UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE BOTÂNICA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

RODRIGO DE QUEIROGA MIRANDA

Avaliação da germinação e do desenvolvimento de Prosopis juliflora (Sw.) sob condições de baixo potencial hídrico do substrato

Recife
2013

RODRIGO DE QUEIROGA MIRANDA

Avaliação da germinação e do desenvolvimento de Prosopis juliflora (Sw.) sob condições de baixo potencial hídrico do substrato

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Vegetal do Departamento de Botânica, Centro de Ciências Biológicas da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Mestre em Biologia Vegetal.
Área de concentração: Ecologia
Orientadora: Profa. Jarcilene Almeida-Cortez
Coorientador: Prof. Marcelo Francisco Pompelli

Recife

2013

Catalogação na fonte
Elaine Barroso
CRB 1728

Miranda, Rodrigo de Queiroga
Avaliação da germinação e do desenvolvimento de *Prosopis juliflora* (Sw.) sob condições de baixo potencial hídrico do substrato/ Rodrigo de Queiroga Miranda– Recife: O Autor, 2013.

103 folhas : il., fig., tab.
Orientadora: Jarcilene Almeida-Cortez
Coorientador: Marcelo Francisco Pompelli
Dissertação (mestrado) – Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Biologia Vegetal, 2013.
Inclui bibliografia

1.Algaroba 2. Caatinga 3. I. Almeida-Cortez, Jarcilene (orientadora) II. Pompelli, Marcelo Francisco (coorientador) III. Título

583.748 CDD (22.ed.) UFPE/CCB- 2013- 183

RODRIGO DE QUEIROGA MIRANDA

"AVALIAÇÃO DA GERMINAÇÃO E DO DESENVOLVIMENTO DE *Prosopis juliflora (Sw.) SOB CONDIÇÕES DE BAIXO POTENCIAL HÍDRICO DO SUBSTRATO*".

BANCA EXAMINADORA:

Dra. Jarcilene Silva Almeida Cortez (Orientadora) – UFPE

Dra. Dra. Emília Cristina Pereira de Arruda - UFPE

Dr. Mauro Guida dos Santos - UFPE

Recife- PE
2013

Para minha mãe, meu pai,
minha irmã e minha namorada.

Agradecimentos

À minha família, namorada e amigos, que sempre me incentivaram e apoiaram.

À minha orientadora Dra. Jarcilene Almeida-Cortez, e meu co-orientador Dr. Marcelo Francisco Pompelli, pelo privilegio de ter sido orientado por ambos, por todo apoio, compreensão, amizade, confiança, imensa dedicação e paciência no desenvolvimento deste trabalho.

Ao meu amigo e professor Dr. Mauro Guida dos Santos por me tirar várias dúvidas e auxiliar direta e indiretamente em todo o desenvolvimento deste trabalho.

Á minha amiga Rafaela Moura Correia por ter me ajudado a conduzir uma considerável parte dos experimentos que compõem esta dissertação.

Ao meus amigos M.Sc. Marciel Teixeira de Oliveira e Camila Dias Barros Medeiros, cujos testes bioquímicos e orientação foram de crucial importância para uma condução precisa das análises bioquímicas que compõem este trabalho.

Ao meus amigos João Antônio de Almeida Granja e Girleyde da Costa Lima por terem me ajudado a conduzir uma significante parte das coletas e análises bioquímicas.

Às minhas amigas Dâmaris Gabriela Alves das Neves e Deborah Alani Silva de Oliveira por terem me ajudado a conduzir várias análises bioquímicas.

Aos meus amigos M.Sc. Hiram Marinho Falcão e M.Sc. Karla Viviane de Figueiredo por todas as dicas e orientação durante os experimentos e o desenvolvimento dessa dissertação.

À minha amiga M.Sc. Rebeca Rivas Costa por me ajudar com a assepsia das sementes utilizadas em todos os experimentos de germinação que compõem esta dissertação.

Aos meus amigos e colegas do LEV (Laboratório Ecofisiologia Vegetal), pelo imenso e extraordinário apoio durante todo o curso.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq (Recursos 473202/2008-5) e a Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco, FACEPE (Recursos APQ-0150-2.03/08) pelo financiamento deste trabalho, e pela bolsa de estudos concebida a mim durante o curso.

Sumário

1. Apresentação

A capacidade que algumas espécies apresentam de colonizar outros ambientes além do seu de ocorrência natural, não é um fenômeno novo e sempre exerceu um papel fundamental na dinâmica da biodiversidade (HURKA; BLEEKER; NEUFFER, 2003). Espécies invasoras são aquelas que estabelecem uma nova área de distribuição na qual proliferam, espalham-se, e persistem em detrimento do ambiente (PRITEKEL et al., 2006; REID et al., 2009). A invasão de novos territórios pode ser acidental ou deliberadamente antrópica ou causada por mudanças climáticas (HURKA; BLEEKER; NEUFFER, 2003).

Um exemplo de espécie invasora que vem recebendo atenção internacional por conta dos impactos negativos em ecossistemas de áreas cultivadas e de matas nativas é a *Prosopis juliflora* (Sw) DC. Na Nigéria, as raízes de *P. juliflora* impedem o fluxo normal da água em áreas do lago Chad (GALLAHER; MERLIN, 2010), problemas similares também foram encontrados no Yemen (LANDERAS et al., 2005). No Havaí, a deficiência na auto regeneração de populações de plantas nativas em zonas semiáridas é prejudicada principalmente devido à extensa cobertura das populações de *P. juliflora* (RICHMOND; MUELLER-DOMBOIS, 1972). Na Australia, *P. juliflora* também é considerada uma planta de comportamento invasivo extremamente agressivo (VAN KLINKEN; CAMPBELL, 2001).

No Brasil, *P. juliflora* foi introduzida no semiárido nordestino na década de 1940 como mais uma opção econômica para a convivência com a seca, por apresentar qualidades como forrageira, produtora de lenha e carvão (ANDRADE; FABRICANTE; OLIVEIRA, 2010; PEGADO et al., 2006). A partir daí, através dos plantios comerciais, regeneração natural e pela falta de manejo adequado, *P. juliflora* começou a invadir e se estabelecer rapidamente em áreas de relevante interesse social e ambiental como a Caatinga, haja vista que esta ocupa principalmente os neossolos flúvicos, as baixadas sedimentares e as manchas de solos mais profundos, onde a água é mais facilmente encontrada, representando grande risco na conservação da biodiversidade daquela região (ANDRADE; FABRICANTE; OLIVEIRA, 2010; PEGADO et al., 2006). Vale ressaltar também que em algumas destas áreas se encontram matas ciliares, que são ambientes legalmente enquadrados como Áreas de Preservação Permanente (APP) (Lei Brasileira 4.771/1965 e 7.803/1989), as quais desempenham funções ambientais e ecológicas estratégicas, por deterem elevado grau de endemismo e apresentarem biodiversidade potencialmente maior que suas adjacências (ANDRADE; FABRICANTE; OLIVEIRA, 2009).

Pensando nesta problemática, a dissertação ora apresentada, tem como objetivo elucidar as principais estratégias de adaptação de *Prosopis juliflora* (Sw) DC a condições de estresse hídrico e osmótico, afim de entender ecofisiologicamente porque essa espécie se tornou uma invasora tão agressiva do ecossistema Caatinga. Para alcançar tal objetivo, este trabalho foi dividido em 3 capítulos, dos quais o primeiro trata da quebra da dormência das sementes da espécie em questão, e tem como objetivo qualificar e quantificar, em laboratório, os efeitos de métodos de escarificação na viabilidade de sementes e plântulas de *P. juliflora*. O segundo capitulo trata das influências do solo salino e da concentração das chuvas em poucos meses na Caatinga sobre a germinação de *P. juliflora*, e tem como objetivo avaliar, em laboratório, os efeitos de cloreto de sódio (NaCl) e polietilenoglicol (PEG 6000) na velocidade de germinação, sincronia de germinação, germinabilidade, e viabilidade de sementes de *P. juliflora*. O terceiro capitulo tem como objetivo caracterizar ecofisiologica e bioquimicamente plantas jovens de *P. juliflora*, verificando as resposta das mesmas quando submetidas a diferentes tratamentos de baixo potencial hídrico do substrato.

2. Fundamentação teórica

2.1. Caracterização da Caatinga

O nordeste brasileiro corresponde a 18% do território nacional, sendo 75% deste, classificado como semiárido e árido (LIMA; BARBOSA; BARBOSA, 2009). A vegetação dominante nessa região é a Caatinga, nome indígena que significa "floresta branca" (SAMPAIO, 1995), e é constituída por um complexo vegetacional que abrange uma área de aproximadamente 800.000 km^2 (SANTOS et al., 2011). Nesta região, predomina na paisagem depressões que variam de 300 a 500m de altitude (SANTOS et al., 2011), e o regime anual de chuvas é concentrado num período de 3 a 4 meses e marcado por forte irregularidade, variando de 240 a 900 mm/ano (PRADO, 2003; SAMPAIO, 1995). As temperaturas médias variam de 23 °C a 27 °C (NASCIMENTO; ALVES, 2008). O solo é altamente salino devido ao alto nível de evaporação e à pobre drenagem, resultando na acumulação de sais na superfície edáfica (DANTAS et al., 2006; SAMPAIO, 1995). Os mapas vegetacionais atualmente disponíveis reconhecem a Caatinga como um mosaico de florestas secas e vegetações arbustivas (LEAL et al., 2005), sendo assim referenciada na literatura como Floresta Sazonal Tropical Seca (TOBY PENNINGTON; PRADO; PENDRY, 2000) ou como Vegetação Arbustiva (OLSON et al., 2001). Apesar da imprecisão na classificação da

Caatinga (PORTILLO-QUINTERO; SÁNCHEZ-AZOFEIFA, 2010; SANTOS et al., 2011), neste domínio destaca-se a vegetação característica de floresta seca (arbusto e árvores) (PRADO, 2003; SAMPAIO, 1995), especialmente nas áreas da depressão sertaneja, onde a maior parte dos indivíduos perdem as folhas, como adaptação à deficiência hídrica (KOZLOWSKI; KRAMER; PALLARDY, 1991; LARCHER, 2003) e apresenta proporção significativa de espécies espinhosas.

2.2. Caracterização da espécie *Prosopis juliflora* (Sw.) DC.

Prosopis L. é um gênero da família Fabaceae (Leguminosae), subfamília Mimosoideae, que é composto por arvores e arbustos de porte médio, os quais ocorrem naturalmente na África, Asia e nas Américas do Norte e do Sul. O mais importante trabalho sobre a taxonomia do gênero foi conduzido por Burkart (1976), que reconheceu 44 espécies. Dentre estas devemos destacar a *Prosopis juliflora* (Sw.) DC. como uma das mais conhecidas espécies do gênero e alvo de estudo deste trabalho.

Nativa do noroeste americano, *P. juliflora* possui uma vasta distribuição incluindo áreas da Venezuela, Colômbia, e Equador na América do Sul, e do Panamá e México na América Central (BURKART, 1976). *P. juliflora* está entre as mais agressivas espécies invasoras de ambientes áridos e semiáridos no mundo (BURGHARDT; ESPERT, 2007; BURKART, 1976; ELFADL; LUUKKANEN, 2003; PASIECZNIK et al., 2001). Sendo conhecida por vários nomes indígenas como Mesquite e Algarroba, os quais podemos destacar como os principais nomes nas Américas do Norte e do Sul respectivamente (D'ANTONI; SOLBRIG, 1977; FELGER, 1977). No Brasil, o nome foi modificado para Algaroba (ANDRADE; FABRICANTE; OLIVEIRA, 2010; PEGADO et al., 2006).

