed treatment, similar to that proposed in the study. Other studies demonstrate similarly, as described by Azeem et al. (2019) in wheat under salt stress, that the concentration of 1000 μM of SA was more efficient than 500 μM in the soaking of the seeds during 12 hours in several evaluated parameters. Still, the different ways to supply this plant hormone can be subject to further studies, which would demonstrate more interesting results that can have practical applications on the crops improving its productivity.

4. Conclusions

The results obtained in this study suggest that the use of SA in soaking soybean seeds is an efficient method to support seedlings under water deficit.

The retention capacity of 30% simulated the water deficit situation, damaging the physiological parameters of the soybean seedlings in the controlled environment and in the greenhouse.

The concentrations of 500 and 1000 μM of SA were efficient when there was stress due to water deficit in the retention capacity of 30% for the variables root length, and length, fresh and dry shoot mass.

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5. CONSIDERAÇÕES FINAIS

Através dos resultados obtidos, observou-se que o ácido salicílico interfere na germinação de sementes de soja de forma positiva, conforme hipóteses do estudo. Das concentrações avaliadas, as superiores a 1000 µM prejudicam os parâmetros da qualidade fisiológica, enquanto concentrações menores, entre 250 e 750 µM no máximo até 1000 µM, mostraram ser o intervalo para utilização. Além disso, o método de embebição da solução de ácido salicílico durante o período de 24 horas na germinação demonstrou não afetar ou diminuir os parâmetros fisiológicos no comparativo do método em papel de germinação durante todo o período. Esse resultado ofereceu um ganho de sete dias em relação ao papel de germinação, ou seja, 24 horas de embebição já foi suficiente para absorção do composto, ao invés de utilizar durante períodos superiores oferecendo riscos aos parâmetros fisiológicos.

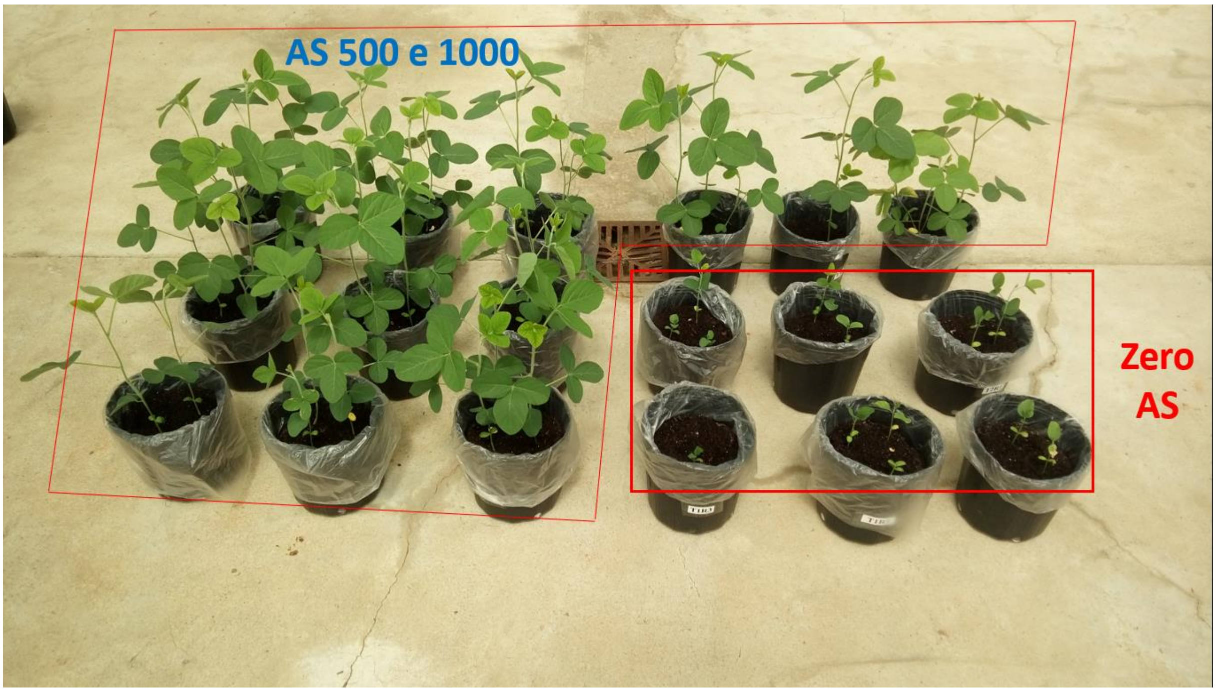
Outro ponto importante foi que para as duas cultivares de soja utilizadas, o comportamento em relação às concentrações de AS e método foi semelhante. Assim, esses dados foram importantes para dar sustento as próximas etapas no segundo capítulo, na tentativa de aproximar-se ao que poderia ocorrer no campo ou simular o estresse por déficit hídrico, frente ao efeito atenuador do AS. Logo, os dois experimentos, um em condições controladas no intervalo de concentrações de AS de 250 a 1000 µM, e o outro em casa de vegetação nas concentrações de 500 e 1000 µM, ambos com diferentes capacidades de retenção para simular o déficit, poderiam revelar a capacidade do AS nessas situações. Também foi utilizado apenas um genótipo, visto que anteriormente não houve diferenças marcantes.

Diante disso, os resultados encontrados no segundo capítulo foram promissores, principalmente na situação da casa de vegetação. Notou-se que a capacidade de retenção de 30% simulou o déficit hídrico por afetar diversos parâmetros fisiológicos nas duas condições de teste. No entanto, quando se utilizou a concentração de 500 e 1000 µM a maioria dos parâmetros fisiológicos como comprimento das raízes, massa fresca e seca da parte aérea demonstraram os maiores valores.

Portanto, o uso de AS na germinação de sementes mostrou-se eficiente no alívio do estresse causado pelo déficit hídrico nas condições realizadas do estudo. No entanto, trabalhos mais aprofundados e em condições de campo poderão oferecer maior segurança, e confirmar as propriedades do composto, desde a semente, como um veículo no processo germinativo ou no tratamento de sementes, sendo esta a principal forma prática do produtor utilizá-lo.

ANEXOS

ANEXO A- Foto que mostra a diferença entre concentrações de AS na capacidade de retenção de 30% e concentrações de AS de zero, 500 e 1000 µM.



ANEXO B- Foto do tratamento na capacidade de retenção de 50% e concentrações de AS de zero, 500 e 1000 µM.

ANEXO C- Foto do tratamento na capacidade de retenção de 70% e concentrações de AS de zero, 500 e 1000 µM.