JEAN MARCEL SOUSA LIRA

HIDROCONDICIONAMENTO E SUAS RELAÇÕES COM O DESENVOLVIMENTO INICIAL DE FEIJOEIRO CV. BRSMG MEDREPÉROLA

LAVRAS-MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fisiologia Vegetal, área de concentração em Crescimento e Desenvolvimento de Plantas, para obtenção do título de Doutor.

Orientador

Dr. Amauri Alves de Alvarenga

Coorientador

Dr. Renato Mendes Guimarães

LAVRAS-MG

2015

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo (a) próprio(a) autor(a).

Lira, Jean Marcel Sousa.
Hidrocondicionamento e suas relações com o desenvolvimento inicial de feijoeiro cv. BRS Madrepérola. / Jean Marcel Sousa Lira. – Lavras : UFLA, 2015.
74 p.

Tese(doutorado)–Universidade Federal de Lavras, 2015.
Orientador(a): Amauri Alves de Alvarenga.
Bibliografia.

1. Hydrotime. 2. \"Memória\" do estresse. 3. Taxa média de germinação. I. Universidade Federal de Lavras. II. Título.

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________________________________em 19 de fevereiro de 2015

Dr. Amauri Alves de Alvarenga..UFLA

Dr.Marcelo Murad Magalhães..EMBRAPA

Dra. Fernanda Carlota Nery..UFSJ

Dr. João Paulo Rodrigues Alves Delfino Barbosa...........................UFLA

Dra Milene Alves de Figueiredo Carvalho.........................EMBRAPA

Orientador
Dr. Amauri Alves de Alvarenga

LAVRAS-MG
2015

AGRADECIMENTOS

Ao ser inominável criador das leis que regem o nosso universo.

Aos meus pais que me ensinaram o respeito ao próximo e a honestidade nas palavras.

A minha irmã, que com sua alegria e amor faz do impossível possível.

Aos meus tios e tias que fazem parte da minha criação e convicções.

Aos meus primos.

Ao meu avô e minhas avós exemplos de caráter e elo entre gerações distintas.

Aos amigos de Aracaju, amigos de infância, amigos para o resto da vida.

Aos amigos da UFS, grupo restauração e Prof. Dr. Robério Anastácio Ferreira, pela primeira orientação e despertar científico.

Ao professor Dr. Amauri Alves de Alvarenga, pela continuidade na minha orientação e na minha formação quanto cientista e ser humano.

Ao Prof. Luiz Edson pelas disciplinas a mim ministradas.

Ao Tanhan, pelo apoio e amizade incondicionais.

À Lena, Barrinha, Manu, Joel, Evaristo e Odorêncio pelo apoio e amizade.

Ao Cláudio e Andréa por terem me recebido em seu lar de coração aberto.

Aos amigos da Fisiologia Vegetal.

Ao Prof. Dr. Renato Mendes Guimarães pela coorientação e conversas produtivas.

Ao Pesquisador Dr. Marcelo Murad pela amizade e competência.

Ao Prof. João Paulo pelo apoio e conhecimento transmitido.

Aos amigos de todas as horas e companheiros de experimento Pedrinho, Gustavo e Túlio.

As amigas Amanda, Kamila e Carla.

A minha amiga, companheira, noiva e mulher Marinês, que divide comigo esta conquista e toda a carga que carregamos juntos ao longo destes cinco anos.

À Universidade Federal de Lavras (UFLA) e ao Programa de Fisiologia Vegetal pela oportunidade.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa concedida.

RESUMO

A BRSMG Madrepérola é uma cultivar de feijão desenvolvida pela UFLA com vários atributos de produtividade, tolerância a patógenos e apelo comercial. No entanto, sob a seca esta cultivar reduz a produtividade. O hidro condicionamento é uma técnica simples e econômica utilizada como alternativa ao combate à seca. Esta técnica pode aumentar o vigor das sementes, mas os efeitos ao longo do desenvolvimento são pouco estudado. Desta forma foi realizado experimento com o objetivo de avaliar os benefícios do hidro condicionamento com diferentes subsequentes secagens (rápida e lenta) na germinação e no estabelecimento das plântulas de BRSMG Madrepérola sob condições de seca através do modelo "hydrotime", para avaliação das sementes, e mecanismos bioquímicos de tolerância à seca para avaliação das plântulas com intuito de observar possível efeito de "memória" do estresse. Contudo, através do modelo de hidrotime foi observado que o hidro condicionamento mais secagens reduziram a porcentagem e aumentaram o tempo de germinação em potenciais hídricos mais negativos. Além disso, o hidro condicionamento aumentou a energia de germinação das sementes e reduziu o conteúdo de prolina livre. Todavia, nas plântulas de BRSMG Madrepérola o hidro condicionamento mais secagem rápida e lenta aumentaram a velocidade e a intensidade de acúmulo de osmóticos e atividade de enzimas do sistema antioxidante nas raízes e folhas. Portanto, o uso do hidro condicionamento não melhora a germinação sob seca, enquanto melhora os mecanismos de tolerância à seca nas plântulas.

Palavras-chave: Hydrotime. "Memória" do estresse. Taxa Média de Germinação

ABSTRACT

The BRSMG Madrepérola is a common bean cultivar which was development by UFLA. This cultivar has many attributes to productivity, disses tolerance and commercial. Hydropriming is a simple and sheep technic used to improved drought tolerance. It can increased seeds vigor, but little is known about the effects on plant growth. Indeed, experiment was realized to measure hydropriming with re-drying (fast and slow) benefits on germination and seedlings establishment of BRSMG Madrepérola under drought. Hydrotime model was used to measure hydropriming effect on seeds and biochemistry process of drought tolerance were used to measure hydropriming effect on seedlings and stress "memory" effect. Hydrotime showed which hydropriming with re-drying reduced germinability and increased time-course of germination under more negatives water potentials. Furthermore, hydropriming increased germination energy of seeds and reduced free proline content. In BRSMG Madrepérola seedlings hydropriming with fast and slow re-drying increased speed and intensity of osmolity accumulation. The hydropriming also increased enzymes activity of antioxidant system in roots and leaves. Thus, hydropriming use not improved germination under drought while in seedlings it promote improve on drought tolerance mechanisms.

Keywords: Hydrotime. Stress "memory". Mean Germination Rate

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

A seca é um dos grandes desafios da agricultura. Estima-se que acima de um terço da produção é perdida devido à escassez de água. Ao longo dos anos diversos pesquisadores buscam plantas tolerantes à seca com manutenção da produtividade.

Diversas pesquisas de melhoramento genético e transgenia tentam obter através de seleção e/ou inserção dos genes de tolerância à seca (ASFAW, et al., 2012). A "memória" do vegetal é um conceito emergente vinculado ao pensamento atual, bastante controverso, de "inteligência vegetal" (BRENNER et al., 2006). Esta memória estaria ligada a capacidade do vegetal em "aprender" e "memorizar" mecanismos de tolerância ao estresse (GARZON, 2007).

Geralmente a aplicação de algum tratamento/estresse funcionaria como "treinamento" condicionando o vegetal a tolerar estresses subsequentes ou futuros. A aplicação de um estresse na tentativa de aumentar a tolerância a outro estresse mais severo é conhecida como tolerância cruzada (WALTER et al., 2013).

Os fatores que afetam a memória e/ou tolerância cruzada são: estádio de desenvolvimento do vegetal que é aplicado o estresse (semente, plântula, juvenil e adulto); duração do estresse (minutos, dias, semanas, meses, anos, etc...); intensidade do estresse (fraco, moderado ou forte) e tipo de estresse (hídrico, térmico, físico, etc...). As sementes são organismos vegetais propícios para aplicação de técnicas de memória e/ou tolerância cruzada por ser o estádio inicial do biociclo da planta.

Nas sementes são utilizados tratamentos para aumentar vigor e melhorar parâmetros de germinação submetida ao estresse abiótico (temperaturas baixas e elevadas, deficiência hídrica e salinidade) (RAHIMI, 2013). Estes tratamentos são conhecidos por condicionamento e podem ser realizados de diversas formas.

O condicionamento pode ser hídrico, químico, hormonal, biológico, sólido, etc. (JISHA; VIJAYAKUMARI; PUTHUR, 2013). Dentre estes, o hidro condicionamento consiste da interrupção da embebição na fase II da germinação sendo bastante utilizado devido à simplicidade, economia e segurança (HAMIDI; PIRASTEH-ANOSHEH; IZADI, 2013).

Os efeitos a curto e longo prazo do condicionamento foram chamados de "memória do condicionamento" por Chen e Arora (2012). Estaria relacionado ao condicionamento de sementes o aumento da síntese de proteínas protetoras de membranas (LEAs e HSPs) e aumento da atividade do sistema antioxidante. Tais efeitos melhorariam a tolerância das sementes ao estresse abiótico. Assim, hidro condicionamento funcionaria como tolerância cruzada, onde a pré-hidratação da semente iniciando a germinação leva ao estresse oxidativo, consequentemente, maior atividade do sistema antioxidante enquanto a secagem para armazenamento aumentaria a síntese de moléculas protetoras (CHEN; ARORA, 2012).

O hidro condicionamento pode ajudar a melhorar a germinação em ambientes com baixa disponibilidade hídrica através do aumento na capacidade de realizar ajuste osmótico (KAUR; GUPTA; KAUR, 2002).

O ajuste osmótico é um importante mecanismo nas células de plantas para tolerância ao estresse hídrico. No entanto, em sementes este mecanismo é atribuído apenas à mobilização de reservas durante fase II da germinação. No entanto foram observado acúmulo de açucares, aminoácidos, prolina, malato, citrato e íons (K+, NO3-, Na+ and Cl-) nas células da semente aumentando a absorção de água e facilitando a germinação (HANDA et al., 1983).

Ajuste osmótico em sementes é um mecanismo ainda pouco estudado. Desta forma, o uso de modelos matemáticos que possam simular potenciais hídricos das sementes seria útil.

O modelo "hydrotime" estima o potencial hídrico através do calculo do potencial hídrico basal ou limite que impede uma fração de sementes germinarem num determinado tempo (WINDAUER; ALTUNA; BENECH-ARNOLD, 2007) podendo ser utilizado na avaliação de ajuste osmótico de sementes. Através do "hydrotime" estimaríamos o efeito do hidro condicionamento no ajuste osmótico das sementes e aumento da germinação em condições de baixo potencial hídrico.

Dentre as culturas que podem ser beneficiadas com o hidro condicionamento na germinação e estabelecimento de plântulas submetidas à seca o feijoeiro comum (*Phaseolus vulgaris* L) por ser plantado em regiões de baixa disponibilidade hídrica.

O feijoeiro é uma planta que sofre os efeitos da seca através da redução da fotossíntese, redução do enchimento de grãos, diminuição no número de vagens, queda do crescimento vegetativo e produtividade (GOMES et al., 2000). Além disso, sementes de feijão submetidas ao estresse hídrico podem ser induzidas a dormência. Obter cultivares de feijoeiro mais tolerante a seca é objetivo das pesquisas. A BRSMG Madrepérola é uma cultivar de feijão do tipo carioca, com grãos de escurecimento tardio, originado do cruzamento entre as cultivares AN 512666-0 e AN 730031 (EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA – EMBRAPA, 2011). Esta cultivar produzida pela UFLA em parceria reduz a produtividade submetida à seca.

O trabalho foi realizado com objetivo de avaliar o efeito do hidro condicionamento na germinação de sementes de feijão cv. BRSMG Madrepérola em diferentes potenciais hídricos através do modelo "hydrotime", além de avaliar o efeito do hidro condicionamento no estabelecimento das plântulas do feijoeiro submetidas à seca através dos mecanismos de tolerância possivelmente ligados a “memória” do condicionamento.

2 REFERENCIAL TEÓRICO

2.1 Estresse hídrico na cultura do feijoeiro

A principal restrição para a produção em vários países é a seca. Os efeitos do estresse hídrico são complexos, com respostas variadas em função da interação dos fatores. Ambos, qualidade e produção do feijão são afetados negativamente por curtos períodos de seca (RAMIREZ-VALLEJO; KELLY, 1998).