Prosopis juliflora possui hábito arbustivo ou arborescente e raramente raramente ultrapassa 10 m de altura (GALLAHER; MERLIN, 2010), e é conhecida por alcançar porte e produtividade máximas em áreas com água em abundância (SCHADE et al., 2003), e também como uma espécie "sempre verde", pois mantem as folhas em períodos de estiagem. *P. juliflora* possui um grande dossel aberto, a casca externa é áspera, repleta de fissuras, de cor avermelhada e bastante rígida devido à abundância de resina (GALLAHER; MERLIN, 2010), os galhos são verde-marrom com espinhos axiais situados em ambos os lados dos nós que podem atingir até 60 mm de comprimento cada (GALLAHER; MERLIN, 2010). O sistema radicular é do tipo pivotante e relativamente profundo, podendo alcançar 20-25 m abaixo do

solo (HAVARD, 1884; HEITSCHMIDT et al., 1988; PASIECZNIK et al., 2001). As folhas são compostas, bipinadas e têm filotaxia alterna, o seu comprimento varia de 5 a 20 cm, e cada folha é composta por 1 a 5 pares de folíolos, e cada um com 6-29 pares de foliólulos (PASIECZNIK; HARRIS; SMITH, 2004). As inflorescências são do tipo racemosas, axilares, cilíndricas com até 15 cm de comprimento (GALLAHER; MERLIN, 2010). Cada uma delas é constituída por centenas de flores amarelas de 0,5 cm de comprimento (GALLAHER; MERLIN, 2010). O cálice e a corola têm cinco lobos, as sépalas tendem a ser fundidas e as pétalas livres, e cada flor tem 10 estames e um único carpelo (GALLAHER; MERLIN, 2010). Os frutos são legumes indeiscentes e podem atingir mais de 25 cm de comprimento (BURKART, 1976), eles são verdes quando imaturos, e amarelos quando maduros, e podem possuir forma reta ou curva. O endocarpo é dividido em rígidos , coráceos e impermeáveis segmentos contendo uma marrom, elíptica semente (MEYER et al., 1971; SOLBRIG; CANTINO, 1975). Cada fruto contêm de 10 a 40 sementes, e estas são frequentemente rígidas e possuem tegumentos impermeáveis (MANGA; SEN, 1995), os quais garantem a dormência e são muito bem adaptados à períodos longos de seca (MARTIN, 1948; TSCHIRLEY; MARTIN, 1960), além de permitirem a dispersão por zoocoria (BEWLEY, 1997; GUTTERMAN, 1994).

2.3. Efeito da escarificação na germinação

Tradicionalmente a dormência é definida como o estado do desenvolvimento da semente no qual mesmo estando viável e sob condições ambientais favoráveis (*e.g.* disponibilidade de água), a germinação não acontece. Esse mecanismo é categorizado de acordo com o pré-requisito para quebrá-lo, como por exemplo o escarificação (dormência tegumentar ou física), envelhecimento acelerado (dormência morfológica), estratificação (dormência fisiológica) ou a exposição à luz (CARDOSO, 2009). A dormência é muito importante ecologicamente, pois promove a distribuição da germinação de sementes no tempo e espaço (EIRA; CALDAS, 2000; FOWLER; BIANCHETTI, 2000; FOWLER; MARTINS, 2001).

A dormência tegumentar é caracterizada quando o tegumento retarda a germinação da semente (FINKELSTEIN, 2006), conferindo-lha resistência e impermeabilidade à absorção de água e/ou de gases, além de impor uma restrição mecânica ao crescimento do embrião (POPINIGIS, 1985). Esse tipo de dormência pode ser superada através da escarificação, termo

que se refere a qualquer tratamento que resulte na ruptura ou no enfraquecimento do tegumento, que pode acontecer basicamente de duas formas: mecanicamente e/ou quimicamente (MARTINS; NAKAGAWA, 2008; MAYER; POLJAKOFF-MAYBER, 1989). A escarificação ocorre naturalmente no meio ambiente devido a vários fatores bióticos e abióticos, nos quais se incluem altas temperaturas (*e.g.* fogo), abrasão mecânica (*e.g.* areia e pedras em cursos d'água), e mudanças químicas no meio ambiente (*e.g.* ingestão por frugívoros e passagem pelo trato digestivo) (VILELA, 2001), sendo este último fator comum em ambientes áridos e semiáridos.

A germinação em sementes com tegumento espesso e/ou com dormência prolongada pode ser facilitada pela passagem das mesmas pelo trato intestinal de animais (LOHAMMAR, 1954). Santamaría et al., 2002 mostraram que a ingestão de sementes por pássaros pode afetar a germinabilidade (% final de sementes germinadas), a a taxa (velocidade) de germinação ou ambos. Em espécies de *Prosopis*, a ingestão parcial ou completa dos frutos por animais selvagens e domésticos pode promover e acelerar a germinação das sementes neles contidas (DANTHU et al., 1996), além de ajudar na dispersão das sementes. Entretanto, muito estudos têm mostrado que os efeitos da ingestão na capacidade germinativa, tempo médio de germinação e sincronia podem variar consideravelmente. A digestão pode aumentar (DANTHU et al., 1996; MIRANDA et al., 2011), diminuir (GÜNSTER, 1994; MIRANDA et al., 2011) ou não afetar (FIGUEROA; CASTRO, 2002; MIRANDA et al., 2011; OTANI, 2004) a germinação se comparada com sementes não ingeridas. Esta variação pode ser explicada por meio de simulação com ácido sulfúrico em laboratório. O ácido sulfúrico cria ou aumenta poros que permitem a entrada de água através do tegumento para o embrião, e acelerando assim a germinação. Na eficiência da escarificação química pode 2 fatores: (i) o ácido não erodiu o tegumento bastante, e assim não permitindo a entrada de água; (ii) o ácido perfurou o tegumento demais, a ponto de entrar em contato com o embrião e assim causar danos, diminuindo o vigor da semente (MIRANDA et al., 2011; UPRETI; DHAR, 1997).

A eficiência da escarificação química também foi constatada para sementes de *Cassia bicapsularis* L., *C. speciosa* Ried., *C. javanica* Schrad. (RODRIGUES; AGUIAR; SADER, 1990) e *C. sieberiana* D.C. (TODD-BOCKARIE et al., 1993); *Senna macranthera* (Colladon) Irwin & Barneby (LEMOS-FILHO et al., 1997) e *S. silvestris* (Vell.) Irwin & Barneby (MARANHO; PAIVA, 2012); *Samanea tubulosa* (Benth.) (GIACHINI et al., 2010); *Centrosema plumieri* (Benth.) (GAMA et al., 2011); *Prosopis juliflora* (Sw.) DC. (MIRANDA

et al., 2011).

2.4. Efeito da temperatura na germinação

Variações na temperatura estão relacionadas a mudanças na porcentagem, velocidade e frequência relativa de germinação ao longo do tempo de incubação (ARAÚJO NETO; AGUIAR; FERREIRA, 2003; POMPELLI; FERNANDES; GUERRA, 2006). Cada espécie apresenta uma faixa de temperatura para germinação (SOCOLOWSKI; TAKAKI, 2004), a qual é composta de um valor ótimo no qual se registra a mais alta germinabilidade no menor espaço de tempo, além de valores máximos e mínimos nos quais geralmente não ocorre germinação (ARAÚJO NETO; AGUIAR; FERREIRA, 2003).

Danos significativos foram registrados em sementes de *Agave asperrima, A. duranguensis* e *A. salmiana* quando submetidas a temperaturas extremas durante a germinação (RAMÍREZ-TOBÍAS et al., 2012). Efeitos similares foram encontrados em sementes de *Lactuca sativa* L. (SCHWEMBER; BRADFORD, 2010). Isto provavelmente aconteceu devido a diminuição ou aumento drástico das taxas metabólicas até o ponto em que processos essenciais para a germinação colapsaram e deixaram de ocorrer (AMARAL; PAULILO, 1992; HENDRICKS; TAYLORSON, 1976), pois a temperatura afeta diretamente a atividade enzimática nas sementes por meio de mudanças na energia de ativação das enzimas e na conformação de proteínas (CARDOSO, 1999; ÖPIK; ROLFE, 2006). Além disso, tanto o frio como o calor podem afetar a conformação da membrana, tornando-a mais rígida ou fluida respectivamente, e assim modificando a sua permeabilidade, promovendo saída de soluto da célula, o que pode ocasionar muitas vezes em morte celular (BEWLEY; BLACK, 1994; MURPHY; NOLAND, 1982; ÖPIK; ROLFE, 2006; VERTUCCI; LEOPOLD, 1987). Por outro lado, dentro de certos limites de temperatura a velocidade de absorção de água e das reações químicas são mais intensas, registrando as maiores germinabilidade e velocidade de germinação (CARVALHO; NAKAGAWA, 2000). Esta é conhecida como faixa de temperatura ótima para a germinação (CARVALHO; NAKAGAWA, 2000; MIRANDA et al., 2011; POMPELLI; FERNANDES; GUERRA, 2006).

Sob um ponto de vista ecológico, em ambientes semiáridos, como é o caso da Caatinga, durante a estação seca quando as condições de temperatura no solo são normalmente supra ótimas, as sementes tendem a entrar num estado de inibição ou dormência térmica até que as condições voltem a ser favoráveis à sua germinação, e ao desenvolvimento

da plântula (HILHORST, 1998; PROBERT, 2000). Pois temperaturas altas, além de reduzir a velocidade de germinação (BEWLEY; BLACK, 1994), diminuem a taxa de crescimento das plântulas,expondo-as por um período maior a fatores adversos (CARVALHO; NAKAGAWA, 2000).

Permanecer inativa durante a estação seca e germinar rapidamente na estação chuvosa, aproveitando a alta umidade do solo e as temperaturas mais amenas, é uma estratégia bastante difundida entre as espécies ocorrentes em ambientes áridos e semiáridos (FINCH-SAVAGE et al., 2010), consequentemente essas espécies normalmente sofrem a alta competição que marca a estação chuvosa nesses locais (WATT; BLOOMBERG; FINCH-SAVAGE, 2011).

2.5. Efeito dos estresses osmótico e salino na germinação

A habilidade de uma semente de germinar sob amplo limite de condições pode ser a manifestação de seu vigor, dependendo entre outros fatores, das condições ambientais encontradas no local onde foi semeada (BEWLEY, 1997). Secas periódicas, por exemplo, podem ser encontradas no campo, e a semente deverá ser hábil para supera-las e germinar com sucesso quando as condições voltarem a serem propícias (FANTI; PEREZ, 1998).

A escassez dos recursos hídricos por longos períodos acarreta na imobilização de nutrientes minerais no solo, fenômeno conhecido como salinização do solo, o qual pode se tornar o principal fator limitante na produção vegetal (CAVALCANTI; RESENDE; BRITO, 2000; KHAN; GULZAR, 2003; PARIDA; DAS, 2005), pois os estresses osmótico e salino, além de afetarem o estabelecimento e crescimento das plântulas (ASCH; DINGKUHN; DORFFLING, 2000; SULTANA; IKEDA; ITOH, 1999), atuam diminuindo a porcentagem e a velocidade de germinação (POMPELLI; FERNANDES; GUERRA, 2006). Este padrão corrobora com os dados obtidos por Fonseca e Perez (2003) que observaram maior tempo requerido para a germinação quando o potencial osmótico foi reduzido para a espécie *Adenanthera pavonina*. O mesmo foi observado para as espécies *Bowdichia virgilioides* (SILVA; AGUIAR; RODRIGUES, 2001), *Peltophorum dubium* (PEREZ; FANTI; CASALI, 2001) e *Senna occidentalis* (DELACHIAVE; PINHO, 2003). Além disso em um estudo com sementes de leguminosas da savana africana (*Combretum apiculatum, Colophospermum mopane, Acacia karroo e A. tortilis*), Choinski e Tuohy (1991) verificaram redução da germinação em potenciais a partir de -0,3MPa. Estes decréscimos nos atributos da germinação de sementes residem no fato de que em condições de salinidade e restrição hídrica ocorrem

um prolongamento da fase estacionária do processo de embebição, diminuindo a velocidade de absorção de água pelas sementes (CAVALCANTE; PEREZ, 1995; HEGARTY, 1977; ZENG; WANG; ZHANG, 2010), e consequentemente reduzindo a atividade enzimática, o que resulta em menor desenvolvimento meristemático e atraso na protrusão da radícula (BEWLEY, 1997; BOYDAK et al., 2003). Bouaziz e Hicks (1990) sugeriram que a salinidade afeta a germinação limitando a mobilização de reservas em muitas espécies, reduzindo a porcentagem de germinação. Entretanto Almansouri et al. (2001) em um experimento de embebição de sementes com PEG e NaCl mostrou que a diferença nos teores de açucares solúveis em relação ao tratamento controle não é significante, sugerindo que o contraste observado nos atributos de germinação das sementes quando embebidas em PEG e NaCl não se deve à mobilização de reservas. Além disso, o sal pode intoxicar a célula (SONG et al., 2005), causando inicialmente diminuição de turgor (HEGARTY, 1977), e em seguida inibindo alguns processos metabólicos (CAMPOS et al., 2012; MOHAMMADKHANI; HEIDARI, 2008).

Assim não é surpreendente que as plantas tenham desenvolvido vários mecanismos de proteção contra os efeitos impostos por solos salinos. Estes mecanismos incluem aumento em proteínas envolvidas no transporte de água (*e.g.* aquaporinas), sequestro e secreção de íons, e aumento de osmólitos ou solutos compatíveis (LEATHERWOOD et al., 2007), os quais muitas vezes são armazenados em vacúolos afim de diminuir o seu potencial osmótico, e permitir absorver água do ambiente mais eficientemente (PARIDA; DAS, 2005; SONG et al., 2005; ZENG; WANG; ZHANG, 2010; ZHANG et al., 2010). Um bom exemplo são as sementes de algumas halófitas perenes que podem germinar em níveis de NaCl iguais ou superiores a 860 mM com a *Salicornia pacifica* (KHAN; WEBER, 1986) e a *Cressa cretica* (KHAN, 1991). No entanto mesmo entre as halófitas, há espécies como *Zygophyllum simplex* que apresenta baixa germinação de sementes acima de 125mM NaCl (KHAN; UNGAR, 1997). No geral, a ótima germinação em sementes de halófitas ocorre frequentemente sob condições de água doce, entretanto, como visto, a resposta pode variar muito dependendo da espécie.

Em habitats semiáridos, onde as condições para que a germinação ocorra podem ser imprevisíveis no tempo e espaço (SCIFRES; BROCK, 1972), a germinação geralmente acontece no período chuvoso, pois as chuvas mobilizam os nutrientes no solo, aumentando o potencial osmótico do substrato e diminuindo os níveis de salinidade (EL-KEBLAWY, 2004;

UNGAR, 1995). Esse curto período influencia fortemente a germinação e o estabelecimento de plântulas, pois estas devem crescer rapidamente para conseguir superar os estresses ambientais severos (MEYER et al., 1971; SCIFRES; BROCK, 1972; SCIFRES; HAHN, 1971).

2.6. Efeito do estresse hídrico na fotossíntese e no sistema antioxidativo

Nas plantas, existe um complexo sistema hidráulico, onde todas as partes funcionais são interligadas por uma fase líquida, permitindo o movimento da água nas folhas, no caule, e nas raízes (DAVIES; WILKINSON; LOVEYS, 2002; JAVOT, 2002; SCHOLANDER; HAMMEL; BRADSTREET, 1960). A teoria mais aceita afirma que esse movimento é causado pela redução do potencial hídrico foliar, resultante da transpiração foliar (*E*) (DAVIES; WILKINSON; LOVEYS, 2002; JARVIS; MCNAUGHTON, 1986; NOORMETS et al., 2001).

A condutância estomática (g_s) é o principal mecanismo fisiológico que as plantas vasculares dispõem para o controlar a transpiração foliar (*E*) (DAVIES; WILKINSON; LOVEYS, 2002; JAVOT, 2002). As principais funções adaptativas dos estômatos são a otimização das trocas gasosas, e redução da desidratação, especialmente em folhas com potencial hídrico abaixo do ponto de cavitação do xilema (JAVOT, 2002). A forma como a taxa de assimilação de CO_2 (P_N) relaciona-se com g_s possui uma importância ecológica, pois quando a P_N e *gs* variam proporcionalmente, numa relação linear, é possível dizer que a concentração interna de CO_2 (C_i) e a eficiência intrínseca do uso da água (EUA_i) mantêm-se constantes, no sentido de otimizarem as trocas gasosas (CHAVES; FLEXAS; PINHEIRO, 2009; SOUZA; RODRIGUES; et al., 2010). Oliveira et al. (2002), trabalhando com imposição de deficiência hídrica em *Bactris gasipaes* Kunth., observaram que, apesar de P_N e g_s variarem proporcionalmente numa relação linear, EUA_i diminuiu com o decréscimo de g_s, indicando que a diminuição de *gs*, durante o estresse, provocou uma redução na eficiência dos processos fotossintéticos. Por outro lado, Rocha e Moraes (1997) encontraram respostas diferentes, nas quais foi observado um aumento no EUA_i, quando g_s e *E* foram menores do que P_N em plantas de *Stryphnodendron adstringens* (Mart.) Coville. Provavelmente, essa espécie esteja mais bem adaptada à deficiência hídrica do que a *B. gasipaes*, uma vez que esta última é uma planta característica de ambiente tropical úmido. Sugere-se, então, que o aumento de C_i, sob baixos valores de potencial hídrico foliar, pode estar relacionado à queda

na atividade de enzimas envolvidas no processo de fixação de CO_2 (MACHADO; MEDINA; GOMES, 1999; MACHADO et al., 2005).

Contudo quando g_s atinge valores muito próximos de 0, espera-se que o C_i comece a diminuir proporcionalmente à g_s, sugerindo que a limitação estomática seria o fator principal limitando o desempenho fotossintético, uma vez que quanto menor a abertura estomática menor a difusão de CO_2 para a câmara subestomática (CHAVES; FLEXAS; PINHEIRO, 2009). Entretanto quando ocorre aumento no C_i associado diminuição de g_s, o decréscimo de P_N indica que esta limitação é devida não somente ao aumento da resistência estomática, mas, também, ao efeito do estresse hídrico sobre as enzimas do ciclo Calvin, ou sobre a capacidade mesofílica (BOTA; MEDRANO; FLEXAS, 2004; CARMO-SILVA et al., 2012; DE SOUZA et al., 2005; FARQUHAR; SHARKEY, 1982; GILBERT; ZWIENIECKI; HOLBROOK, 2011; NOORMETS et al., 2001; PERRY; KRIEG; HUTMACHER, 1983). Porém esta afirmação nem sempre é verdadeira, devido à ocorrência de fechamento não uniforme dos estômatos (DOWNTON; LOVEYS; GRANT, 1988). Alguns autores atribuem a manutenção aproximadamente constante de C_i, durante o estresse hídrico, a valores desuniformes de g_s (GUNASEKERA; BERKOWITZ, 1992). No entanto, esta heterogeneidade não é comum a todas as espécies, principalmente quando o estresse é imposto lentamente (GUNASEKERA; BERKOWITZ, 1992).

Além disso já é bem documentado que sob condições de baixos níveis de g_s, onde há um aumento nas taxas de fotorrespiração, e, consequentemente, na produção de espécies reativas ao oxigênio (ERO) nos cloroplastos (SMIRNOFF, 1993), os quais, em altos níveis, também podem ser considerados como um fator de limitação não estomatal de P_N. As ERO são conhecidas por causarem danos ao DNA, e aos lipídios presentes nas membranas celulares, por meio de um processo chamado de peroxidação (IMLAY, 2003).

O conteúdo de malondialdéido (MDA) tem sido apontado como um bom indicador de dano oxidativo (MØLLER; JENSEN; HANSSON, 2007). De fato, o MDA é um dos produtos finais resultantes da peroxidação de lipídios por ERO. Em contra partida, as plantas dispõem de enzimas de proteção contra ERO (*e.g.* superoxido dismutase - SOD) (MØLLER; JENSEN; HANSSON, 2007; POMPELLI et al., 2010). Neste trabalho foram analisados conteúdos de SOD e MDA, pois estes são, resumidamente, a primeira defesa e o resultado final, respectivamente, resultantes da presença de ERO na planta. A SOD captura ERO no tecidos, e produz H_2O_2, o qual, por sua vez, é o primeiro produto do ciclo antioxidativo e um indicador

da eficacia da SOD (CENTRITTO et al., 2005; POMPELLI et al., 2010).

Outro efeito do deficit hídrico na fotossíntese é a exposição da planta a energia excessiva, pois níveis ótimos de luz em plantas bem hidratadas se tornam excessivos para plantas sofrendo privação de água (LUNA et al., 2005; SOUZA; MEIADO; et al., 2010). Sob tais condições, a energia captada em excesso não se dissipa com segurança e pode se tornar danosa ao fotossistema II (PSII), e consequentemente, aos centros de reação funcionais (DEMMIG-ADAMS; ADAMS, 1992; SOUZA; MEIADO; et al., 2010).

3. Referências bibliográficas

ALMANSOURI, M.; KINET, J.; LUTTS, S. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). **Plant and Soil**, v. 231, n. 2, p. 243–254, jun. 2001.

AMARAL, L. I. V; PAULILO, M. T. F. Efeito da luz, temperatura, reguladores de crescimento e nitrato de potássio na germinação de *Miconia cinnamomifolia* (DC) Naudin. **Insula**, v. 21, p. 59-86, 1992.

ANDRADE, L. A. DE; FABRICANTE, J. R.; OLIVEIRA, F. X. DE. Invasão biológica por *Prosopis juliflora* (Sw.) DC.: impactos sobre a diversidade e a estrutura do componente arbustivo-arbóreo da caatinga no Estado do Rio Grande do Norte, Brasil. **Acta Botanica Brasilica**, v. 23, n. 4, p. 935-943, dez. 2009.

ANDRADE, L. A. DE; FABRICANTE, J. R.; OLIVEIRA, F. X. DE. Impactos da invasão de *Prosopis juliflora* (sw.) DC. (Fabaceae) sobre o estrato arbustivo-arbóreo em áreas de Caatinga no Estado da Paraíba, Brasil. **Acta Scientiarum. Biological Sciences**, v. 32, n. 3, p. 249-255, 15 set. 2010.

ARAÚJO NETO, J. C.; AGUIAR, I. B.; FERREIRA, V. M. Efeito da temperatura e da luz na germinação de sementes de *Acacia polyphylla* DC. **Revista Brasileira de Botânica**, v. 26, n. 2, p. 249-256, jun. 2003.

ASCH, F.; DINGKUHN, M.; DORFFLING, K. Salinity increases CO2 assimilation but reduces growth in field-grown, irrigated rice. **Plant and Soil**, v. 218, n. 1-2, p. 1-10, 2000.

BEWLEY, J. D. Seed germination and dormancy. **The Plant Cell**, v. 9, n. 7, p. 1055-1066, 1997.

BEWLEY, J. D.; BLACK, M. **Seeds: physiology of development and germination**. New York: Plenum Press, 1994. p. 445

BOTA, J.; MEDRANO, H.; FLEXAS, J. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? **New Phytologist**, v. 162, n. 3, p. 671-681, jun. 2004.

BOUAZIZ, A.; HICKS, D. R. Consumption of wheat seed reserves during germination and early growth as affected by soil water potential. **Plant and Soil**, v. 128, n. 2, p. 161-165, nov. 1990.

BOYDAK, M. et al. Effects of water stress on germination in six provenances of *Pinus brutia*

seeds from different bioclimatic zones in Turkey. **Turkish Journal of Agriculture and Forestry**, v. 27, n. 2, p. 91-97, 2003.

BURGHARDT, A. D.; ESPERT, S. M. Phylogeny of *Prosopis* (Leguminosae) as shown by morphological and biochemical evidence. **Australian Systematic Botany**, v. 20, n. 4, p. 332, 2007.

BURKART, A. A monograph of the genus *Prosopis* (Leguminosae subfam. Mimosoideae). (Part 1 and 2). Catalogue of the recognized species of *Prosopis*. **Journal of the Arnold Arboretum**, v. 57, p. 219–249 / 450–525, 1976.

CAMPOS, M. L. DE O. et al. Photosynthesis and antioxidant activity in *Jatropha curcas* L. under salt stress. **Brazilian Journal of Plant Physiology**, v. 24, n. 1, p. 55-67, 2012.

CARDOSO, V. Thermal-dependence of the germination of *Catharanthus roseus* seeds. **Revista Brasileira de Fisiologia Vegetal**, v. 11, n. 1, p. 41-49, 1999.

CARDOSO, V. J. M. Conceito e classificação da dormência em sementes. **Oecologia Brasiliensis**, v. 13, n. 4, p. 619-631, 2009.

CARMO-SILVA, A. E. et al. Decreased CO2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. **Environmental and Experimental Botany**, v. 83, p. 1-11, nov. 2012.

CARVALHO, N. M.; NAKAGAWA, J. **Sementes: ciência, tecnologia e produção**. 4 ed. ed. Jaboticabal: FUNEP, 2000. p. 429

CAVALCANTE, A. D. M. B.; PEREZ, S. C. J. G. D. A. Effects of water and salty stress on germination of *Leucaena Leucocephala* (Lam.) de Wit seeds. **Pesquisa Agropecuaria Brasileira**, v. 30, n. 2, p. 281-289, 1995.

CAVALCANTI, N. DE B. .; RESENDE, G. M. .; BRITO, L. T. DE L. **Extrativismo vegetal como falta de absorção de mão-de-obra e geração de renda: o caso do umbuzeiro (Spondias tuberosa, Arruda Câmara)**. Petrolina: Embrapa, 2000.

CENTRITTO, M. et al. Effects of partial rootzone drying (PRD) on adult olive tree (*Olea europaea*) in field conditions under arid climate. **Agriculture, Ecosystems & Environment**, v. 106, n. 2-3, p. 303-311, 2 abr. 2005.

CHAVES, M. M.; FLEXAS, J.; PINHEIRO, C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. **Annals of botany**, v. 103, n. 4, p. 551-560, fev. 2009.

CHOINSKI, J.; TUOHY, J. Effect of water potential and temperature on the germination of four species of African savanna trees. **Annals of Botany**, v. 68, n. 3, p. 227, 1991.

D'ANTONI, H. L.; SOLBRIG, O. T. Algarrobos in South American cultures past and present. In: SIMPSON, B. B. (Ed.). **Mesquite: Its biology in two desert scrub ecosystems**. Stroudsburg: Dowden, Hutchinson, and Ross, 1977. p. 189–199.