A seca é um dos principais estresses abióticos que afetam a produção de feijão. Estima-se que períodos de seca resultem na redução de 45% da produtividade do feijoeiro no Brasil (COMPANHIA NACIONAL DE ABASTECIMENTO-CONAB, 2013).

No Brasil, a região Nordeste possui a maior área plantada de feijão com 410,4 mil hectares, mas tem a menor produtividade. Produtividade do feijão no nordeste foi (564 kg hectare^{-1}) menor que norte (853 kg hectare^{-1}), centro-oeste (2.544 kg hectare^{-1}), sul (1.033 kg hectare^{-1}) e sudeste (2.512 kg hectare^{-1}) (CONAB, 2013).

A menor produtividade na região nordeste é devido ao clima semi-árido caracterizado por grande variação temporal e espacial das chuvas e altas temperaturas durante todo ano (AZEVEDO; SILVA; RODRIGUES, 1998).

As áreas produtoras de feijão são frequentemente sujeitas à secas intermitentes, que ocorrem quando o feijoeiro é semeado em ambientes secos e completa todo seu ciclo de crescimento em estresse hídrico. Enquanto à seca terminal afeta o florescimento e o estádio de preenchimento de grão (ROSALES et al., 2012).

Na seca intermitente a semeadura é limitada devido à baixa disponibilidade hídrica do meio, consequentemente, as sementes de feijão têm reduzida germinabilidade, velocidade da germinação, estabelecimento das plântulas, comprimento da raiz, razão raiz/parte aérea e massa seca total (DE MORAES; MENEZES; PASQUALLI, 2005; CUSTODIO; SALOMAO; NETO, 2009). Além disso, as sementes submetidas a potenciais hídricos mais negativos aumentam o tempo de germinação tornando-se dormentes.

A manutenção da seca durante o estádio vegetativo ocasiona diminuição no acúmulo da biomassa da parte aérea e redução no número de fitômeros (ROSALES-SERNA et al., 2004). Sob estresse hídrico a planta de feijão reduz o potencial hídrico nos tecidos levando a queda dos processos de condutância estomática, transpiração e taxa fotossintética líquida (SILVA et al., 2010). Com a redução dos processos ocorre perda significativa de produtividade.

Em situação de estresse hídrico é observado no feijoeiro reduções no índice de colheita (razão da produção de sementes pela produção de biomassa), número de sementes e vagens, produção de sementes, peso e qualidade das sementes (FRAHM et al., 2004; PADILLA-RAMIREZ et al., 2005; RAMIREZ-VALLEJO; KELLY, 1998; TERAN; SINGH 2002).

Ao longo das décadas o feijoeiro vem passando por diferentes estratégias de melhoramento para conferir tolerância à seca terminal e intermitente. Com isso, as principais características a serem obtidas em plantas tolerantes incluem redução na taxa de transpiração, ajuste osmótico celular, melhor controle estomático, maior eficiência do uso da água e sistema radicular mais desenvolvido (MUNOZ-PEREA, et al., 2007).

Dentre as características pretendidas o ajuste osmótico é o mais controverso e promissor, pois pode agir em todo biociclo do feijoeiro. Desta forma, o mecanismo de acúmulo de osmólitos a nível celular se torna importante para feijoeiros mais tolerantes à seca.

2.2 Ajustamento osmótico

O mecanismo de ajustamento osmótico consiste da manutenção do turgor celular pela síntese de osmólitos que reduzem o potencial osmótico e aumentam a absorção de água pela célula. Osmólitos incluem moléculas de baixo peso molecular, compostos altamente solúveis tais como açúcares e aminoácidos (PINTO-MARIJUAN; MUNNE-BOSCH, 2013).

Os compostos envolvidos no ajuste osmótico diferem entre espécies, no entanto, tipos de moléculas orgânicas específicas são recorrentes nos trabalhos de ajustamento osmótico. Açúcares solúveis, betainas, polióis e prolina são acumulados no citoplasma de células vegetais em altas concentrações sem prejudicar a fisiologia da célula (SANCHEZ, et al., 1998).

Em plantas de feijoeiro vem sendo observado os efeitos benéficos do acúmulo de prolina e açúcares solúveis em plantas submetidas ao estresse hídrico e salino. Estes compostos conferem maior tolerância a maiores concentrações de sais e menor potencial hídrico do meio (JIMÉNEZ-BREMONT et al., 2006).

Os osmólitos acumulam nas células das raízes, caule e folhas realizando o ajustamento em condições de estresse. Em estresse hídrico este mecanismo é importante na manutenção do turgor celular e absorção de água, mesmo em potenciais muito negativos. Quanto mais negativo o potencial há um aumento progressivo da concentração de osmólitos na célula (JHA et al., 2014).

A ação dos osmólitos e o mecanismo de ajuste osmótico são observados nos diversos órgãos da planta, no entanto pouco discutido no nível de sementes. Nestas, o ajuste osmótico ocorre na germinação sendo associado a hidrolise do material de reserva comandada pelo eixo embrionário através da atividade de enzimas hidrolíticas (KARUNAGARAN; RAO, 1991).

Em sementes de leguminosas a atividade de proteases e α-amilase aumentam nos estádios iniciais da germinação desempenhando um papel importante no ajustamento osmótico das células dos cotilédones mantendo a absorção de água pela semente na fase II da germinação (YOMO; VARNER, 1973; MOROHASHI, et al., 1989).

A redução do potencial osmótico nas células da semente provoca a diminuição do potencial hídrico permitindo uma maior absorção de água do meio externo. Contudo, em meios com potencial hídrico mais negativo as sementes ajustariam seu potencial osmótico (reduzindo ainda mais o potencial hídrico) para conseguir absorver água e completar a germinação (WELBAUM, 1998).

O mecanismo de ajuste osmótico é bastante controverso na germinação de sementes (JONES AND ARMSTRONG, 1971; GEPSTEIN AND ILAN, 1981), mas algumas técnicas que promovem melhorias na germinação em condições adversas e aumentam vigor das sementes podem estar relacionada ao melhor ajuste do potencial osmótico e, consequentemente, maior e/ou mais rápida absorção de água.

O condicionamento de sementes é bastante utilizado para germinação em ambientes com baixa disponibilidade hídrica. Entre os tipos de condicionamento o hidro condicionamento aumenta a velocidade de germinação e a germinabilidade das sementes em ambientes com baixo potencial hídrico (WINDAUER; ALTUNA; BENECH-ARNOLD, 2007).

2.3 Hidro condicionamento

O hidro condicionamento de sementes é baseado na possibilidade de hidratar a semente de algumas maneiras em que o conteúdo de água seja suficiente para iniciar os eventos da germinação, mas não suficiente para permitir a protrusão da radícula (MORADI; YOUNESI, 2009).

Existem várias maneiras de condicionar uma semente baseado na pré-embebição. O método em que a embebição não é controlada, mas interrompida antes de completar a germinação é denominado de hidro condicionamento. No entanto, o método em que embebição é controlada é denominado osmocondicionamento. No primeiro, embebição é retardada apenas quando as sementes são retiradas da água, enquanto no osmocondicionamento a embebição é retardada quando o potencial hídrico da solução se torna mais negativo do que o potencial hídrico da semente (TAYLOR et al., 1998).

O hidro condicionamento envolve imersão das sementes na água antes da semeadura (JANMOHAMMADI; DEZFULI; SHARIFZADEH, 2008). A absorção da água durante o hidro condicionamento é governada exclusivamente pela afinidade dos tecidos da semente pela água. Como o potencial hídrico do meio não é fator limitante, sementes podem eventualmente germinar. Para evitar que isso ocorra o tempo de imersão das sementes na água e a temperatura de incubação são fatores essenciais no hidro condicionamento (MORADI; YOUNESI, 2009).

O uso do hidro condicionamento tem promovido o aumento nas variáveis de germinação sob ambiente ótimo e adverso em diversas espécies, como trigo (*Triticum aestivum*), alfafa (*Medicago sativa* L), feijão (*Phaseolus vulgaris* L.) e girassol (*Helianthus annuus* L.) (HAMIDI; PIRASTEH-ANOSHEH; IZADI, 2013; AMOOAGHAIE, 2011; MAROUFI; FARAHANI, 2011; MOGHANIBASHI et al., 2012).

Em sementes de feijão o hidro condicionamento tem sido utilizado para melhorar o desempenho na germinação de sementes, estabelecimento inicial de plântulas e produção de grãos no campo (GHASSEMI-GOLEZANI et al., 2010a, 2010b; MAROUFI; FARAHANI, 2011). Contudo, estes trabalhos foram realizados em condições ideais e pouco se conhece sobre os efeitos do hidro condicionamento em sementes e plântulas em baixa disponibilidade hídrica.

Os mecanismos sub celulares induzidos pelo hidro condicionamento que podem ajudar sementes e plântulas a tolerar à seca são: reativação da atividade do ciclo celular; aumento da atividade da dismutase do íon superóxido (SOD), peroxidase do ascorbato (APX) e catalase (CAT); aumento na concentração de proteínas relacionadas à tolerância a dessecação (HSPs e LEAs); aumento da síntese de RNA, principalmente de proteínas envolvidas na produção de energia e mecanismos de defesa química; aumento da síntese de rRNA, devido a maior integridade dos ribossomos; reparo da molécula de DNA; aumento da concentração de adenosina trifosfato (ATP); aumento da razão ATP/ADP; e melhoria na integridade das membranas mitocondriais (VARIER; VARI; DALANI, 2010).

O sucesso da técnica de condicionamento (hidro condicionamento) depende da secagem. Esta é a fase final do processo permitindo as sementes ser armazenadas e usadas posteriormente. Contudo, tem sido atribuída à secagem perda dos efeitos benéficos do condicionamento. Desta forma, tipos de secagem têm sido testados com intuito de manter os ganhos obtidos no condicionamento (DEKKERS et al., 2015).

Os trabalhos com secagem de sementes condicionadas têm utilizado da secagem lenta e rápida. A secagem lenta geralmente é realizada em temperatura e umidade relativa ambiente com as sementes postas em contato direto com o ar perdendo água lentamente para o meio. Já a secagem rápida pode ser realizada em sílica gel ou estufa de circulação forçada, ambas em ambiente fechado com umidades relativas abaixo do ambiente. Neste caso, as sementes sofrem uma perda de água mais rápida (KERMODE, 1997).

A técnica de condicionamento (hidro condicionamento) tem sido útil e promissora não apenas para as sementes como também para todo o biociclo da cultura. Os benefícios desta poderiam ser observados em diferentes fases do desenvolvimento da planta através da maior rapidez e/ou intensidade dos mecanismos celulares promovidos pelo condicionamento. Este comportamento tem sido referido como "memória do condicionamento" ou "memória do estresse" funcionando como tolerância cruzada.

2.4 "Memória" do Condicionamento

A "memória" do condicionamento em sementes representa uma irreversível ativação dos mecanismos de germinação iniciados durante o condicionamento; e a tolerância cruzada ativada pelo estresse abiótico gerado pelo condicionamento (CHEN; ARORA, 2012).

Quando condicionadas sementes exibem maior quantidade de proteínas do tipo II (proteínas de aproximadamente 20 kDa) que são mais abundantes em sementes germinando. Esta quantidade de proteínas pode ser reduzida através da secagem. Todavia, sementes condicionadas e secas quando reidratadas exibem maior rapidez em sintetizar e acumular proteínas do tipo II (CHEN e ARORA, 2011, 2012).

O hidro condicionamento inicia atividades relacionadas à germinação, momentaneamente interrompidas quando as sementes hidro condicionada passam por secagem. Neste caso, quando as sementes que foram hidro condicionadas e secas são reidratadas retomam as atividades do ponto de interrupção com melhorias nos parâmetros de germinação. Este processo é chamado de "memória do condicionamento". O ganho obtido na germinação através do hidro condicionamento pode levar ao aumento na tolerância ao estresse destas sementes (CHEN; ARORA, 2012).