DANTAS, J. A. et al. Efeito da salinidade sobre o crescimento e composição mineral de seis clones de *Pennisetum*. **Revista Ciência Agronômica**, v. 37, n. 1, p. 97-101, 2006.

DANTHU, P. et al. Effet du passage par le tractus digestif des ruminants domestiques sur la germination des graines de légumineuses ligneuses des zones tropicales sèches. **Revue d'elevage et de Medecine Veteri- naire des Pays Tropicaux**, v. 49, n. 3, p. 235–242, 1996.

DAVIES, W. J.; WILKINSON, S.; LOVEYS, B. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. **New Phytologist**, v. 153, n. 3, p. 449-460, mar. 2002.

DE SOUZA, C. R. et al. Impact of deficit irrigation on water use efficiency and carbon isotope composition (delta13C) of field-grown grapevines under Mediterranean climate. **Journal of experimental botany**, v. 56, n. 418, p. 2163-72, ago. 2005.

DELACHIAVE, M. E. A.; PINHO, S. Z. DE. Germination of *Senna Occidentalis* link: seed at different osmotic potential levels. **Brazilian Archives of Biology and Technology**, v. 46, n. 2, p. 163-166, mar. 2003.

DEMMIG-ADAMS, B.; ADAMS, W. W. Photoprotection and Other Responses of Plants to High Light Stress. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 43, n. 1, p. 599-626, jun. 1992.

DOWNTON, W. J. S.; LOVEYS, B. R.; GRANT, W. J. R. Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. **New Phytologist**, v. 110, n. 4, p. 503-509, dez. 1988.

EIRA, M. T. S.; CALDAS, L. S. Seed dormancy and germination as concurrent processes. **Revista Brasileira de Fisiologia Vegetal**, v. 12, n. 1, p. 85-104, 2000.

EL-KEBLAWY, A. Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*. **Seed Science and Technology**, v. 32, n. 3, p. 873–878, 2004.

ELFADL, M. A.; LUUKKANEN, O. Effect of pruning on *Prosopis juliflora*: considerations for tropical dryland agroforestry. **Journal of Arid Environments**, v. 53, n. 4, p. 441-455, abr. 2003.

FANTI, S. C.; PEREZ, S. C. J. G. A. Efeitos do estresse hídrico salino e térmico no processo germinativo de sementes de *Andenanthera pavonina* L. **Revista Brasileira de Sementes**, v. 20, n. 1, p. 167-177, 1998.

FARQUHAR, G. D.; SHARKEY, T. D. Stomatal Conductance and Photosynthesis. **Annual Review of Plant Physiology**, v. 33, n. 1, p. 317-345, jun. 1982.

FELGER, R. S. Mesquite in Indian cultures of southwestern North America. In: SIMPSON, B. B. (Ed.). **Mesquite: Its biology in two desert scrub ecosystems**. Stroudsburg: Dowden, Hutchinson, and Ross, 1977. p. 151–176.

FIGUEROA, J. A.; CASTRO, S. A. Effects of bird ingestion on seed germination of four woody species of the temperate rainforest of Chiloe island, Chile. **Plant Ecology**, v. 160, n. 1, p. 17-23, jan. 2002.

FINCH-SAVAGE, W. E. et al. Towards a genetic understanding of seed vigour in small-seeded crops using natural variation in *Brassica oleracea*. **Plant Science**, v. 179, n. 6, p. 582-589, dez. 2010.

FINKELSTEIN, R. Abscisic Acid: A seed maturation and antistress signal. In: TAIZ, L.; ZEIGER, E. (Eds.). **Plant Physiology**. 4. ed. Sunderland: Sinauer Associates, 2006. p. 539–558.

FONSECA, S. C. L.; PEREZ, S. C. J. G. DE. Ação do polietileno glicol na germinação de sementes de *Adenanthera pavonina* L. e o uso de poliaminas na atenuação do estresse hídrico sob diferentes temperaturas. **Revista Brasileira de Sementes**, v. 25, n. 1, p. 1-6, jul. 2003.

FOWLER, A. J. P.; BIANCHETTI, A. **Dormência em sementes florestais**. Colombo: Embrapa Florestas, 2000. p. 27

FOWLER, J. A. P.; MARTINS, E. G. **Manejo de sementes de espécies florestais**. Colombo: Embrapa Florestas, 2001. p. 76

GALLAHER, T.; MERLIN, M. Biology and Impacts of Pacific Island Invasive Species. 6. *Prosopis pallida* and *Prosopis juliflora* (Algarroba, Mesquite, Kiawe) (Fabaceae). **Pacific Science**, v. 64, n. 4, p. 489-526, out. 2010.

GAMA, J. S. N. et al. Superação de dormência em sementes de *Centrosema plumieri* Benth. **Revista Brasileira de Sementes**, v. 33, n. 4, p. 643-651, 2011.

GIACHINI, R. M. et al. Influência da escarificação e da temperatura sobre a germinação de sementes de *Samanea tubulosa* (Benth.) Barneby & J.W. Grimes (sete cascas). **Acta Amazonica**, v. 40, n. 1, p. 75-80, mar. 2010.

GILBERT, M. E.; ZWIENIECKI, M. A; HOLBROOK, N. M. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. **Journal of experimental botany**, v. 62, n. 8, p. 2875-87, maio. 2011.

GUNASEKERA, D.; BERKOWITZ, G. A. Heterogenous stomatal closure in response to leaf water deficits is not a universal phenomenon. **Plant physiology**, v. 98, n. 2, p. 660-665, fev. 1992.

GÜNSTER, A. Seed bank dynamics — longevity, viability and predation of seeds of serotinous plants in the central Namib Desert. **Journal of Arid Environments**, v. 28, n. 3, p. 195-205, nov. 1994.

GUTTERMAN, Y. Strategies of seed dispersal and germination in plants inhabiting deserts. **The Botanical Review**, v. 60, n. 4, p. 373-425, out. 1994.

HAVARD, V. The mezquit. **The American Naturalist**, v. 18, n. 5, p. 451-459, 1884.

HEGARTY, T. W. Seed aActivation and seed germination under moisture stress. **New Phytologist**, v. 78, n. 2, p. 349-359, mar. 1977.

HEITSCHMIDT, R. K. et al. Some observations from the excavation of honey mesquite root systems. **Journal of Range Management**, v. 41, n. 3, p. 227-231, 1988.

HENDRICKS, S. B.; TAYLORSON, R. B. Variation in germination and amino Acid leakage of seeds with temperature related to membrane phase change. **Plant physiology**, v. 58, n. 1, p. 7-11, jul. 1976.

HILHORST, H. W. M. The regulation of secondary dormancy. The membrane hypothesis revisited. **Seed Science Research**, v. 8, n. 2, p. 77-90, 19 set. 1998.

HURKA, H.; BLEEKER, W.; NEUFFER, B. Evolutionary Processes Associated with Biological Invasions in the Brassicaceae. **Biological Invasions**, v. 5, n. 4, p. 311-322, 2003.

IMLAY, J. A. Pathways of oxidative damage. **Annual review of microbiology**, v. 57, p. 395-418, jan. 2003.

JARVIS, P. G.; MCNAUGHTON, K. G. Stomatal Control of Transpiration: Scaling Up from Leaf to Region. **Advances in Ecological Research**, v. 15, n. 1, p. 1-49, 1986.

JAVOT, H. The Role of Aquaporins in Root Water Uptake. **Annals of Botany**, v. 90, n. 3, p.

301-313, 5 ago. 2002.

KHAN, M. Studies on germination of *Cressa cretica* L. seeds. **Pakistan Journal of Weed Science Research**, v. 4, n. 1, p. 89–98, 1991.

KHAN, M. A.; GULZAR, S. Light, salinity, and temperature effects on the seed germination of perennial grasses. **American journal of botany**, v. 90, n. 1, p. 131-4, jan. 2003.

KHAN, M. A.; UNGAR, I. A. Alleviation of Seed Dormancy in the Desert Forb *Zygophyllum simplex* L. from Pakistan. **Annals of Botany**, v. 80, n. 4, p. 395-400, out. 1997.

KHAN, M. A.; WEBER, D. J. Factors influencing seed germination in *Salicornia pacifica* var. utahensis. **American Journal of Botany**, v. 73, n. 8, p. 1163–1167, 1986.

KOZLOWSKI, T. T.; KRAMER, P. J.; PALLARDY, S. G. **The physiological ecology of woody plants**. New York: Academic Press, Inc., 1991. p. 248-302

LANDERAS, G. et al. Identification of *Prosopis juliflora* and *Prosopis pallida* Accessions Using Molecular Markers. **Biodiversity and Conservation**, v. 15, n. 5, p. 1829-1844, 13 jun. 2005.

LARCHER, W. **Physiological plant ecology: ecophysiology and stress physiology of functional groups**. 4rd. ed. Berlin / London: Springer Verlag, 2003. p. 533

LEAL, I. R. et al. Changing the Course of Biodiversity Conservation in the Caatinga of Northeastern Brazil. **Conservation Biology**, v. 19, n. 3, p. 701-706, jun. 2005.

LEATHERWOOD, W. R. et al. Carbonhydrate Content and Root Growth in Seeds Germinated Under Salt Stress: Implications for Seed Conditioning. **Journal of the American Society for Horticultural Science**, v. 132, n. 6, p. 876-882, 2007.

LEMOS-FILHO, J. P. DE et al. Germinação de sementes de *Senna macranthera*, *Senna multijuga* e *Stryphnodendron polyphyllum*. **Pesquisa Agropecuária Brasileira**, v. 32, n. 4, p. 357-361, 1997.

LIMA, L. C. M. .; BARBOSA, M. C. A. .; BARBOSA, D. C. A. **Calendário didático de floração de espécies lenhosas da caatinga de Pernambuco, com base em coleções do Herbário UFP - Geraldo Mariz**. Recife: Editora Universitária da UFPE, 2009.

LOHAMMAR, G. Matsmältningens inverkan på Potamogeton-frönas groning. **Fauna och Flora (Stockholm)**, v. 49, n. 1-2, p. 17–32, 1954.

LUNA, C. M. et al. Drought controls on H2O2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. **Journal of experimental botany**, v. 56, n. 411, p. 417-23, jan. 2005.

MACHADO, E. C. et al. Respostas da fotossíntese de três espécies de citros a fatores ambientais. **Pesquisa Agropecuária Brasileira**, v. 40, n. 12, p. 1161-1170, dez. 2005.

MACHADO, E. C.; MEDINA, C. L.; GOMES, M. D. M. D. A. Teor de água no substrato de crescimento e fotossíntese em laranjeira "Valência". **Bragantia**, v. 58, n. 2, p. 217-226, 1999.

MANGA, V.; SEN, D. N. Influence of seed traits on germination in *Prosopis cineraria* (L.) MacBride. **Journal of Arid Environments**, v. 31, n. 3, p. 371-375, nov. 1995.

MARANHO, Á. S.; PAIVA, A. V. DE. Superação de dormência tegumentar em sementes de *Senna silvestres* (Vell.) H. S. Irwin & Barneby. **Biotemas**, v. 25, n. 2, p. 25-31, 18 maio. 2012.

MARTIN, S. C. Mesquite Seeds Remain Viable After 44 Years. **Ecology**, v. 29, n. 3, p. 393, jul. 1948.

MARTINS, C. C.; NAKAGAWA, J. Germinação de sementes de *Stryphnodendron adstringens* (Mart.) Coville de diferentes origens submetidas a tratamentos para superação de dormência. **Revista Árvore**, v. 32, n. 6, p. 1059–1067, 2008.

MAYER, A. M.; POLJAKOFF-MAYBER, A. **The germination of seeds**. 4 ed. ed. New York: Pergamon Press, 1989. p. 270

MEYER, R. et al. **Morphology and Anatomy of Honey Mesquite**. Washington: USDA Technology Bulletin, 1971. p. 186

MIRANDA, R. Q. et al. Germination of *Prosopis juliflora* (Sw) DC seeds after scarification treatments. **Plant Species Biology**, v. 26, n. 2, p. 186-192, 24 maio. 2011.

MOHAMMADKHANI, N.; HEIDARI, R. Water stress induced by polyethylene glycol 6000 and sodium chloride in two maize cultivars. **Pakistan journal of biological sciences: PJBS**, v. 11, n. 1, p. 92-97, 1 jan. 2008.

MØLLER, I. M.; JENSEN, P. E.; HANSSON, A. Oxidative modifications to cellular components in plants. **Annual review of plant biology**, v. 58, p. 459-81, jan. 2007.

MURPHY, J. B.; NOLAND, T. L. Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. **Plant physiology**, v. 69, n. 2, p. 428-31, mar. 1982.

NASCIMENTO, S. S. DO; ALVES, J. J. A. Ecoclimatologia do Cariri Paraibano. **Revista Geográfica Acadêmica**, v. 2, n. 3, p. 28-41, 2008.

NOORMETS, A. et al. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO2 and/or O3. **Plant, Cell and Environment**, v. 24, n. 3, p. 327-336, mar. 2001.

OLIVEIRA, M. A. J. DE et al. Fotossíntese, condutância estomática e transpiração em pupunheira sob deficiência hídrica. **Scientia Agricola**, v. 59, n. 1, p. 59-63, mar. 2002.

OLSON, D. M. et al. Terrestrial Ecoregions of the World: A New Map of Life on Earth. **BioScience**, v. 51, n. 11, p. 933-938, 2001.

ÖPIK, H.; ROLFE, S. A. **The Physiology of Flowering Plants**. 4. ed. Cambridge: Cambridge University Press, 2006. p. 404

OTANI, T. Effects of macaque ingestion on seed destruction and germination of a fleshy-fruited tree, *Eurya emarginata*. **Ecological Research**, v. 19, n. 5, p. 495-501, set. 2004.

PARIDA, A. K.; DAS, A. B. Salt tolerance and salinity effects on plants: a review. **Ecotoxicology and environmental safety**, v. 60, n. 3, p. 324-349, mar. 2005.

PASIECZNIK, N. . M.; HARRIS, P. J. C.; SMITH, S. J. **Identifying Tropical Prosopis Species: A Field Guide**. Coventry, UK: International Research Department, HDRA, 2004. p. 29

PASIECZNIK, N. M. et al. **The *Prosopis juliflora* - *Prosopis pallida* Complex**. Coventry, UK: HDRA, 2001. p. 162

PEGADO, C. M. A. et al. Efeitos da invasão biológica de algaroba: *Prosopis juliflora* (Sw.) DC. sobre a composição e a estrutura do estrato arbustivo-arbóreo da caatinga no Município de Monteiro, PB, Brasil. **Acta Botanica Brasilica**, v. 20, n. 4, p. 887-898, dez. 2006.

PEREZ, S. C. J. G. A.; FANTI, S. C.; CASALI, C. A. Salt stress and salt-temperature interaction effects on the germination of *Peltophorum dubium* seeds. **Journal of Tropical Forest Science**, v. 13, n. 1, p. 44-61, 2001.

PERRY, S. W.; KRIEG, D. R.; HUTMACHER, R. B. Photosynthetic rate control in cotton : photorespiration. **Plant physiology**, v. 73, n. 3, p. 662-665, nov. 1983.

POMPELLI, M. F. et al. Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. **Biomass and Bioenergy**, v. 34, n. 8, p. 1207-1215, ago. 2010.

POMPELLI, M.; FERNANDES, D.; GUERRA, M. Germination of *Dyckia encholirioides* (Gaudichaud) Mez var. encholirioides under saline conditions. **Seed Science and Technology**, v. 34, n. 3, p. 759–763, 2006.

POPINIGIS, F. **Fisiologia da semente**. 2 ed. ed. Brasília: ABRATES, 1985. p. 298

PORTILLO-QUINTERO, C. A.; SÁNCHEZ-AZOFEIFA, G. A. Extent and conservation of tropical dry forests in the Americas. **Biological Conservation**, v. 143, n. 1, p. 144-155, jan. 2010.

PRADO, D. E. As Caatingas da América do Sul. In: LEAL, I. R.; TABARELLI, M.; SILVA, J. M. C. (Eds.). **Ecologia e conservação da Caatinga**. Recife: Editora Universitária da UFPE, 2003. p. 3-76.

PRITEKEL, C. et al. Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. **Applied Soil Ecology**, v. 32, n. 1, p. 132-141, maio. 2006.

PROBERT, R. J. The role of temperature in the regulation of the seed dormancy and germination. In: FENNER, M. (Ed.). **Seeds: The ecology of regeneration in plant communities**. 2. ed. New York: CABI Publishing, 2000. p. 261–292.

RAMÍREZ-TOBÍAS, H. M. et al. Seed germination temperatures of eight Mexican Agave species with economic importance. **Plant Species Biology**, v. 27, n. 2, p. 124-137, 12 maio. 2012.

REID, A. M. et al. Does invasive plant management aid the restoration of natural ecosystems? **Biological Conservation**, v. 142, n. 10, p. 2342-2349, out. 2009.

RICHMOND, T. DE A.; MUELLER-DOMBOIS, D. Coastline ecosystems on Oahu, Hawaii. **Plant Ecology**, v. 25, n. 1, p. 367-400, 1972.

ROCHA, A. M. S.; MORAES, J. A. P. V. Influência do estresse hídrico sobre as trocas gasosas em plantas jovens envasadas de *Stryphnodendron adstringens* (Mart.) Coville. **Revista Brasileira de Fisiologia Vegetal**, v. 9, n. 1, p. 41-46, 1997.

RODRIGUES, E. H. A.; AGUIAR, I. B.; SADER, R. Quebra de dormência de sementes de três espécies do gênero *Cassia*. **Revista Brasileira de Sementes**, v. 12, n. 12, p. 17-27, 1990.

SAMPAIO, E. V. S. B. Overview of the Brazilian Caatinga. In: BULLOCK, S. H.; MOONEY, H. A.; MEDINA, E. (Eds.). **Seasonally dry forests**. London: Cambridge University Press, 1995. p. 35-63.

SANTAMARÍA, L. et al. Effect of passage through duck gut on germination of fennel pondweed seeds. **Archiv fur Hydrobiologie**, v. 156, n. 1, p. 11-22, 1 dez. 2002.

SANTOS, J. C. et al. Caatinga: the scientific negligence experienced by a dry tropical forest. **Tropical Conservation Science**, v. 4, n. 3, p. 276-286, 2011.

SCHADE, J. D. et al. The influence of *Prosopis* canopies on understorey vegetation: Effects of landscape position. **Journal of Vegetation Science**, v. 14, n. 5, p. 743-750, 9 out. 2003.

SCHOLANDER, P. F.; HAMMEL, H. T.; BRADSTREET, E. D. Pressure in Vascular Plants. 1960.

SCHWEMBER, A. R.; BRADFORD, K. J. Quantitative trait loci associated with longevity of lettuce seeds under conventional and controlled deterioration storage conditions. **Journal of experimental botany**, v. 61, n. 15, p. 4423-36, out. 2010.

SCIFRES, C. J.; BROCK, J. H. Emergence of honey mesquite seedlings relative to planting depth and soil temperature. **Journal of Range Management**, v. 25, n. 3, p. 217–219, 1972.

SCIFRES, C. J.; HAHN, R. R. Response of honey mesquite seedlings to top removal. **Journal of Range Management**, v. 24, n. 4, p. 296-298, 1971.

SILVA, L. M. DE M.; AGUIAR, I. B. DE; RODRIGUES, T. DE J. D. Seed germination of *Bowdichia virgilioides* Kunth, under water stress. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 5, n. 1, p. 115-118, abr. 2001.

SMIRNOFF, N. The role of active oxygen in the response of plants to water deficit and desiccation. **New Phytologist**, v. 125, n. 1, p. 27-58, set. 1993.

SOCOLOWSKI, F.; TAKAKI, M. Germination of *Jacaranda mimosifolia* (D. Don - Bignoniaceae) seeds: effects of light, temperature and water stress. **Brazilian Archives of Biology and Technology**, v. 47, n. 5, p. 785-792, set. 2004.

SOLBRIG, O. T.; CANTINO, P. D. Reproductive adaptations in *Prosopis* (Leguminosae, Mimosoideae). **Journal of the Arnold Arboretum**, v. 56, n. 2, p. 185-210, 1975.

SONG, J. et al. Strategies for adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed-germination stage. **Annals of botany**, v. 96, n. 3, p. 399-405, out. 2005.

SOUZA, B. D.; RODRIGUES, B. M. et al. Ecophysiology parameters of four Brazilian Atlantic Forest species under shade and drought stress. **Acta Physiologiae Plantarum**, v. 32, n. 4, p. 729-737, 9 jan. 2010.

SOUZA, B. D.; MEIADO, M. V. et al. Water relations and chlorophyll fluorescence responses of two leguminous trees from the Caatinga to different watering regimes. **Acta Physiologiae Plantarum**, v. 32, n. 2, p. 235-244, 19 set. 2010.

SULTANA, N.; IKEDA, T.; ITOH, R. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. **Environmental and Experimental Botany**, v. 42, n. 3, p. 211-220, dez. 1999.

TOBY PENNINGTON, R.; PRADO, D. E.; PENDRY, C. A. Neotropical seasonally dry forests and Quaternary vegetation changes. **Journal of Biogeography**, v. 27, n. 2, p. 261-273, mar. 2000.

TODD-BOCKARIE, A. H. et al. Pretreatment to overcome seed coat dormancy in *Cassia sieberiana*. **Seed Science and Technology**, v. 21, n. 2, p. 383-398, 1993.

TSCHIRLEY, F. H.; MARTIN, S. C. Germination and longevity of velvet mesquite seed in

soil. **Journal of Range Management**, v. 13, n. 2, p. 94–97, 1960.

UNGAR, I. Seed germination and seed-bank ecology in halophytes. In: KIGEL, J.; GALILI, G. (Eds.). **Seed development and germination**. New York: Marcel Dekker Inc., 1995. p. 599–629.

UPRETI, J.; DHAR, U. Study on seed germination of a leguminous liana - *Bauhinia vahlii* Wight and Arnott. **Seed Science and Technology**, v. 25, n. 2, p. 187-194, 1997.

VAN KLINKEN, R. D.; CAMPBELL, S. D. The biology of Australian weeds. 37. *Prosopis* L. species. **Plant Protection Quarterly**, v. 16, n. 1, p. 2-20, 2001.

VERTUCCI, C. W.; LEOPOLD, A. C. Water binding in legume seeds. **Plant physiology**, v. 85, n. 1, p. 224-231, jan. 1987.

VILELA, A. The effect of seed scarification and soil-media on germination, growth, storage, and survival of seedlings of five species of *Prosopis* L. (Mimosaceae). **Journal of Arid Environments**, v. 48, n. 2, p. 171-184, jun. 2001.

WATT, M. S.; BLOOMBERG, M.; FINCH-SAVAGE, W. E. Development of a hydrothermal time model that accurately characterises how thermoinhibition regulates seed germination. **Plant, cell & environment**, v. 34, n. 5, p. 870-876, maio. 2011.

ZENG, Y. J.; WANG, Y. R.; ZHANG, J. M. Is reduced seed germination due to water limitation a special survival strategy used by xerophytes in arid dunes? **Journal of Arid Environments**, v. 74, n. 4, p. 508-511, abr. 2010.

ZHANG, H. et al. The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. **Annals of botany**, v. 106, n. 6, p. 1027-35, dez. 2010.

Capitulo I

Germination of *Prosopis juliflora* (Sw) DC seeds after scarification treatments

(Este artigo foi publicado na revista *Plant Species Biology* em 24 de Fevereiro de 2011)

PLANT SPECIES BIOLOGY

Plant Species Biology (2011) 26, 186–192 doi: 10.1111/j.1442-1984.2011.00324.x

NOTES AND COMMENTS

Germination of *Prosopis juliflora* (Sw) DC seeds after scarification treatments

RODRIGO Q. MIRANDA, MARCELA T. P. OLIVEIRA, RAFAELA M. CORREIA, JARCILENE S. ALMEIDA-CORTEZ and MARCELO F. POMPELLI

Plant Physiology Laboratory, Federal University of Pernambuco, Department of Botany, CCB, Recife, Pernambuco 50670901, Brazil

Abstract

Invasive plant species are the second most important threat to global biodiversity loss after land-use change. Invasive species can modify native community composition, deplete species diversity and affect ecosystem processes. The Caatinga is one of the most human-affected Brazilian ecosystems owing to non-sustainable use of its natural resources. *Prosopis juliflora* is an important invasive plant species in the Caatinga ecosystem. Seed germination is a critical stage in plant life cycles and is a major factor in the establishment and success of invasive plant species. Among the factors that affect seed germination and dormancy, coat-imposed seems to be the most important for *P. juliflora*. In *Prosopis* species, the ingestion of fruits by wild and domestic animals may promote and accelerate germination, enhancing the dispersal of seeds and fruits of these species. We investigated the germination capacity of *P. juliflora* seeds after artificial mechanical and chemical scarification and analyzed the changes in seedling vigor caused by the scarification treatments. Germination rate, germination time (TMG) and germination synchrony (*E*) differed significantly with the length of the scarification treatments in H_2SO_4 for both seeds with endocarps and seeds without endocarps (non-endocarp seeds). Sulfuric acid affected plant survival more strongly than germination rate, particularly in non-endocarp seeds.

Keywords: frugivory, plantlet regeneration, ruminants, seed dormancy, sulfuric acid.