A exposição à pré-embebição e secagem subsequente podem ser considerados um estresse pré-germinativo levando às sementes a “memória do estresse”. Neste caso, hidro condicionamento seria o estresse causado à semente na fase pré-germinativa utilizado para adquirir maior tolerância ao estresse na germinação e outros estádios do desenvolvimento. Desta forma, o hidro condicionamento funcionaria como tolerância cruzada para planta (BRUCE et al., 2007).

A tolerância cruzada consiste da ação do estresse inicial (geralmente menos severo) no organismo com intuito deste adquirir maior tolerância a estresses mais severos e/ou de outro tipo (GENOUD; MÉTRAUX 1999). Desta forma, o estresse inicial deixaria a semente em estado de “alerta” ativando mecanismos de defesa sem levar à exaustão ou danos irreversíveis. Com isso, a semente submetida ao estresse posterior passaria do estado “alerta” para “resistência” (KRANNER et al., 2010).

O hidro condicionamento causa estresse inicial para sementes. Quando hidro condicionadas a embebição por completo da semente em água sem aeração pode promover a via anaeróbica e seus subprodutos levando a um estresse. Além disso, ao iniciar o processo germinativo o hidro condicionamento provoca o aumento na formação de espécies reativas de oxigênio (WATTANAKULPAKIN et al., 2014).

As sementes submetidas ao hidro condicionamento passam pela secagem que também é tida como estresse. A secagem de sementes que toleram a perda parcial de água resulta em injúrias que vão variar de acordo com o modo de secagem (lenta ou rápida) (SOEDA, et al 2005).

Os possíveis mecanismos da “memória” do estresse induzidos pelo hidro condicionamento estão relacionados ao acúmulo de moléculas protetoras e maior atividade do sistema antioxidante. O aminoácido prolina é uma das moléculas relacionadas à tolerância cruzada e o aumento da atividade das enzimas dismutase do íon superóxido, ascorbato peróxidase e Catalase promovem maior remoção de espécies reativas de oxigênio (EROs) (JISHA; VIJAYAKUMARI; PUTHUR, 2013). •.

Todavia, os efeitos do hidro condicionamento como tolerância cruzada para imprimir “memória” ao estresse hídrico em sementes de feijão são desconhecidos. Tal estratégia também poderia conferir a “memória” do estresse na fase de plântulas. Para avaliar o efeito da tolerância cruzada nas sementes de BRSMG Madrepérola o modelo de hydrotime é uma ferramenta.

Existem grandes variações no potencial hídrico das sementes devido a genótipo, ambiente e condição fisiológica que influenciam a absorção de água e, consequentemente, a germinação. Desta forma, o modelo hydrotime é a maneira para descrever a relação entre potencial hídrico (ψ) e taxa de germinação estimando o potencial hídrico basal da semente (BRADFORD; STILL, 2004).

2.5 Modelo Hydrotime

De forma similar utilizada ao modelo de graus dias o modelo de germinação de sementes hydrotime tem sido desenvolvido para modelar o efeito dos baixos potenciais hídricos sobre a germinação (MESGARAN et al., 2013).

O modelo hydrotime utiliza funções para descrever respostas da germinação ao potencial hídrico $\theta_H = [\psi - \psi_{b(g)}]$ tg onde θ_H é a constante do hydrotime (MPa h^{-1}) requerida pelas sementes para germinação, ψ é o potencial hídrico do meio (MPa), $\psi_{b(g)}$ é o potencial hídrico basal que determina se uma determinada fração da população de sementes germinará no potencial hídrico imposto pelo meio, e tg é o tempo (horas) para ocorrer a germinação de determinada fração da população de sementes (BRADFORD; STILL, 2004; WINDAUER; ALTUNA; BENECH-ARNOLD, 2007).

O modelo assume que ψ_b varia entre as frações da população seguindo uma distribuição normal com valor médio $\psi_{b(50)}$ e desvio padrão $\sigma\psi_b$ enquanto, θ_H é considerado constante para a população de sementes (BRADFORD, 1990). Estas afirmativas permitem relacionar o potencial hídrico e a taxa de germinação (GRg = 1/tg, o inverso do tempo de germinação) da fração (g) da população de sementes através da função $GRg = 1/tg = [\psi - \psi_{b(g)}] / \theta_H$.

Os parâmetros que definem o modelo hydrotime caracterizam a velocidade e vigor das sementes avaliadas. A constante hydrotime (θ_H) indica a velocidade global da germinação; ψ_b indica a inerente tolerância das sementes; e $\sigma\psi_b$ indica a uniformidade da germinação. Desta forma, velocidade, tolerância ao estresse e uniformidade das sementes podem ser quantificados pela modelo (BRADFORD; STILL, 2004).

O modelo também assume que ψ_b difere entre as frações da população de sementes avaliadas. Estas variações seguiriam a distribuição normal, podendo então o ψ_b de toda a população ser caracterizado pelo potencial basal médio $\psi_{b(50)}$, potencial basal atingido por 50% das sementes da população, e seu desvio padrão $\sigma\psi_b$ (BRADFORD, 2002).

A vantagem do hydrotime é que os três parâmetros possibilitam a quantificação e previsão do comportamento de germinação, mas somente para uma única temperatura em que os parâmetros foram determinados (BOCHENEK; GOLASZEWSKI; GIELWANOWSKA, 2010).

A aplicação do modelo para avaliar vigor das sementes é bastante consistente. Estas análises geradas do comportamento das sementes em estresse hídrico permitem a classificação de lotes, cultivares e a eficácia de tratamentos de condicionamento de acordo com o potencial atingido para o sucesso da germinação. (BRADFORD; STILL 2004).

Contudo, utilizar o modelo hydrotime para avaliar a tolerância das sementes de feijão cv BRSMG Madrepérola ao estresse hídrico não foi testada.

Além disso, o modelo não foi testado para avaliar o efeito do hidro condicionamento sobre as sementes de BRSMG Madrepérola em relação a baixos potenciais hídricos.

2.6 Cultivar BRSMG Madrepérola

Os grãos do feijoeiro comum (*P. vulgaris*) são um dos principais alimentos na dieta brasileira permitindo alto conteúdo de proteínas (25%). Ele é rico em lisina, mas pobre em aminoácidos com enxofre (ABREU; BIAVA, 2005). A maioria do feijão cultivado no Brasil é do grão tipo carioca (ARAUJO; RAMALHO; ABREU, 2012).

BRSMG Madrepérola foi desenvolvida pela Universidade Federal de Lavras é uma cultivar de feijão do grão carioca comum com características de escurecimento tardio e adequado para cultivo no estado de Minas Gerais. A obtenção de cultivar foi através da hibridização entre as linhagens AN 512666-0 e AN 730031 como parentais (CARNEIRO, et al., 2012).

O biociclo da cultivar (emergência até maturidade fisiológica) é completado em 88 dias na estação de frio e 83 dias para períodos de alta pluviosidade e períodos de seca. Desta forma, a BRSMG Madrepérola é considerada uma cultivar do tipo semi-precoce (EMBRAPA, et al. 2011).

A produção de grãos da cultivar BRSMG Madrepérola é alta em períodos com temperaturas menores e períodos com alta disponibilidade hídrica, mas sofre uma perda significativa em períodos de baixa disponibilidade hídrica. Esta cultivar produz cerca de 6% mais grãos em relação às cultivares Pérola e BRSMG Talismã em períodos com alta disponibilidade hídrica e 11% em períodos com baixa temperatura (CARNEIRO, et al., 2012).

Os grãos da cultivar BRSMG Madrepérola são do tipo “Carioca” (levemente bege com estrias marrons) e mantem esta coloração por mais tempo sem escurecer em comparação com a linhagem VC-3 BRSMG Majestoso, considerada uma cultivar de escurecimento tardio (SILVA et al., 2008). Além disso, a cv BRSMG Madrepérola possui menor tempo de cozimento e maior conteúdo de proteína em relação à cv Pérola e BRSMG Talismã (EMBRAPA, et al. 2011).

BRSMG Madrepérola tem mostrado resistência ao vírus do mosaico comum e para alguns tipos de anthracnose (EMBRAPA, et al. 2011). Por estas características a cv BRSMG Madrepérola é promissora para o estado de Minas e outras regiões, no entanto, devido aos menores valores de produção em períodos de baixa disponibilidade hídrica faz se necessários mais estudos e uso de técnicas que possam amenizar e/ou superar este efeito.

3. CONCLUSÃO

A cultivar BRSMG Madrepérola tem apresentado alta produtividade de grãos, principalmente, no estado de Minas Gerais. Além disso, possui alta qualidade de grãos, resistência a doenças e características desejáveis para os consumidores. No entanto, c.v. BRSMG Madrepérola mostra redução na produção de grãos em períodos de seca. Este comportamento compromete o uso da cultivar em regiões mais secas, como norte de Minas e nordeste do Brasil (área mais plantada de feijão).

O hidro condicionamento pode ser usado como um método simples, econômico e seguro para aumentar a capacidade de germinação de sementes e estabelecimento das plântulas em condições de seca.

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SEGUNDA PARTE

ARTIGO 1 (Submetido a revista Caatinga)

HYDROPRIMING AS A PRE-TREATMENT FOR COMMON BEAN GERMINATION UNDER WATER STRESS

ABSTRACT - The objective of the study was to evaluate the effectiveness of seed hydropriming and re-drying on germination of BRSMG Madrepérola beans under drought stress. The experiment was completely randomized with two factors and five replications. The first factor was seed priming: control (untreated), hydropriming with slow drying or hydropriming with fast drying. The second factor was different water potential: 0.0, -0.2, -0.4 or -0.6 MPa. ANOVA was used to examine the effects and interactions of seed priming and water potential on germination and biochemical responses. Hydrotime, energy of germination (EG), proline free content, antioxidant activity and dormancy breakdown were measured. Hydropriming did not reduce the seeds' threshold potential, but fast drying reduced the hydrotime constant. Time of germination increased with lower potential and the control seeds had lower EG. Higher proline content was observed in control seeds and water had a major effect on dormancy breakdown of seeds under -0.6 MPa water potential. Thus, hydropriming reduced drought tolerance in BRSMG Madrepérola seeds.

Keyword: Hydrotime. MGR. *Phaseolus vulgaris*.

HIDRO CONDICIONAMENTO NA GERMINAÇÃO DE SEMENTES DE FEIJOEIRO CULTIVAR BRSMG MADREPÉROLA EM DIFERENTES POTENCIAIS HÍDRICOS

RESUMO – O objetivo deste trabalho foi avaliar o efeito do hidro condicionamento e subsequente secagem na germinação de sementes de feijão comum da cultivar BRSMG Madrepérola submetidas à seca. O delineamento experimental foi inteiramente casualizado realizado em fatorial com 2 fatores e 5 repetições. O primeiro fator foi constituído do hidro condicionamento (controle (não condicionadas), hidro condicionadas com secagem lenta e hidro condicionadas com secagem rápida) enquanto o segundo fator foram os diferentes potenciais hídricos (0.0, -0.2, -0.4 -0.6 MPa). Uma ANOVA foi realizada para examinar os efeitos e interações do hidro condicionamento com diferentes potenciais hídricos sobre a germinação e respostas bioquímicas. Foi realizado o teste de Tukey ($p \leq 0.05$). Foram avaliados Hydrotime, Taxa de Germinação (MGR), prolina, atividade antioxidante e quebra de dormência. Foi observado que o hidro condicionamento não reduziu o potencial limite, mas a secagem rápida reduziu a constante do hydrotime. O tempo de germinação aumenta com a redução do potencial e o controle obteve maior MGR e conteúdo de prolina, enquanto água superou a quiescência no potencial de -0,6 MPa. Assim, hidro condicionamento reduz a tolerância à seca em sementes de BRSMG Madrepérola.

Palavras-chave: Hydrotime. MGR. *Phaseolus vulgaris*.