Received 8 October 2010; accepted 25 November 2010

Introduction

Invasive plant species modify native community composition, deplete species diversity and affect ecosystem processes (Pritekel *et al.* 2006; Reid *et al.* 2009). Studies of past invasive-species introductions have revealed that the impacts of invasion are complex and can permanently alter the structure and function of communities and cause local extinctions and changes in ecosystems (Reid *et al.* 2009).

The semiarid Caatinga occupies the region between the Amazon Forest (south of the equator) and the Atlantic Forest. It covers approximately 10% of the Brazilian arid and semiarid region. The Caatinga climate and its seasonality are related to climatic oscillations (Monteiro *et al.* 2006). The average regional annual rainfall is less than 800 mm and is distributed entirely during a short rainy season (i.e. May–August), resulting in xerophytic and caducifolious vegetation (Queiroz 2006).

The Caatinga is one of the most human-affected Brazilian ecosystems owing to non-sustainable use of its natural resources. Extensive areas have been replaced with pastures, agriculture fields or lands undergoing desertification, transforming the Caatinga into one of the most threatened ecosystems in the world. *Prosopis juliflora* (Sw) DC is a shrub native to Central and South America that has become an invasive species in many countries with arid and semiarid climates (Andrade *et al.* 2009). *Prosopis juliflora* exhibits disordered growth in some areas of Brazil, threatening the conservation of woody-species diversity in

Correspondence: Marcelo F. Pompelli
Email: mpompelli@yahoo.com.br

this ecosystems (Pegado *et al.* 2006). Notably, colonization of the Caatinga by exotic species may lead to competition with native species, affecting the floristic composition and structure of local communities (Andrade *et al.* 2009).

Fruits of *P. juliflora* are indehiscent legumes measuring 10–40 cm in length and 15–20 mm in width, containing an average of 20 seeds per pod. The endocarp is divided into rigid, leathery segments with one brown, elliptical seed in each segment (Meyer *et al.* 1971). The seeds frequently have hard, impermeable seminal integuments (Manga & Sen 1995), which ensure dormancy and are well adapted to long-term survival (Martin 1948; Tschirley & Martin 1960) and dispersal strategies (Guttermann 1994; Bewley 1997). In coat-imposed dormancy, the seed coat and other tissues that enclose the embryo (such as the endosperm, pericarp and extrafloral organs) prevent germination until they are removed or damaged, after which the embryo can readily germinate in the presence of water and oxygen (Finkelstein 2006). These tissues may be removed by mechanical, physical and/or chemical pretreatment (Martins & Nakagawa 2008).

It has been suggested that gut passage can trigger germination in seeds with a thick coat and/or with prolonged dormancy, such as the fruits of *Potamogeton* pondweed species (Lohammar 1954). Several studies have shown that seed ingestion by birds can affect the germinability (final germination %), the rate (speed) of germination or both (Santamaría *et al.* 2002 and references therein). In *Prosopis* species, ingestion of fruits by wild and domestic animals may promote and accelerate germination (Danthu *et al.* 1996), enhancing the dispersal of seeds and fruits of these species. In most studies simulating the effects of these processes, seed germination is strongly enhanced by seed scarification (mechanical removal of the soft epicarp plus mesocarp) (Santamaría *et al.* 2002). Some information is available about *P. julifora* germination (Manga & Sen 1995; Villagra 1995), although little is known about the benefits and drawbacks of particular scarification methods for individual *Prosopis* species. The aim of the present study was to describe the germination capacity of *P. juliflora* seeds after mechanical and chemical scarification and to analyze the resulting changes in seedling vigor.

Materials and methods

Study area

Fruits of *P. juliflora* were collected from the Experimental Station of the Agronomic Institute of Pernambuco, located in Caruaru (8°14′18″S, 35°55′20″W; 550 m a.s.l.), approximately 140 km from Recife in the semiarid Caatinga region. The annual average temperature in the region is approximately 24°C and precipitation is less than 600 mm/year, with rains concentrated in June and July.

Seed scarification and germination

Fruits of *P. juliflora* were randomly collected by hand from at least 20 individual plants within natural populations. The fruits were dried in the shade at ambient temperatures for 2–3 days. The seeds were stored at 4 ± 2°C, as recommended by Pompelli *et al.* (2010).

The experimental treatments consisted of chemical (sulfuric acid) and mechanical scarification. The seeds were divided into two groups: (i) seeds with intact endocarps (endocarp seeds); and (ii) seeds from which the endocarps were removed manually (non-endocarp seeds). Endocarp seeds were immersed in H_2SO_4 for 30, 60 or 90 min, and non-endocarp seeds were immersed in H_2SO_4 for 5, 20 or 40 min. To assure uniform coverage, the solution was mixed continuously during the acid treatment. Mechanical scarification was accomplished by rubbing the seeds against No. 50 sandpaper (Norton, Norton Saint-Gobain, Brazil). After each treatment, the seeds were promptly rinsed three times with sterile distilled water to remove acid or integument residues. The seeds were sterilized in a 1% solution of NaOCl for 10 min and then rinsed three times with sterile distilled water.

The resultant seeds were transferred into 110 mm × 110 mm × 35 mm germination boxes lined with a triple layer of filter paper and moistened with 5 mL of sterile distilled water plus Mycostatin solution (100 mg/L) (Bristol-Myers Squibb Pharmaceutics, New York, NY, USA) to prevent fungal growth. The germination boxes were then placed in a growth chamber where the temperature was maintained at 25 ± 0.5°C with a 12 h photoperiod provided by Sylvania cool-white fluorescent lamps, which have a light intensity of 40 µmol photons m^{-2} s^{-1}. Seed germination was evaluated daily and seeds were considered to have germinated when the radicle extended at least 0.5 mm out of the seed (Bewley 1997). After 40 days, the germination time (TMG) and germination synchrony (E) were recorded and expressed in days and bits, respectively (Ranal & Santana 2006). Germinated plantlets were transferred to polyethylene bags (80 cm^3) filled with soil and sand (3:1), where they remained for 60 days.

Seedling survival and allometry

For the first 30 days, regenerated seedlings of *P. juliflora* were grown in a growth chamber with a temperature of 25 ± 3°C (67.5 ± 8.4% relative humidity) and a 12 h photoperiod. After this they were transferred to a greenhouse for an additional 30 days (28 ± 2°C and 78.3 ± 8.9% relative humidity). Seedlings were counted after each 30-day period to determine their survival rate. After 60 days, the height, accumulated shoot and root biomass, and leaf area of each seedling were measured. To measure leaf area, 100 leaflets per treatment were imaged using a scanner

Treatment	Germinability (%)	TMG (days)	*E* (bits)
Endocarp seeds			
T1	8.5 ± 2.63d	22.46 ± 2.90b	1.78 ± 0.37ab
T2	9 ± 2.08d	17.89 ± 4.32b	1.94 ± 0.37ab
T3	29 ± 1.73c	6.91 ± 0.40b	2.47 ± 0.20a
T4	85 ± 8.39b	2.47 ± 0.15a	1.35 ± 0.11bc
T5	86.5 ± 5.50b	1.99 ± 0.07a	1.03 ± 0.17cd
Non-endocarp seeds			
T6	21.5 ± 3.86c	16.78 ± 0.96b	2.48 ± 0.17a
T7	100 ± 0a	2.03 ± 0.01a	0.42 ± 0.04d
T8	99.5 ± 0.50a	1.28 ± 0.07a	0.83 ± 0.12cd
T9	99.5 ± 0.50a	1.51 ± 0.06a	1.26 ± 0.05bcd
T10	99 ± 0.58a	2.04 ± 0.04a	0.72 ± 0.19cd

Values are mean ± standard error ($n = 4$). T1 to T5 represent endocarp seeds (control, mechanically scarified, H_2SO_4 for 30, 60 or 90 min, respectively) and T6 to T10 represent non-endocarp seeds (control, mechanically scarified, H_2SO_4 for 5, 20 or 40 min, respectively). Means followed by different letters within columns are significantly different (Student–Newman–Keuls' test; $P \leq 0.05$).

Table 1 Germination rate, germination time (TMG) and germination synchrony (*E*) of *Prosopis juliflora* seeds after scarification

(Genius 1200 × 1200 dpi), and the images were analyzed using the software Image-Pro Plus (version 4.5, Media Cybernetics, Silver Spring, MD, USA). The total leaf area was obtained by multiplying the mean leaflet area by the total number of leaflets counted in each treatment.

Statistical analysis

For seed germination, each treatment was composed to four replicates of 50 seeds. The germination percentages were transformed using the formula $Arcsin\sqrt{\frac{x}{100}}$, where x is the germination percentage (Ranal & Santana 2006). A Shapiro–Wilk test was used to test for normality (Shapiro & Wilk 1965) and a Brown–Forsyth test was used to test for equal variances (Brown & Forsyth 1974) using the software Statistica version 7.0 (StatSoft, Tulsa, OK, USA). All data are presented as means ± standard errors (SE). The results were analyzed with a mixed-model ANOVA, and means were compared using a Student–Newman–Keuls' test (SNK) in Statistica software.

Results

The effects of the scarification treatments on germination rate, germination time (TMG) and germination synchrony (*E*) were significant for both endocarp and non-endocarp seeds of *P. juliflora* (SNK; $P \leq 0.05$) (Table 1). Independently of treatment, non-endocarp seeds exhibited a much higher germination rate (44%) than endocarp seeds. There were no significant differences in germination rate or TMG among the scarification treatments for non-endocarp seeds. However, scarification of non-endocarp seeds with H_2SO_4 showed a trend toward a higher TMG after the longest treatment duration (Table 1). We observed an inverse pattern for endocarp seeds, for which TMG gradually decreased with longer durations of treatment with H_2SO_4. Mechanical scarification did not affect any germination attributes of endocarp seeds, but increased the germination rate of non-endocarp seeds in the same was as in the other scarification treatments. Germination synchrony was more acute (*i.e.* homogeneous) in non-endocarp seeds (34%) (Fig. 1, right panel); however, endocarp seeds treated with H_2SO_4 for 30 or 60 min showed a similar pattern (Fig. 1d,e).

As shown in Table 2, the H_2SO_4 treatments did not significantly influence seedling growth in either endocarp or non-endocarp seeds. However, non-endocarp seeds tended to have higher mean values. In contrast, the survival rate of seedlings in both groups decreased with increasing duration of exposure to H_2SO_4 (Fig. 2). Independently of the duration of the H_2SO_4 treatment, non-endocarp seeds exhibited lower survival rates than their endocarp counterparts. Mechanical scarification caused malformation of seedlings and therefore low survival rates (Fig. 2).

Discussion

Prosopis species have attracted attention because of their great ability to survive in inhospitable environments and their capacity to provide fuel, timber, fodder and edible pods (El-Keblawy & Al-Rawai 2005). *Prosopis juliflora* is highly aggressive and resprouts well, so that it often crowds out native vegetation (Tiwari 1999; Pegado *et al.* 2006; Andrade *et al.* 2009). However, it shows great potential for use as a multipurpose tree in different parts of the world in contrast to several other native and exotic species (Shukla *et al.* 1990; Deans *et al.* 2003).