INTRODUCTION

Drought is one the main constraints on production of crops and it may become more frequent and severe due to the impacts of anthropogenic climate change. Reductions in rainfall and increases in dry season length are associated with a decrease in agricultural yield of many crops. In common beans, dry season intensity and length is a major constraint on productivity in several countries. In Brazil, where beans are an important staple in the daily diet, productivity is reduced by around 45% due to drought (CONAB, 2013). Indeed, lack of water has been observed to reduce germinability, germination speed and seedling establishment in beans (Custodio et al., 2009). However, these negative effects can potentially be alleviated or overcome by adopting hydropriming methods (Windauer et al., 2007).

Hydropriming consists of soaking seeds in water before sowing while carefully controlling soak time, temperature and light (Moradi and Younesi, 2009). After hydropriming, re-drying is very important to promote retention of hydropriming's benefits in stored seeds. Increased vigor after hydropriming has been observed in many species, such as wheat, alfalfa, common bean and sunflower (Hamidi et al., 2013). It is thought that hydropriming triggers subcellular mechanisms such as cellular cycles, enzymatic antioxidant systems (superoxide dismutase-SOD, ascorbate peroxide-APX and catalase-CAT), increased energy production, syntheses of RNA, syntheses of heat shock proteins (HSPs) and late embryogenesis proteins (LEAs), and DNA conservation (Varier et al., 2010). In addition, hydropriming can activate osmolyte synthesis (e.g., proline and soluble sugar), leading to osmotic adjustment. This process allows the seeds to absorb water in phase II of germination. In common bean seeds, hydropriming has been used to increase germinability, seedling establishment and yield under normal environmental conditions (Ghassemi-Golezani et al., 2010a, 2010b). However, there is a lack of knowledge about the effect of hydropriming on bean seeds under drought conditions. The hydrotime model is one means of measuring hydropriming's effects under drought in common bean seeds.

The hydrotime model utilizes functions which describe germination responses to water potential: $\theta_H = [\psi - \psi_{b(g)}]$ tg, where θ_H is the hydrotime constant (MPa h^{-1}) required by the seeds for germination, ψ (MPa) is the water potential of the environment, $\psi_{b(g)}$ (MPa) is threshold water potential for germination fraction specific to the seed lot, and tg (hours) is the time to germination specific to the seed lot (Windauer et al., 2007; Bradford and Still, 2004.). By applying the hydrotime model, it is thus possible to estimate speed, tolerance and uniformity of germination in seeds exposed to different water potentials (Bradford, 2002). However, this model has not been applied to common bean seeds treated with hydropriming and, more specifically, has never been applied to the BRSMG Madrepérola common bean cultivar.

The BRSMG Madrepérola cultivar was developed at Federal University of Lavras (UFLA) in collaboration with other institutions. It is a common bean cultivar with late darkening that was obtained through hybridization of strains AN 512666-0 and AN 730031 (Carneiro, et al., 2012). This cultivar has increased productivity at low temperatures in comparison with Pérola and BRSMG Talismã common bean cultivars. However, under drought conditions the BRSMG Madrepérola cultivar has lower productivity than other cultivars (Carneiro et al., 2012; Embrapa, 2011). In this context, the objective of the present study was to evaluate the effects of seed hydropriming and re-drying on germination of the BRSMG Madrepérola common bean cultivar under drought stress.

MATERIALS AND METHODS

The experiment was conducted at the Plant Growth and Development Laboratory of Federal University of Lavras (UFLA), Lavras, MG, Brazil in 2014. The experiment was completely randomized in a 3x4 factorial arrangement with five replications. The factors were seed priming (control (untreated), hydropriming with slow re-drying (Hydro+SD) or hydropriming with fast re-drying (Hydro+FD) and five different water potentials (0.0, -0.2, -0.4, -0.6 MPa).

BRSMG Madrepérola seeds were submitted to hydropriming in deionized water, without aeration, at 20 °C and in the dark, for 14 hours (Moradi and Younesi, 2009). The treated seeds were dried slowly at room temperature and relative humidity for 48 hours or dried quickly using gerboxes containing silica gel with seeds on metal mesh (to avoid contact) for 24 hours.

For the hydrotime model, five replicates of 20 seeds were placed in 9 cm diameter Petri dishes on two germination blotters saturated with deionized water or solutions of PEG 6000 with -0.2 MPa, -0.4 MPa or -0.6 MPa at constant temperature of 25 °C in light in a BOD chamber for 480 hours. Seed germination was recorded daily as radicle protrusion to 2 mm, and germinated seeds were removed. Seeds that had not germinated were transferred to fresh solutions at 48-hour intervals. Ungerminated seeds under -0.6 MPa were used for the breakdown dormancy experiment: seeds were transferred to 5 cm diameter Petri dishes on two germination blotters saturated with deionized water, abscisic acid (ABA) (10 µM) with PEG 6000 (-0.6 MPa), gibberellic acid (GA) (10 µM) with PEG 6000 (-0.6 MPa) or only PEG 6000 (-0.6MPa), after which germinability was measured.

Germination time data were analyzed by repeated probit regression using the hydrotime model (Bradford, 1990; Gummerson, 1986) with sigmoidal regression of three parameters. Mean germination rates (MGR) were calculated according to Ranal et al. (2009) and used to calculate linear regression on different potentials.

For biochemical tests, three replicates of 10 seeds were placed in 5 cm diameter Petri dishes on two germination blotters saturated with solutions of PEG 6000 at -0.6 MPa at constant temperature of 25 °C in light in a BOD chamber for 480 hours. The ungerminated seeds were frozen immediately in liquid nitrogen, placed in air-tight plastic bags and stored frozen at -80 °C until analysis of their enzymatic antioxidant activity and proline content.

The activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were measured by the method of Biemelt, Keetman and Albrecht (1998). Ground dry seed material (0.2 g) was mixed with 100 mM of potassium phosphate buffer (pH = 7.8), EDTA (0.1 mM) and ascorbic acid (1 mM). Extracts were centrifuged at 13,000 g at 4 °C for 20 min and the supernatant was collected and used for enzyme assays.

SOD activity was measured following Giannopolitise and Ries (1977), adapted for tomato seed analyses. The assay mix consisted of 50 mM of potassium phosphate buffer (pH = 7.8), 14 mM of methionine, 75 µM of nitro blue tetrazolium, 2 µM of riboflavin, 100 mM of EDTA and 20 µL of enzyme extract. The reaction was initiated by adding riboflavin and submission to fluorescent light (15 W), and was allowed to continue for 40 min. The assay was ended by switching off the light and covering the tubes with a black cloth. The absorbance was measured at 560 nm using a BioTek Epoch microplate spectrophotometer. One unit of SOD activity was defined as the amount of the enzyme required to reach 50% inhibition of the reaction in the “minus enzyme extract” control, which should have a higher absorbance compared to the samples with enzyme extract.

CAT activity was measured using the protocol of Havir and McHale (1987). The assay mix consisted of 200 mM of potassium phosphate buffer (pH 7.0), 20 µl of enzyme extract, and 120 µl of 12.5 mM H_2O_2 solution. One unit of CAT activity was defined as the degradation of 1 µM of H_2O_2 during one minute at 240 nm (using extinction coefficient $\varepsilon = 36\ mM^{-1}\ cm^{-1}$).

APX activity was measured using Nakano and Asada’s (1981) modified protocol for tomato seeds. The assay mix was composed of 50 mM of potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbic acid (ASA), 0.1 mM of EDTA, and 20 µl of enzyme extract. H_2O_2 (0.1 mM) was added to initiate the reaction. One unit of APX was defined as the conversion of

ASA (1 µM) into monodehydroascorbate at 290 nm, during one minute (ε=2.8 mM^{-1} cm^{-1}).

Free proline was assayed spectrophotometrically using the method of Bates et al. (1973). Seed samples (100 mg FW) were homogenized in 3% (w/v) aqueous sulfosalicylic acid and centrifuged for 30 min at 14,000 g. Then, 2 ml of acid-ninhydrin reagent and 2 ml of glacial acetic acid were added to the supernatant. The mixture was then boiled for 1 h. After extraction with toluene, free proline was quantified at 520 nm.

Analysis of variance (ANOVA) was used to examine the effects and interactions of seed priming and water potential on final germination and biochemical responses. Differences were assessed by the Tukey and least significant difference (LSD) tests at $p \leq 0.05$, calculated using RStudio 3. 1. 1. (R Core Team, 2014). All biochemical data are reported as mean ± standard error of the mean for three replicates.

RESULTS AND DISCUSSION

The interaction between priming treatments and different water potentials significantly influenced germination and biochemical variables. A reduction in water potential increased germination time and reduced germinability in all treatments (Figure 1). The Hydro+SD treatment reduced germination time with 0.0 MPa (Figure 1-A) while the Hydro+FD treatment induced early germination with -0.2 MPa but did not improve germinability (Figure 1-B). Observed germination time with -0.4 MPa increased seven-fold in relation to the control in all treatments and reduced germinability in the Hydro+SD treatment (9%) and the Hydro+FD treatment (24.75%) (Figure 1-C). With -0.6 MPa, germination time increased to 168 hours in the control and Hydro+FD treatments, while seeds in the Hydro+SD treatment had germination increased to 288 hours (Figure 1-D). The germination of control seeds with water potential under -0.6 MPa declined by 70%, while Hydro+SD and Hydro+FD treatments reduced germination by 80% when compared to 0.0 MPa conditions.

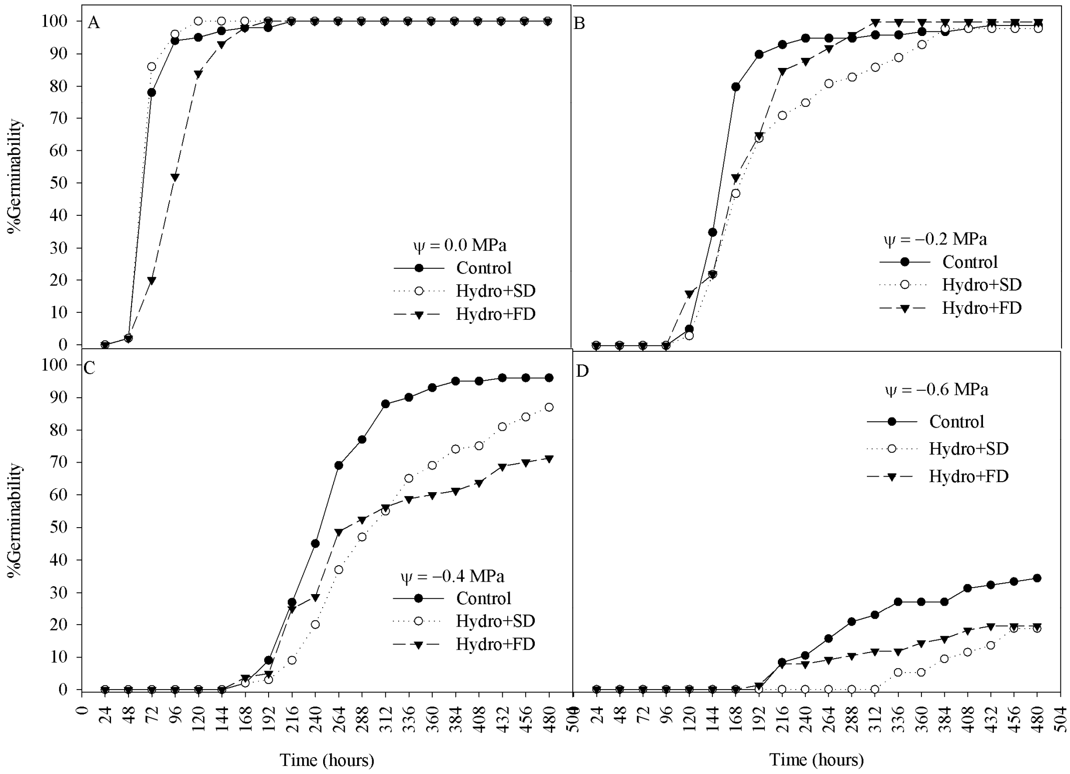

Figure 1. Germination times of BRSMG Madrepérola bean seeds that received different hydropriming treatments (●, Control; ○, Hydro+SD; ▼, Hydro+FD.) and were exposed to different water potentials (0.0 MPa, -0.2 MPa, -0.4 MPa or -0.6 MPa).