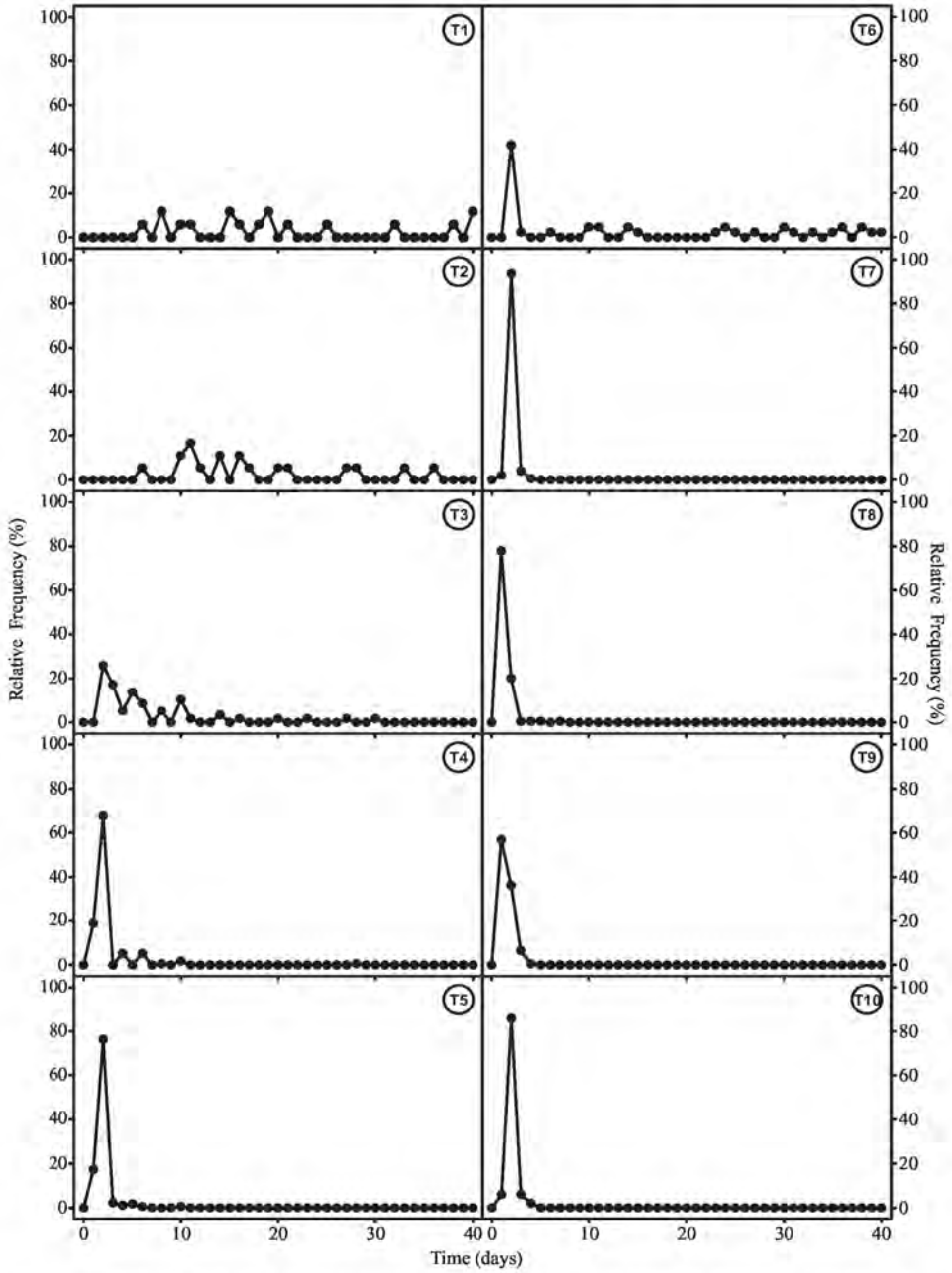


Fig. 1 Relative frequencies of *Prosopis juliflora* seeds subjected to scarification. Values represent averages of four replicates of 50 seeds each. T1 to T5 represent endocarp seeds (control, mechanically scarified, H_2SO_4 for 30, 60 or 90 min, respectively) and T6 to T10 represent non-endocarp seeds (control, mechanically scarified, H_2SO_4 for 5, 20 or 40 min, respectively).

Table 2 Growth and vigor of *Prosopis juliflora* seedlings after the seed scarification treatments

Treatment	Shoot height (cm)	Shoot biomass (mg)	Root biomass (mg)	Shoot : Root	Leaf area (cm^2)
Endocarp seeds					
T1	7.64 ± 0.42b	48.93 ± 3.64ns	20.83 ± 3.93ns	2.08 ± 0.26ns	15.36 ± 2.98a
T2	7.68 ± 0.66b	49.20 ± 4.62ns	28.37 ± 0.64ns	1.91 ± 0.12ns	15.15 ± 0.36a
T3	9.21 ± 0.46ab	50.95 ± 4.92ns	22.44 ± 1.75ns	2.33 ± 0.19ns	11.19 ± 0.71abc
T4	9.76 ± 0.34ab	49.55 ± 1.94ns	23.82 ± 1.12ns	2.08 ± 0.08ns	12.33 ± 0.58abc
T5	9.11 ± 0.38ab	51.34 ± 3.49ns	23.96 ± 1.51ns	2.28 ± 0.25ns	9.43 ± 0.59c
Non-endocarp seeds					
T6	9.27 ± 0.49ab	64.71 ± 5.55ns	27.35 ± 1.86ns	2.56 ± 0.21ns	13.55 ± 0.83abc
T7	8.46 ± 0.35ab	46.64 ± 3.33ns	24.51 ± 1.37ns	2.01 ± 0.11ns	12.61 ± 0.62abc
T8	9.45 ± 0.41ab	54.41 ± 4.01ns	25.49 ± 1.92ns	2.28 ± 0.18ns	12.98 ± 0.72abc
T9	10.05 ± 0.42a	57.80 ± 3.59ns	29.77 ± 2.80ns	2.13 ± 0.15ns	10.18 ± 0.46bc
T10	9.31 ± 0.56ab	55.55 ± 4.08ns	22.97 ± 3.56ns	3.05 ± 0.44ns	14.13 ± 0.76ab

T1 to T5 represent endocarp seeds (control, mechanically scarified, H_2SO_4 for 30, 60 or 90 min, respectively) and T6 to T10 represent non-endocarp seeds (control, mechanically scarified, H_2SO_4 for 5, 20 or 40 min, respectively). Means followed by different letters within columns are significantly different (Student–Newman–Keuls' test; $P \leq 0.05$). Values represent means ± standard errors of 15 replicates. ns, not significant.

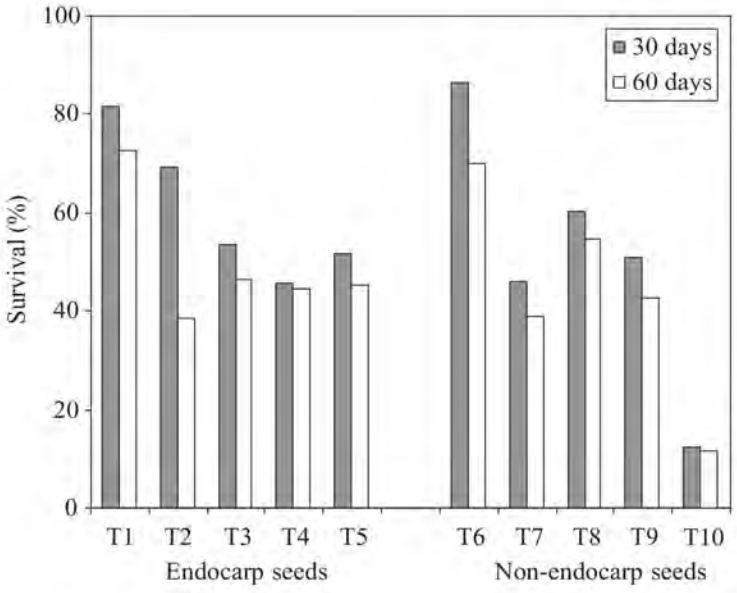


Fig. 2 Survival rate of *Prosopis juliflora* seeds subjected to scarification after 30 (filled columns) or 60 days (open columns). T1 to T5 represent endocarp seeds (control, mechanically scarified, H_2SO_4 for 30, 60 or 90 min, respectively) and T6 to T10 represent non-endocarp seeds (control, mechanically scarified, H_2SO_4 for 5, 20 or 40 min, respectively).

In semiarid habitats, where appropriate conditions for germination may be unpredictable in space and time, germination usually occurs when conditions are most favorable for the survival of seedlings (Grappin *et al.* 2000). Therefore, rapid and synchronous germination is likely to be adaptive. In the present study, we showed that *P. juliflora* seeds may germinate rapidly (Table 1) and synchronously (Fig. 1), particularly when treated with H_2SO_4. In natural ecosystems, *P. juliflora* seeds may be scarified by acids in the digestive tracts of ruminant animals. It has been reported that ingestion by mammals favors the germination of *Prosopis flexuosa* seeds (Campos & Ojeda 1997). However, many studies have shown that the effects of digestion on germination capacity, germination time and germination synchrony can vary considerably. Digestion can increase (Danthu *et al.* 1996), decrease (Günster 1994) or not affect (Figueiroa & Castro 2002; Otani 2004) germination compared with non-ingested seeds. Furthermore,

germination of *P. juliflora* is influenced by the semiarid climate of the Caatinga (Scifres & Brock 1972), where rainfall occurs over only two or three months. Rapid germination of this species may be promoted by scarification during passage through the ruminant gut (Vilela & Ravetta 2001). A short period for seed germination and seedling establishment probably has a strong influence on the success of *P. juliflora* in the Caatinga ecosystem because established seedlings can readily replace top growth (Scifres & Hahn 1971) and may withstand considerable environmental stress (Meyer *et al.* 1971).

In nature, various biotic and abiotic factors can produce seed scarification, including extreme temperatures (*e.g.* fire or chilling), changes in the chemical environment (*e.g.* seed ingestion by frugivores and passage through the digestive tract) and mechanical abrasion of the stony endocarp by sand and rocks in watercourses (Vilela & Ravetta 2001 and references therein). Our results show that for *P. juliflora*, scarification with sulfuric acid promotes seed germination more than mechanical scarification (Table 1), as reported previously for *P. juliflora* (Souza *et al.* 1983; Bastos *et al.* 1992) and *Prosopis chilensis* (Arce & Balboa 1988). In the present study, we observed malformation of seedlings caused by abrasion damaging the cotyledons. This damage may explain the reduced survival rate of plants submitted to mechanical scarification (Vilela & Ravetta 2001).

Although H_2SO_4 promotes seed germination, it can adversely affect embryo development. Sulfuric acid may create or enlarge pores in the seed, enabling water to enter the seed and directly contact the embryo and thus accelerate the germination process. Many researchers have described differing effects of acid scarification; in some cases sulfuric acid does not increase seed germination because it negatively affects the seeds. The lower effectiveness of some chemical scarification treatments compared with mechanical scarification can be explained by two factors: (i) the endocarp or seed coat did not erode enough to break dormancy after a short time of exposure to sulfuric acid; or (ii) the acid penetrated enough to kill or damage the embryo (Upreti & Dhar 1997; Pompelli 2006).

For non-endocarp seeds of *P. juliflora*, 5 min of sulfuric acid treatment is sufficient to promote full germination, whereas for endocarp seeds, the germination rate increases with the time of exposure to H_2SO_4 (Table 1). However, seeds exposed to sulfuric acid for longer time periods tend to produce malformed seedlings. The toxic effect of H_2SO_4 on embryos and germination has been described previously in *P. juliflora* (Bastos *et al.* 1992) and *Dyckia encholirioides* (Pompelli 2006), whose seeds were strongly and negatively influenced by increasing H_2SO_4 concentration and duration of exposure.

Colonization and establishment of an invasive plant species represent a form of disturbance (Pritekel *et al.* 2006). Disturbance is a natural and often essential occurrence in most ecosystems. Although communities differ in their resistance and resilience to pressures caused by chronic disruptions, certain patterns have emerged (Bengtsson 2002). Disturbances often affect the availability of nutrients and the rates of uptake by plants, the structure of the plant community, food-web architecture, and important trophic and symbiotic interactions, resulting in changes in the flow of energy and nutrients (Moore *et al.* 2003). Numerous studies have shown alterations in community structure and ecosystem function following the invasion of exotic species. It is clear that the colonization of *P. juliflora* in the Caatinga ecosystem has caused a series of changes in the ecological dynamics. However, the current level of invasion of this ecosystem by *P. juliflora* makes it necessary to study more advanced concepts involving plant ecophysiology in an attempt to establish a methodology to control its expansion. In our laboratory, several studies focusing on plant ecophysiology have been initiated. We believe that in the coming years we will be able to develop methodologies based on ecophysiology that can be combined with those based on ecological and population dynamics to finally control the expansion of this invasive species. The present study of seed germination is the first step toward this goal.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico—CNPq (the Brazilian Research Council) for project grant (473202/2008-5) and productivity grant (proc n°314532/2009-9) to J. S. Almeida-Cortez and the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support of this research.

References

Andrade L. A., Fabricante J. R. & Oliveira F. X. (2009) Invasão biológica por *Prosopis juliflora* (Sw.) DC.: impactos sobre a diversidade e a estrutura do componente arbustivo-arbóreo da caatinga no estado do Rio Grande do Norte, Brasil. *Acta Botanica Brasilica* **23**: 935–943.

Arce P. & Balboa O. (1988) Some aspects of the biology of *Prosopis* growing in Chile. In: Habit M. A. (ed.). *The Current State of Knowledge on* Prosopis juliflora. Food and Agriculture Organization, Rome, pp. 313–322.

Bastos G. Q., Nunes R. S. & Figueiredo-Cruz G. M. (1992) Reavaliação de quebra de dormência em sementes de algaroba (*Prosopis juliflora* [SW] DC). *Revista Brasileira de Sementes* **14**: 17–20.

Bengtsson J. (2002) Disturbance and resilience in soil animal communities. *European Journal of Soil Biology* **38**: 119–125.

Bewley J. D. (1997) Seed germination and dormancy. *The Plant Cell* **9**: 1055–1066.

Brown M. B. & Forsyth A. B. (1974) Robust tests for equality of variances. *Journal of the American Statistical Association* **69**: 364–367.

Campos C. M. & Ojeda R. A. (1997) Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. *Journal of Arid Environments* **35**: 707–717.