The hydrotime model analysis indicated that for the Hydro + FD treatment, the θ_H constant was reduced (Table 1) and the hydropriming treatment did not shift $\psi_{b(g)}$ towards more negative values (Table 1).

Table 1. Estimated hydrotime parameters for BRSMG Madrepérola bean seeds subjected to hydropriming treatment with slow drying (Hydro+ SD), fast drying (Hydro + FD) and control (unprimed) under different water potentials.

Treatment	Θ_H (MPa.h^{-1})	$\Psi_{b(50)}$ (MPa)	$\sigma_{\Psi b}$ (MPa)
Control	89.29	-0.76	0.09
Hydro+SD	81.97	-0.67	0.09
Hydro+FD	57.47	-0.58	0.09

The hydropriming increased threshold water potential of seeds, mainly in the Hydro+FD treatment. This led to an increase in delay and a reduction in germinability of hydroprimed seeds at lower water potential (-0.4 and -0.6 MPa). When compared with other common beans cultivars, BRSMG Madrepérola has intermediate threshold of water potential (Cardoso & Bianconi, 2013).

The difference between environmental water potential and threshold water potential of seeds is related to the time needed for germination and germinability (Bradford and Still, 2004). There was no difference observed between the Hydro+FD treatment and the control in terms of germination time with 0.0, -0.4 and -0.6MPa, but with -0.2 MPa, the seeds needed less time to germinate than the control. The faster germination was due to the lower θ_H constant. Cardoso and Bianconi (2013) showed that reduced θ_H is not related to Ψ_b reduction in common beans seeds. Seeds with smaller θ_H germinated faster under lower negative water potential (Ramírez-Tobías et al., 2014).

MGR was reduced as a function of declining water potential (Figure 2). Larger reductions in MGR were observed in the Hydro+SD treatment, followed by control and Hydro+FD treatments. MGR in the Hydro+SD treatment was reduced by 89.07% from 0.0 MPa to -0.6 MPa, while the control and Hydro+FD treatments were reduced by 85.35% and 86.62%, respectively.

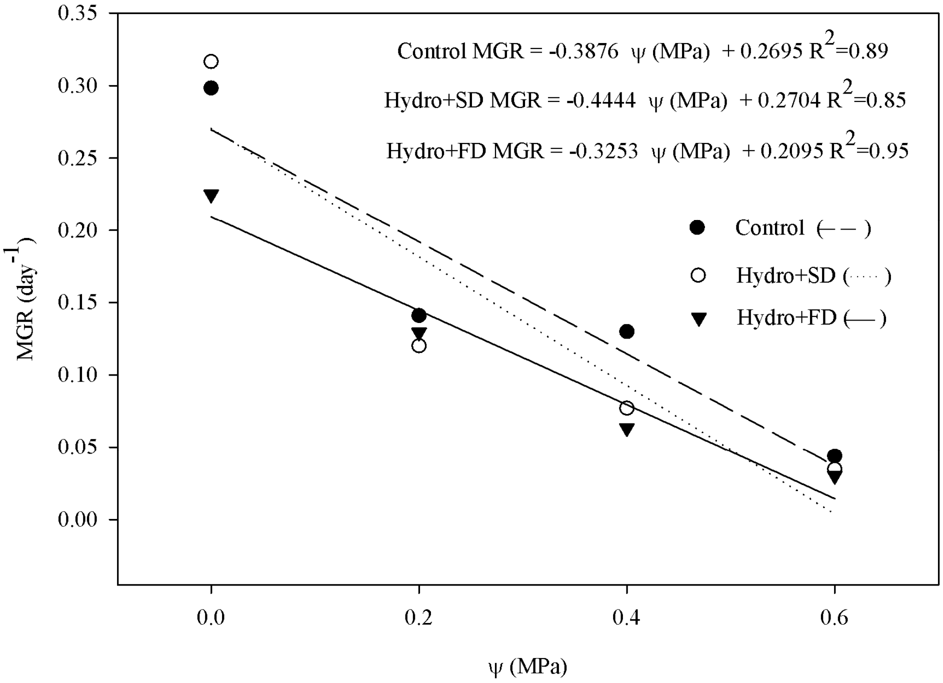

Figure 2. Linear regression of BRSMG Madrepérola seeds that received different hydropriming treatments (●, Control; ○, Hydro+SD; ▼, Hydro+FD.) under different water potentials (0.0 MPa, -0.2 MPa, -0.4 MPa or -0.6 MPa).

With 0.0 MPa, MGR was higher in the Hydro+SD treatment in relation to the control and the Hydro+FD treatment. With -0.2, -0.4 and -0.6 MPa, the control showed higher MGR than the others treatments. The highest reduction observed in hydroprimed seeds was associated with less vigor, which reduced germination speed and germinability with more negative water potentials (Hamidreza et al., 2013). Seeds which do not germinate with negative water potential are associated with less water uptake in phase II of seed germination because they cannot reduce osmotic potential thought the osmotic adjustment mechanism (Veselova and Veselovsky, 2006; Jones and Armstrong, 1977).

The Hydro+FD seeds had lower concentrations of free proline than other treatments with -0.6 water potential (Figure 3). Proline is important for osmotic adjustment in many plant organs, but its role in seeds is still uncertain. Jones and Armstrong (1977) showed osmotic adjustment in seeds' imbibition and sugar as osmolity for water uptake in seeds. The role of proline in seeds may be unclear, but an explanation for its reduction in the Hydro+FD treatment might be that fast re-dry causes denaturation of proteins (Abreu et al., 2014).

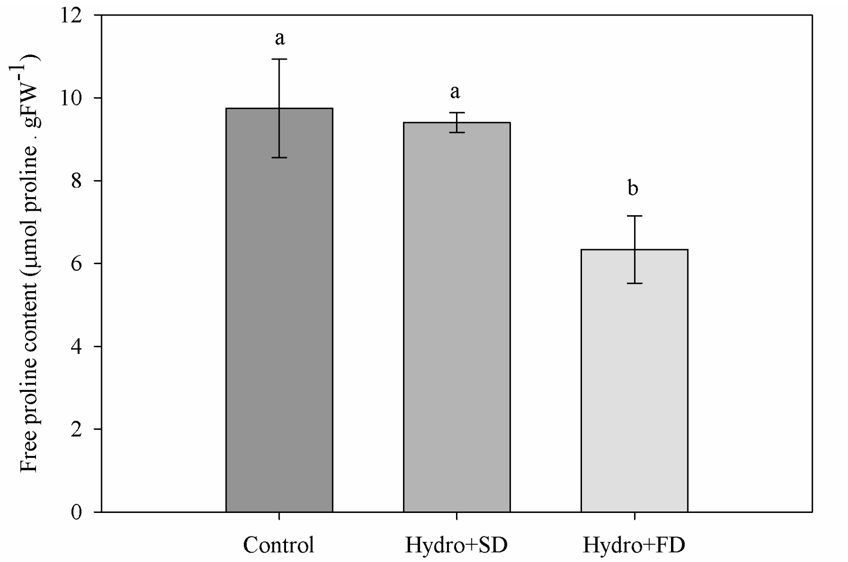

Figure 3. Proline concentration of BRSMG Madrepérola seeds that received different hydropriming treatments (●, Control; ○, Hydro+SD; ▼, Hydro+FD.) under water potential of -0.6 MPa. *Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$) (n=3).

Damage caused to proline biosynthesis enzymes, such as Δ^1 -pyrroline-2-carboxylate reductase, Δ^1 -pyrroline-5-carboxylate synthetase or Δ^1 -pyrroline-5-carboxylate reductase, can reduce free proline content (Delauney and Verma, 1993). However, the Hydro+SD seeds did not differ from control in proline content, although they did have lower germinability with -0.6 MPa. This may be caused by damage to antioxidant enzymes, as observed for Hydro+SD and Hydro+FD seeds, where there were no observable SOD, CAT and APX activity. In control seeds, SOD activity was observed. Hydropriming for longer durations (> 9 hours) could reduce the activity of antioxidant enzymes (Wattanakulpakin et al., 2012). Furthermore, the avoidance oxidative damage in dry seeds is more likely related to AOS scavenging by antioxidant compounds (Baily, 2004).

The seeds that did not germinate with -0.6 MPa were observed to be in a quiescent state, which could only be overcome by water (Figure 4).

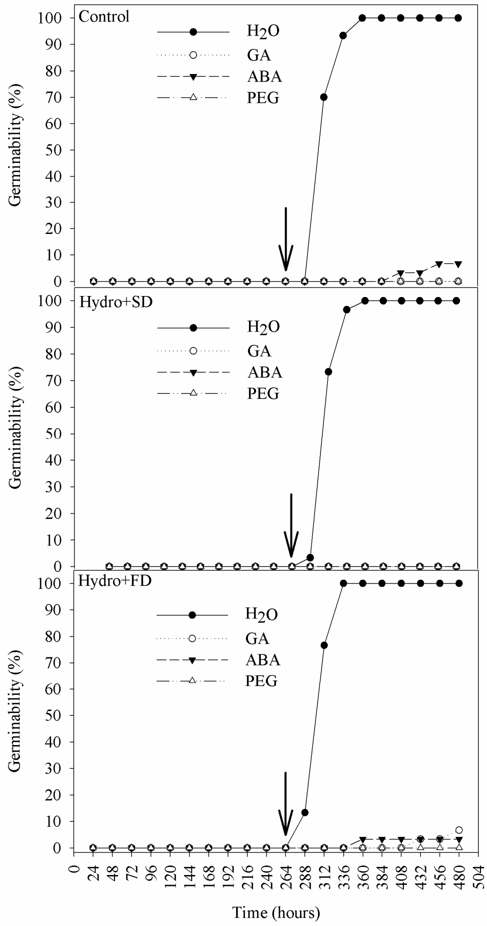

Figure 4. Germination recovery of BRSMG Madrepérola seeds that received different hydropriming treatments (●, Control; ○, Hydro+SD; ▼, Hydro+FD.) under water potential of -0.6 MPa using deionized water, GA (10 µM), ABA (10 µM) andPEG

Hydroprimed seeds had germination peak 24 hours before that of the control seeds. Although only a few seeds germinated, ABA breakdown of quiescence was observed in control and Hydro+FD seeds, while GA-promoted breakdown was observed in Hydro+FD seeds. Phytohormones had no effect on Hydro+SD seeds and contrary to what was observed by Kaur et al. (1998) in chickpea seeds, in BRSMG Madrepérola seeds gibberellic acid also did not improve germination under low water potential.

CONCLUSION

The hydropriming treatment reduced drought tolerance in BRSMG Madrepérola seeds due to a reduction of free proline content and antioxidant enzyme activity.

ACKNOWLEDGMENTS

We thank Lavras Federal University (UFLA) for providing the structure. This work was supported by CNPq (Conselho Nacional de Desenvolvimento Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais).

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ARTIGO 2. (Submetido a revista Australian Journal of Crop Science)

Influence of hydropriming on bean seedling establishment and drought tolerance

Abstract

Recurrent droughts are a growing problem for common bean crops in Brazil and drought tolerant cultivars are necessary to maintain yield. Hydropriming is a simple, economic and short-term treatment that may improve bean seedling establishment under drought stress. The objective of the present study was to evaluate seed hydropriming and re-drying "memory" of priming on osmolyte accumulation and enzymatic antioxidant system in in seeds of BRSMG Madrepérola common bean seedlings under drought stress. The experiment was a factorial with two factors arranged in a completely randomized design with five replications. The first factor was seed priming (control (untreated), hydropriming with slow drying and hydropriming with fast drying) and the second was irrigation suspension for 2, 4, 6 days, rehydrated after 6 days. In the control, water lost by evapotranspiration was replaced on a daily basis. An ANOVA was used to examine the effects and interactions of seed priming and different water potential on final germination and biochemical responses. Plant growth, total soluble sugars, free proline content, SOD activity, CAT activity and APX activity were measured in roots and leaves. Hydropriming improved RGR, NAR, LAR and LWF growth parameters in seedlings under 70% of yield capacity. Seeds with hydropriming plus fast drying had faster sugar accumulation after 2 and 4 days of drought in leaves, and higher free proline content in leaves after 6 days of drought. Hydropriming plus slow drying increased SOD and APX activity in roots. Hydropriming with slow and fast drying improved speed and intensity of osmolity accumulation and enzymatic antioxidant system under drought conditions.

Keywords: BRSMG Madrepérola common bean, “memory” of priming, osmolity accumulation, antioxidant system, re-drying, growth analyses

1 Introduction

BRSMG Madrepérola is a common bean cultivar with high grain yield that is resistant to common mosaic virus and which has a high protein content (Carneiro, et al., 2012; Embrapa, 2011; Silva et al., 2008). However, this cultivar shows reduced grain yield under drought leading to attempts to develop drought resistance by selecting cultivars for high yield under water stress (Frahm et al., 2004). Despite considerable efforts, slow progress has been made on improving abiotic stress tolerance (Beaver and Osorno, 2009).

Seed priming has been observed to more rapid and/or stronger, activation of the various defense responses to abiotic stress (Bruce at al., 2007), potentially enhancing protection without the costs associated with expression of stress genes (Van Hulten et al., 2006). One such method, Hydropriming, is effective at inhibiting pre-germination metabolisms without radicle protrusion (Farooq et al., 2009), and is considered a simple, economic and short-term approach to obtain drought tolerance (Kaya et al., 2006). This technique promotes activation of subcellular mechanisms in seeds, increasing germination and vigor (Hesabi et al., 2014). It has also been observed to enhance seedling growth under normal and drought conditions (Jisha and Puthur, 2014), the latter being driven by priming “memory” - a cross-tolerance activated by abiotic stresses and generated by priming or re-drying (Chen and Arora, 2012). Growth of plants is enhanced by seed-priming treatments because they prepare the plant to respond to imminent abiotic stress more quickly or aggressively (Jisha et al., 2013).

Osmotic adjustment is one of the major plant defenses against drought. Accumulation of molecules called osmolytes confers protection to membranes, prevents enzyme denaturation and avoids water leakage (Slama et al., 2015). Proline appears to be the most widely distributed osmolyte accumulated under stress conditions in plants (Delauney and Verma, 1993), mainly as a consequence of reduced degradation and increased synthesis (Verbruggen and Hermans, 2008). In contrast, total soluble sugar (another common osmolyte) is accumulated due to a carbon reallocation caused by drought (Kerepesi and Galiba, 2000).

Another subcellular mechanism improved by hydropriming is the enzymatic antioxidant system. In hydroprimed seeds, scavenging of reactive oxygen species (ROS) becomes faster and more rigorous promoting an increase in drought tolerance and overall growth (Chen and Arora, 2012). The objective of the present study was therefore to evaluate the seed hydropriming and re-drying “memory” effect on osmolyte accumulation and the enzymatic antioxidant system in BRSMG Madrepérola bean seedlings established under drought conditions.

Results and Discussion

Hydropriming improved five growth parameters in BRSMG seedlings (Fig 1). Hydro+SD treatment increased RGR by 57.14% and resulted in a two-fold increase in NAR in relation to the control (Fig 1-A, 1-B). Hydro+FD increased LAR and LWF by 17.22% (Fig 1-C and –D) when compared with control. SLA was the only parameter not affected by hydropriming. Hydroprimed seedlings also increased to root dry matter as indicated by root-shoot allometry (Fig 1-F).

The benefits of hydropriming common bean seeds on seedling establishment, plant growth and productivity have previously been demonstrated by Ghassemi-Golezani et al. (2010a; 2010b). Nevertheless, re-drying effects have not been frequently investigated. Different types of drying change were observed to growth relationships: when seeds were slow dried there was an increase in RGR and NAR, indicating an improvement in the process of photosynthesis (Ngueyn et al., 2015). Both slow and fast drying increased leaf investment in area (LAR) and mass (LWF), but only slow drying caused significant gains in photosynthesis. Indeed, fast drying may even damage membrane structures in seeds with associated reductions in seedling photosynthesis efficiency. Seeds with low vigor produce plants with lower productivity (Soares et al., 2013). The higher root dry mass observed in hydropriming treatments benefits BSRMG Madrepérola seedlings since, in common bean seedlings, large roots systems improve water and nutrient uptake and enhance seedling establishment under both normal water conditions and drought (Lynch, 2011).

Soluble sugar content varied in relation to ambient conditions, organs and the interactions between them ($p<0.01$). Initially, seeds treated with Hydro+FD had higher total soluble sugar levels in root than the Control and the Hydro+SD treatment(figure 2-A). Hydropriming promoted faster sugar accumulation in roots after 4 days of drought, but only Hydro+FD produced higher sugar levels in roots after 6 days of drought. The Control did not show any changes in sugar levels in roots. Soluble sugar content in the leaves of the Hydro+FD treatment had faster sugar accumulation after 2 and 4 days of drought (Fig 2-B).

Seedlings of control increased sugar levels in the leaves only after 6 days of drought, while the Hydro+SD treatment seedlings maintained the same sugar level in all conditions. After rehydration, total soluble sugar levels in roots and leaves dropped in all treatments. Soluble sugar concentration in the leaves of control seedlings increased more than in roots under prolonged drought (Fig 2-C). The Hydro+FD seedlings accumulated sugar in leaves after 4 days of drought, but changes in sugar accumulation in roots were only observed after 6 days of drought (Fig 2-D). After 4 days of drought the soluble sugar content in roots was higher than in leaves in the Hydro+SD seedlings (figure 2-E).

Soluble sugar accumulation is an important mechanism against drought stress because is linked with osmotic adjustment. The Hydro+FD treatment was associated with faster soluble carbohydrate accumulation which contributes to turgor maintenance and membrane protection under drought stress (Munns and Weir, 1981). It has been observed that oligosaccharides levels increased in fast dried seeds (Buitink et al., 2003)and it is possible that this effect can be maintained in seedlings under stress condition. Thus, seedlings in the Hydro+FD treatment may be more tolerant to drought by quickly accumulating soluble sugars in roots. The high sugar content in Hydro+FD root seedling after 6 days of drought may be associated to photo-assimilated translocation. In common beans, drought tolerance has been associated with higher osmotic adjustment in root systems through sugar accumulation (Cuellar-Ortiz et al., 2008). Hydropriming treatments change sugar accumulation behavior according to stress intensity, which may significantly contribute to acclimation of seedlings in response to stress.

Free proline content also significantly varied by treatment, ambient conditions, organs and interaction between them ($p<0.05$). Hydropriming did not increase proline content in roots (Fig 3-A). Nevertheless, the leaves of Hydro+SD seedlings had higher free proline content after 6 days of drought (Fig 3-B). The proline levels increased with stress intensity, but hydropriming did not appear to increase the rate of proline accumulation.

When seedlings of all treatments returned to normal water conditions (e.g. were rehydrated), proline content decreased. Under drought conditions, seedlings accumulated higher amounts of proline in roots than leaves (Fig 3-C,-D and -E). In the Hydro+SD treatment after 6 days of drought, there were similar levels of proline in roots and leaves (Fig 3-E). Indeed, this treatment only increased proline content in leaves after 6 days of drought. Free proline accumulation under drought is an important stress response to osmotic adjustment (Verbruggen and Hermans, 2008). Nevertheless, hydropriming did not promote an increase in the speed and intensity of proline accumulation - possibly because of the higher accumulation of soluble sugars in hydroprimed seedlings. It has been observed that plants may reduce or increase osmolity under drought stress (Hare et al., 1998). Nevertheless, proline accumulation under severe drought is clearly an important characteristic in drought-tolerant common beans cultivars+ (Rosales et al., 2013) and the `memory` of slow re-drying in hydroprimed seeds could be expected to increase proline accumulation in leaves under severe stress conditions.

SOD, CAT and APX activity significantly varied in relation to the treatments, ambient conditions, organs and interactions between them ($p<0.05$). Hydro+FD roots had increased to SOD activity after 4 days of drought, while Hydro+SD had increased to SOD activity after 6 days of drought (Fig 4-A). In contrast to soluble sugars and proline, SOD activity did not increase in roots with stress severity. The Hydro+SD treatment increased the speed and intensity of SOD activity in leaves (Fig 4-B). Nevertheless, SOD activity did not decrease when seedlings were rehydrated. In the control seedlings, higher SOD activity was observed in leaves (Fig 4-C), while the highest SOD activity in the Hydro+FD treatment after 4 days of drought was observed in roots (Fig 4-D). Leaves of seedlings in the Hydro+SD treatments had higher SOD activity than roots (Fig 4-E).

The higher SOD activity in the roots of Hydro+FD seedlings was related to drought tolerance. Under low water content common bean seedlings increased super oxide production - the SOD enzyme is major scavenge mechanism (Turkan et al., 2005). Priming with re-drying may create cross tolerance in seeds and stress “memory” in seedlings (Chen and Arora, 2012). The Hydro+SD treatment increased SOD activity in leaves under drought. Seedlings which retain a low super oxide levels in leaves under drought are more tolerant because they are protected against chloroplast membrane damage and, consequently, reduction in photosynthesis (Baxter et al., 2014).

The Hydro+FD treatment increased the speed and intensity of CAT activity in bean roots (Fig 5-A). Hydropriming did not increase CAT activity in leaves, although the Hydro+FD treatment promoted higher CAT activity after 4 days of drought (Fig 5-B). The Hydro+SD treatment had no observable effect on CAT activity under drought while the hydropriming treatments increased CAT activity in roots more than leaves (Fig 5-C, 5-D and 5-E).

A more rapid and intense response of the enzymatic antioxidant system under drought stress could result in lower cellular damage and, consequently, higher tolerance to stress (Caverzan et al., 2012). Thus, the Hydro+FD treatment is effective in increasing drought tolerance in BRSMG Madrepérola bean seedlings through improved CAT activity in the roots: the levels of hydrogen peroxide in the roots of seedlings under drought may be high, and higher efficiency of the CAT enzyme means less damage to roots (Horta et al., 2014). The higher CAT activity observed in Hydro+FD seedlings after 4 days of drought also indicates protection of subcellular structures under intermediary stress level. When leaves are under intermediate drought stress there is an increase in photorespiration and hydrogen peroxide production (Miller et al., 2010). CAT activity in peroxisomes is the major defense against hydrogen peroxide accumulation in leaves (Mittler, 2002.). Nevertheless, hydropriming seems to enhance CAT activity in roots more than leaves. The increased of CAT activity in roots promoted by hydropriming could potentially be used to improve drought tolerance since roots are a first organs affected by drought.

Hydropriming also increased APX activity in roots of BRSMG Madrepérola seedlings under field conditions (Fig 6-A). After 2 days of drought the Hydro+SD seedlings increased APX activity in roots while APX activity in the roots of Hydro+FD seedlings was reduced. The Hydro+FD seedlings had a higher rate of APX activity in leaves, with higher activity after 2 days of drought (Fig 6-B). The Control showed higher APX activity after 6 days of drought. There was a change in APX activity in roots to leaves between 4 to 6 days of drought (Fig 6-C). After 2 days of drought the Hydro+FD treatment increased APX activity in leaves and reduced activity in roots (Fig 6-D). In the Hydro+SD treatment, APX activity only increased in roots after 4 days of drought and subsequent rehydration (Fig 6-E).

The increase in APX activity in roots may be associated with a cross tolerance effect promoted by early imbibition (hydropriming) and dissection tolerance mechanisms (slow dry) (Chen and Arora, 2012). Hydropriming with re-drying in seeds could induce a “memory” used in stress defense in many stages of plant growth (Jisha et al., 2013). Thus, the faster APX activity in leaves after 2 days of drought in the Hydro+FD treatment may be due to the stress “memory” gained by seeds during hydropriming (Bruce et al., 2007). The Hydro+SD and Hydro+FD treatments also caused an increase in rate or intensity of the enzymatic antioxidant system, but as observed previously, each enzyme varies according to organ and condition of seedlings. Thus, fast drying appears to increase SOD and CAT activity in roots and APX activity in leaves while slow drying promotes SOD and APX activity in leaves of the BRSMG Madrepérola common bean.

Hydropriming with slow and fast drying of bean seeds improved the rate and intensity of osmolity accumulation and enzymatic antioxidant system activity under drought stress, indicating that a hydropriming "memory" effect may be involved. Under non-stress conditions hydropriming with slow and fast drying seems to be able to improved growth of BRSMG Madrepérola seedlings.

Materials and Methods

Locality and plant material

The experiment was conducted at growth room of Plant Physiology section, Federal University of Lavras (UFLA), Lavras-MG in 2014. BRSMG Madrepérola seeds were submitted to hydropriming in deionized water, without aeration, at 20°C and in the absence of light, for 14 hours (Moradi & Younesi, 2009). The treated seeds were slow dried (Hydro+SD) at room temperature and relative humidity for 48 hours and fast dried (Hydro+FD) using gearboxes containing silica gel with seeds on metallic mash to avoid contact for 24 hours. Untreated seeds (control) were not hydroprimed.

After drying seeds were germinated in plastic dark pots of 200 ml with a commercial soil substrate (turf, cattle manure, limestone, phosphate and vermiculite) and washed sand in a proportion of 1:1. The Cation Exchange Capacity (CTC) was 202.00 mmolc/Kg and Water Retention Capacity (WRC) was 60.00% w/w.

The pots with seeds remained in room with temperature at 25±2 °C, relative humidity 50±2 %, 60 μmol.m-^{2}.s-^{1} of photosynthetic activity radiation, 12 hours of photoperiod and substrate in 70% field capacity.

The treatments were imposed after the appearance of the first pair of leaves.

Drought treatment

The drought treatments were imposed through suspension of irrigation for 2, 4, 6 days and were rehydrated after 6 days. In control seedlings the loss water for evapotranspiration was replaced on a daily basis. Rehydration of seedlings was controlled through weighing using a semi-analytic balance (±0.01 g).

Plant growth analysis

Growth was calculated by dividing the difference in ln-transformed plant weight at two harvests by the time difference between those harvests (Poorter, 1989). The harvests were performed twelve days after sowing and twenty four days after sowing with five seedlings in each treatment. After harvesting, seedlings were separated into roots, shoots and leaves and were dried in a forced air oven at 70°C. Leaf dry matter (LDM), shoot dry matter (SDM), root dry matter and leaf area (LA) were used to calculated relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA), leaf weight fraction (LWF), leaf area ratio (LAR) and root-shoot allometry thought of AOB tools software (Hunt et al., 2002). Overall plant growth analyses were performed with plants at field capacity of 70% (non-drought) and from different hydropriming treatments (Control, Hydro+SD and Hydro+FD).

Total soluble sugar content

Extraction of total soluble sugar was performed after homogenization of 0.2 g of dry roots and leaves samples placed in a 5 mL solution of potassium phosphate buffer (0.1 M; pH 7.0) following a bath for 30 min at 40 °C and centrifuged at 10,000 g for 20 minutes at 4 ºC. From the supernatant extract, the contents of soluble compounds were obtained with the aid of a spectrophotometer. Total soluble sugars (TSS) were determined by reaction with anthrone (Yemm and Willis, 1954).

Proline content

Free proline content was assayed spectrophotometrically using the method of Bates et al. (1973). Roots and leaf samples (100 mg FW) were homogenized in 3% (w/v) aqueous sulfosalicylic acid and centrifuged for 30 min at 14,000 g. Then, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added to the supernatant. The mixture was then boiled for 1 h. After extraction with toluene, free proline was quantified at 520 nm.

Antioxidant system activity

The extracts of the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were obtained by a method of Biemelt, Keetman and Albrecht (1998). Ground fresh root and leaves (0.2 g) were mixed with 100 mM of potassium phosphate buffer (pH = 7.8), EDTA (0.1 mM) and ascorbic acid (1 mM). Extracts were centrifuged at

13,000 g at 4 °C for 20 min. Supernatant was collected and used for the enzyme assay.

SOD activity was measured according to the methods of Giannopolitise and Ries (1977), adapted for tomato seed analyses. The assay mix included: 50 mM of potassium phosphate buffer (pH = 7.8), 14 mM of methionine, 75 µM of nitro blue tetrazolium, 2 µM of riboflavin, 100 nM of EDTA and 20 µl of enzyme extract. Reaction was initiated by adding riboflavin and placing under fluorescent light (15 W), and was allowed to continue for 40 minutes, when was ended by switching off the light and covering the tubes with a black cloth. Subsequently, the absorbance of the assay mix was measured at 560 nm by a spectrophotometer BIOTEK, EPOCH model. One unit of SOD activity was defined as the amount of the enzyme required to reach 50% inhibition of the reaction in the "minus enzyme extract" control - which should have a higher absorbance compared to the samples with enzyme extract.

CAT activity was measured using the protocol of Havir and McHale (1987). The assay mix included 200 mM of potassium phosphate buffer (pH 7.0), 20 µl of enzyme extract, and 120 µl of 12.5 mM H_2O_2 solution. One unit of CAT activity was defined as the degradation of 1 µM of H2O2 during one minute at 240 nm (using extinction coefficient ε= 36 mM^{-1} cm^{-1}).

APX activity was measured using the protocol of Nakano and Asada (1981) modified for tomato seeds. The assay mix was composed of 50 mM of potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbic acid (ASA), 0.1 mM of EDTA, 20 µl of enzyme extract. H_2O_2 (0.1 mM) was added to initiate the reaction. One unit of APX was defined as the conversion of ASA (1 μM) into the monodehydroascorbate at 290 nm, during one minute (ε=2.8 mM^{-1} cm^{-1}).

Statistical analysis

The experimental design was completely randomized, with 3 replicates per treatment. An ANOVA was used to examine the effects and interactions of seed priming treatment (control, Hydro+SD and Hydro+FD), drought levels (0, 2, 4, 6 and rehydrated) and plant organ (leaves and root) on total soluble sugar, proline content, superoxide dismutase, catalase and ascorbate peroxidase activity. A Tukey test was used and the least significant difference (LSD) at $p \leq 0.05$ and calculated using the RStudio 3. 1. 1. (R Core Team 2014). All plant growth data was reported as mean ± standard error of the mean for five replicates calculated by the AOB tool.

Conclusions

Hydropriming of BRSMG Madrepérola common bean seeds with slow and fast drying protocols increased the rate and intensity of osmolity accumulation and the enzymatic antioxidant system in seedlings under drought conditions. A hydropriming "memory" effect may be involved in this result.

Acknowledgments

We acknowledgment the UFLA (University of Lavras, Lavras, MG) for structure. This work was support by CNPq (Conselho Nacional de

Desenvolvimento Tecnológico) and by FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais).

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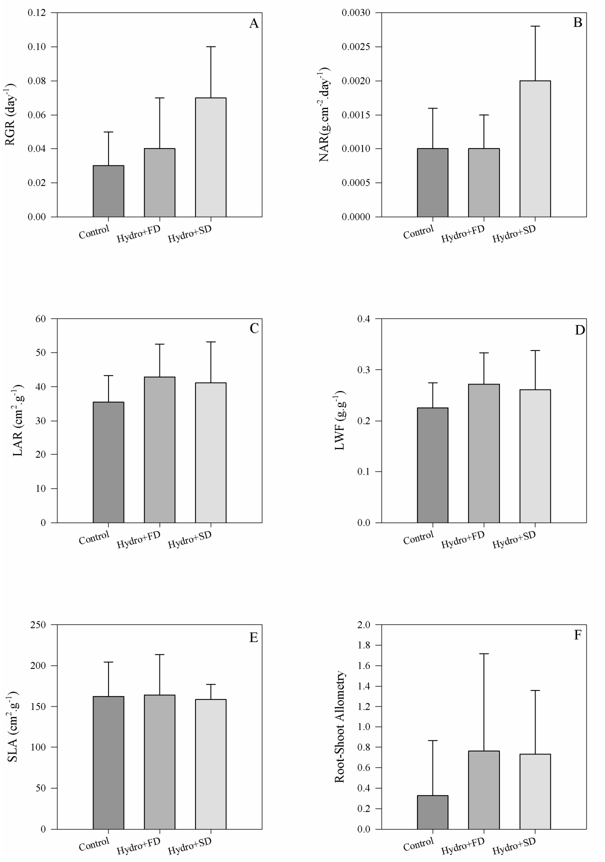

Fig. 1. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on BRSMG Madrepérola bean seedlings growing at 70% field capacity: A- relative growth rate (RGR); B- rate assimilatory net (NAR); C- ratio area leaf (LAR); D- leaf weight fraction (LWF), E- specific leaf area (SLA) and F- root-shoot allometry. * Means are followed by standard error bars (SE) (n= 5).

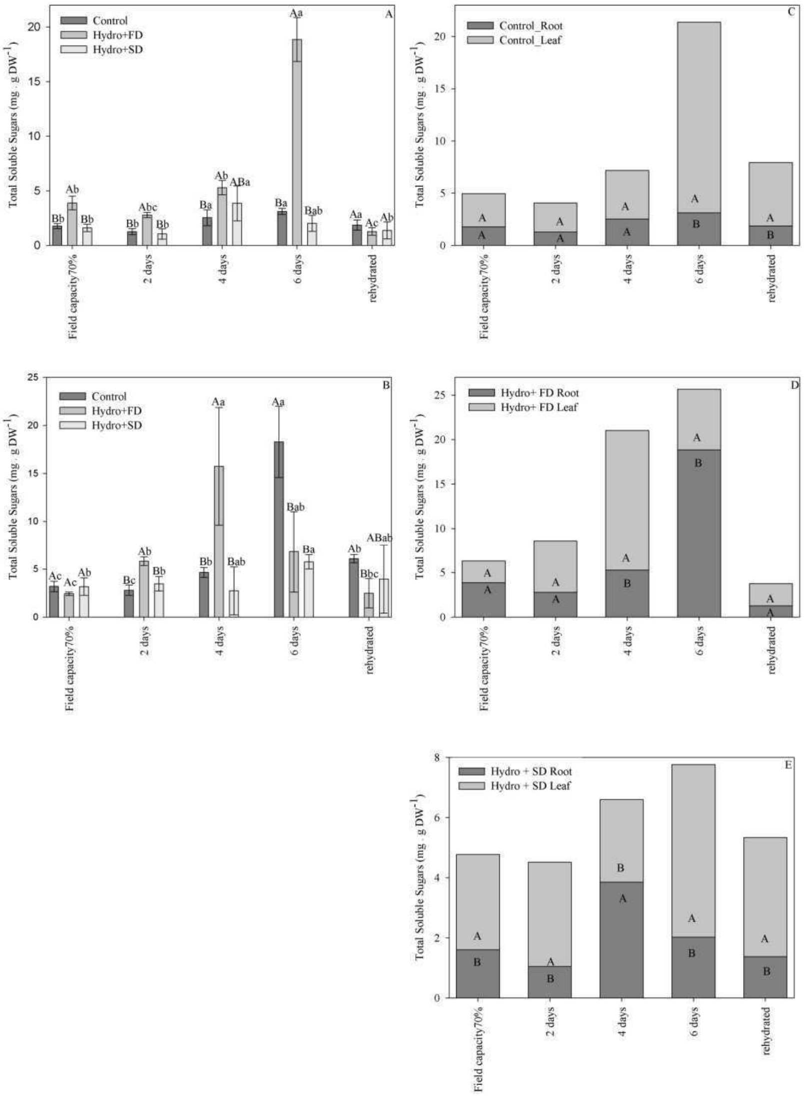

Fig. 2. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on total soluble sugars in BRSMG Madrepérola bean seedlings: A- roots; B- leaves; C- Control ratio root-leaf; D- Hydro+FD ratio root-leaf, E- Hydro+SD ratio root-leaf. Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$). Uppercase compared between treatments and lowercase compared between conditions.

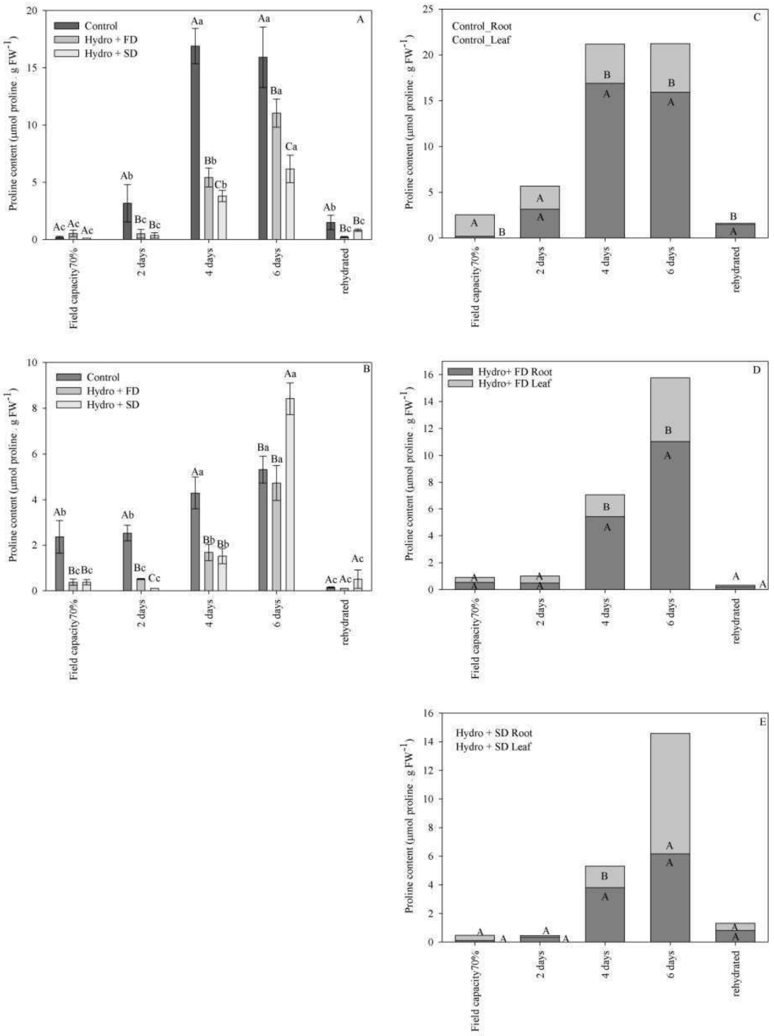

Fig. 3. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on free proline content in BRSMG Madrepérola bean seedlings: A- roots; B- leaves; C- Control ratio root-leaf; D- Hydro+FD ratio root-leaf, E- Hydro+SD ratio root-leaf. Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$). Uppercase represents comparisons between treatments and lowercase comparisons between conditions.

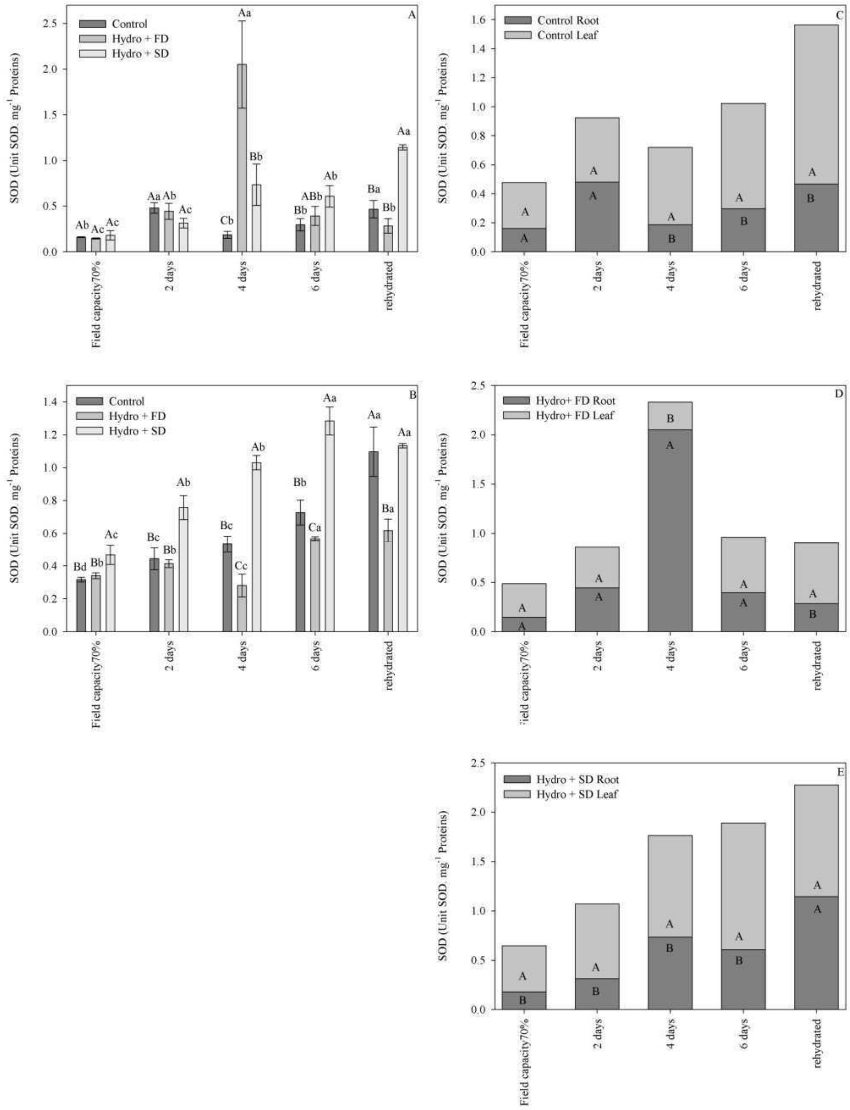

Fig. 4. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on SOD activity in BRSMG Madrepérola bean seedlings: A- roots; B- leaves; C- Control ratio root-leaf; D- Hydro+FD ratio root-leaf, E- Hydro+SD ratio root-leaf. Means followed by the same letter in columns do not differ by the Tukey test ($p \leq 0.05$). Uppercase

represents comparisons between treatments and lowercase comparisons between conditions.

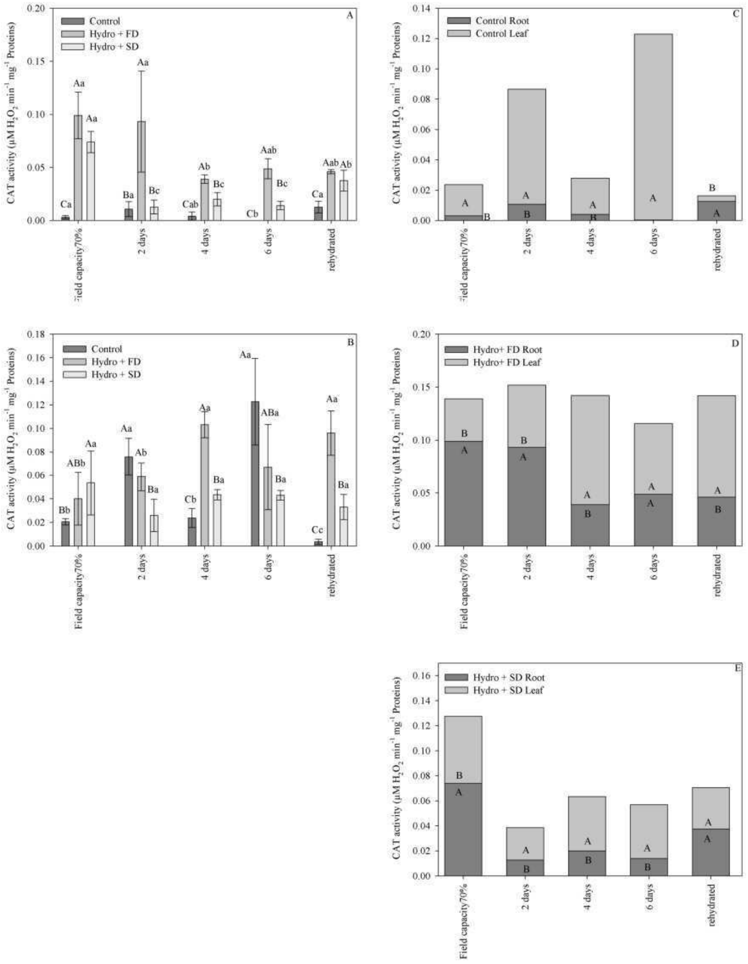

Fig. 5. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on CAT activity in BRSMG Madrepérola bean seedlings; B- leaves; C- Control ratio root-leaf; D- Hydro+FD ratio root-leaf, E- Hydro+SD ratio root-leaf. Means followed by the same letter in columns do not differ by the Tukey test (p≤0.05). Uppercase represents comparisons between treatments and lowercase comparisons between conditions.

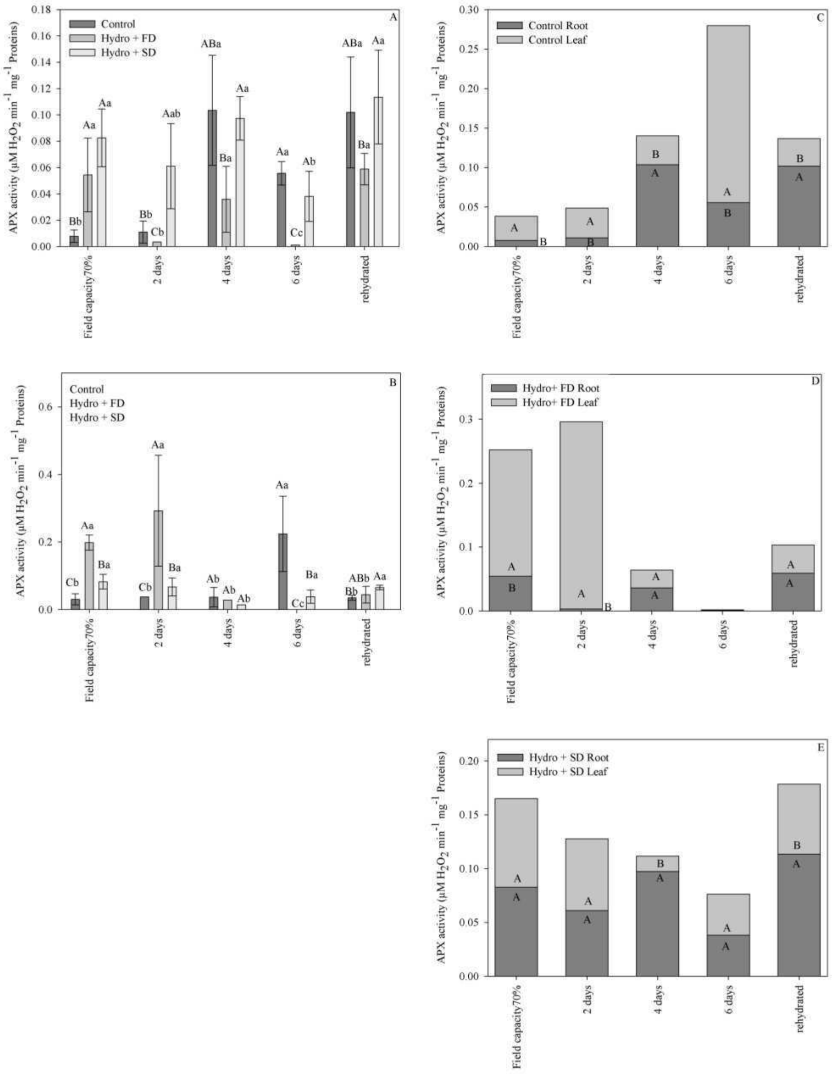

Fig. 6. Effect of different hydropriming treatments (Control, Hydro+SD and Hydro+FD.) on APX activity in BRSMG Madrepérola seedlings: A- roots; B- leaves; C- Control ratio root-leaf; D- Hydro+FD ratio root-leaf, E- Hydro+SD ratio root-leaf. Means followed by the same letter in columns do not differ by the Tukey test (p≤0.05).