Danthu P., Ickowicz A., Friot D., Manga D. & Sarr A. (1996) Effet du passage par le tractus digestif des ruminants domestiques sur la germination des graines de légumineuses ligneuses des zones tropicales sèches. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux* **49**: 235–242.

Deans J. D., Diagne O., Nizinski J., Lindley D. K., Seck M., Ingleby K. & Munro R. C. (2003) Comparative growth, biomass production, nutrient use and soil amelioration by nitrogen-fixing tree species in semi-arid Senegal. *Forest Ecology and Management* **176**: 253–264.

El-Keblawy A. & Al-Rawai A. (2005) Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *Journal of Arid Environments* **61**: 555–565.

Figueiroa J. A. & Castro S. A. (2002) Effects of bird ingestion on seed germination of four woody species of the temperate rainforest of Chiloé Island, Chile. *Plant Ecology* **160**: 17–23.

Finkelstein R. (2006) Abscisic acid: a seed maturation and anti-stress signal. In: Taiz L. & Zeiger E. (eds). *Plant Physiology*, 4th edn. Sinauer Associates, Sunderland, pp. 539–558.

Grappin P., Bouinot D., Sotta B., Miginiac E. & Jullien M. (2000) Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210**: 279–285.

Günster A. (1994) Seed bank dynamics—longevity, viability and predation of seeds of serotinous plants in the central Namib Desert. *Journal of Arid Environments* **28**: 195–205.

Guttermann Y. (1994) Strategies of seed dispersal and germination in plants inhabiting deserts. *Botanical Review* **60**: 373–425.

Lohammar A. G. (1954) Matsmältningens inverkan på *Potamogeton* frönas groning. *Fauna och Flora (Stockholm)* **1-2**: 17–32.

Manga V. K. & Sen D. N. (1995) Influence of seed traits on germination in *Prosopis cineraria* (L.) MacBride. *Journal of Arid Environments* **31**: 371–375.

Martin S. C. (1948) Mesquite seeds remain viable after 44 years. *Ecology* **29**: 393.

Martins C. C. & Nakagawa J. (2008) Germinação de sementes de *Stryphnodendron adstringens* (Mart.) Coville de diferentes origens submetidas a tratamentos para superação de dormência. *Revista Árvore* **32**: 1059–1067.

Meyer R. E., Morton H. L., Haas R. H., Robison E. D. & Riley T. E. (1971) *Morphology and Anatomy of Honey Mesquite*. USDA Technology Bulletin, Washington.

Monteiro J. M., Albuquerque U. P., Lins-Neto E. M. F., Araújo E. L. & Amorim E. L. C. (2006) Use patterns and knowledge of medicinal species among two rural communities in Brazil's semi-arid northeastern region. *Journal of Ethnopharmacology* **105**: 173–186.

Moore J. C., McCann K., Setälä H. & de Ruiter P. C. (2003) Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* **84**: 846–857.

Otani T. (2004) Effects of macaque ingestion on seed destruction and germination of a fleshy-fruited tree, *Eurya emarginata*. *Ecological Research* **19**: 495–501.

Pegado C. M. A., Andrade L. A., Felix L. P. & Pereira L. M. (2006) Efeitos da invasão biológica de algaroba—*Prosopis juliflora* (Sw.) DC. sobre a composição e a estrutura do estrato arbustivo-arbóreo da caatinga no Município de Monteiro, PB, Brasil. *Acta Botanica Brasilica* **20**: 887–898.

Pompelli M. F. (2006) Germinação de *Dyckia encholirioides* var *encholirioides* (Bromeliaceae, Pitcairnioideae). *Floresta e Ambiente* **13**: 1–9.

Pompelli M. F., Ferreira D. T. R. G., Cavalcante P. P. G. S., Salvador T. L., Hsie B. S. & Endres L. (2010) Environmental influence on the physico-chemical and physiological properties of *Jatropha curcas* seeds. *Australian Journal of Botany* **58**: 421–427.

Pritekel C., Whittemore-Olson A., Snow N. & Moore J. C. (2006) Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. *Applied Soil Ecology* **32**: 132–141.

Queiroz L. P. (2006) The Brazilian Caatinga: phytogeographical patterns inferred from distribution data of the Leguminosae. In: Pennington R. T., Lewis G. & Ratter J. A. (eds). *Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography and Conservation*. CRC Press, New York, pp. 121–157.

Ranal M. A. & Santana D. G. (2006) How and why to measure the germination process? *Revista Brasileira de Botânica* **29**: 1–11.

Reid A. M., Morin L., Downey P. O., French K. & Virtue J. G. (2009) Does invasive plant management aid the restoration of natural ecosystems? *Biological Conservation* **142**: 2342–2349.

Santamaría L., Charalambidou I., Figuerola J. & Green A. J. (2002) Effect of passage through duck gut on germination of fennel pondweed seeds. *Archiv für Hydrobiologie* **156**: 11–22.

Santana D. G. & Ranal M. A. (2004) *Análise da Germinação*. Editora da Universidade de Brasília, Brasília.

Scifres C. J. & Brock J. H. (1972) Emergence of honey mesquite seedlings relative to planting depth and soil temperature. *Journal of Range Management* **25**: 217–219.

Scifres C. J. & Hahn R. R. (1971) Response of honey mesquite seedlings to top removal. *Journal of Range Management* **24**: 396–398.

Shapiro S. S. & Wilk M. B. (1965) An analysis of variance test for normality (complete samples). *Biometrika* **52**: 591–611.

Shukla N. K., Khanduri A. K., Krishan L. & Mohan L. (1990) Physical and mechanical properties of some exotic species. *Indian Forester* **116**: 140–147.

Souza S. M., Lima P. C. F. & Araújo M. S. (1983) Sementes de algaroba; métodos e custos de beneficiamento. *Revista Brasileira de Sementes* **5**: 51–61.

Tiwari J. W. K. (1999) Exotic weed *Prosopis juliflora* in Gujarat and Rajasthan, India-boon or bane. *Tigerpaper* **26**: 21–25.

Tschirley F. H. & Martin S. C. (1960) Germination and longevity of velvet mesquite seed in soil. *Journal of Range Management* **13**: 94–97.

Upreti J. & Dhar U. (1997) Study on seed germination of a leguminous liana—*Bauhinia vahlii* Wight and Arnott. *Seed Science and Technology* **25**: 187–194.

Vilela A. E. & Ravetta D. A. (2001) The effect of seed scarification and soil-media on germination, growth, storage, and survival of seedlings. *Journal of Arid Environments* **48**: 171–184.

Villagra P. E. (1995) Temperature effects on germination of *Prosopis argentina* and *P. alpataco* (Fabaceae, Mimosoideae). *Seed Science and Technology* **23**: 639–646.

Capitulo II

Germination of *Prosopis juliflora* (Sw.) D.C seeds at different osmotic potentials and temperatures

(Este manuscrito foi enviado para a revista *Plant Species Biology* em 29 de Outubro de 2012)

Germination of *Prosopis juliflora* (Sw.) D.C seeds at different osmotic potentials and temperatures

Rodrigo de Queiroga Miranda[1], Rafaela Moura Correia[1], Jarcilene Silva de Almeida-Cortez[2], Marcelo Francisco Pompelli[1*]

[1] *Plant Ecophysiology Laboratory, Federal University of Pernambuco, Department of Botany, CCB, Recife, Pernambuco, Brazil, 50670901.*

[2] *Plant-Animal Interactions Laboratory, Federal university of Pernambuco, Recife, Pernambuco, Brazil*

* Correspondence author

M.F. Pompelli

Tel.: +55 81 2126 8844; Fax +55 81 2126 7803. Email address: mpompelli@yahoo.com.br (M.F. Pompelli)

Running title: Germination of *P. juliflora* under osmotic stress

Abstract

The effects of osmolytes, osmotic potential (Ψ_s), temperature and their interactions on the germinability, germination rate and other germination parameters of the invasive shrub *Prosopis juliflora*, which grows in the semiarid environmental conditions of the Caatinga in northeast Brazil, were evaluated. To study the effects of polyethylene glycol (PEG) and NaCl stress and temperature on germination, two separate experiments were carried out at the Plant Ecophysiology Laboratory of the Federal University of Pernambuco in 2011. The overall germinability decreased significantly with increases in both PEG (one-way ANOVA, $F_{4,75}$ = 111.21, $P \leq 0.001$) and NaCl (one-way ANOVA, $F_{4,75}$ = 12.82, $P \leq 0.001$); however, the effects were more accentuated with PEG than NaCl. PEG-treated seeds maintained their germinability, even when they were subjected to a Ψ_s = -1 MPa after being rinsed and allowed to germinate on deionized water. In contrast, NaCl-treated seeds usually lost their ability to germinate; this fact was possibly linked to the accumulation of Na^+ and Cl^- in the cells, which may have contributed to a loss of membrane function that led to the death of the embryos. Although numerous studies describing seed germination in the presence of osmolytes have been conducted, studies that show the interactions between osmolytes, osmotic potentials and

temperature are scarce. The present study is the first one to describe these interactions for *Prosopis juliflora* seeds.

Keywords: Caatinga, exotic species, mesquite, NaCl, osmotic stress, PEG, salinity, seed germination, temperature

Introduction

More than 900 million ha (~20% of the total agricultural land) worldwide are affected by salt (Zhang *et al.* 2010). The affected area is nearly one-fifth of the world's irrigated land, resulting in the loss of 10 million ha of otherwise arable land each year (Boyer 1982). Soil salinity is one of the most important constraints that limits crop production in arid and semiarid regions (Parida & Das 2005), resulting in reductions in crop yields by as much as 50% (Boyer 1982). Moreover, salinity impairs seed germination and reduces nodule formation, plant development, flowering and fruit development (Khan & Gulzar 2003). Plants have diverse cellular mechanisms to protect against specific ion effects and osmotic stresses imposed by saline soils. These mechanisms include increases in proteins involved in water transport (*e.g.*, aquaporins), ion sequestration and secretion and increases in osmolytes or compatible solutes (Leatherwood *et al*., 2007 and references therein).

NaCl is the predominant salt that causes salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation. Salinity affects seed germination through osmotic effects (Almansouri *et al.* 2001), ion toxicity (Song *et al.* 2005) or a combination of the two (Meiado *et al.* 2010), and the effects of excess sodium ions (Na^+)can critically affect biochemical processes (Apse *et al.* 1999). Salt and water stress can reduce germination by limiting water absorption by the seeds (Hegarty 1977, Zeng *et al.* 2010), affecting the mobilization of stored reserves (Bouaziz & Hicks 1990) or directly affecting the structural organization or the synthesis of proteins in germinating embryos.

Seedlings are the most vulnerable stage in the life cycle of plants, and germination determines when and where seedling growth begins (Günster 1994). In saline environments, adaptation of plants to salinity during germination and the early seedling stages is crucial for the establishment of a species (Ungar 1978, Ungar 1995, Ungar 1982). Successful seedling establishment depends on the frequency and amount of precipitation as well as on the ability of the species' seeds to germinate and grow while soil moisture and osmotic potentials decrease. Most seeds are deposited near the surface of saline soil, where the concentration of

salt is usually higher than it is below the surface (Dantas *et al.* 2006). Seed germination occurs after monsoon rains, which cause a reduction in temperature and soil salinity (Almansouri *et al.* 2001, Khan & Gulzar 2003). In this sense, seeds germinate rapidly at low NaCl concentrations but remain ungerminated at high NaCl concentrations (Song *et al.* 2005). This response may produce a persistent bank of viable seeds in saline environments that can maintain the population over time, and it may be an important strategy for seed dispersal (Ungar 1995). Therefore, rapid germination may be an adaptive strategy for the seeds of species to take advantage of transient favorable conditions during the germination stage to ensure seedling establishment.

Reductions in the germination percentage and delays in the onset of germination in saline conditions are well documented. However, salinity stress seldom occurs in isolation and its effects on seed germination may be modified by interactions with other environmental parameters, such as temperature (Pompelli *et al.* 2006, Meiado *et al.* 2010) and light (Khan & Gulzar 2003, Pompelli *et al.* 2006). Although numerous studies have described seed germination in the presence of osmolytes, studies that show the interactions between osmolytes, osmotic potentials and temperature are scarce. The principal aim of the present study was to compare the effects of drought and salt stress on the germination of *Prosopis juliflora* seeds at different temperatures. The present study was initiated to differentiate osmotic effects from toxic effects by comparing NaCl with the metabolically inactive osmotic agent polyethylene glycol 6000 (PEG 6000). To determine the osmotic effects, we measured seed imbibition and the germination of seeds soaked in NaCl and PEG solutions at various osmotic potentials and temperatures.

Materials and Methods

Study area and species studied

The Caatinga, a South American Indian name that means ‘white forest’, is a forest that covers a 760,000 km^2 area of northeast Brazil (Sampaio 1995). Since 2003, the Caatinga has been recognized as one of ‘Earth’s last wild places’ and was classified as one of the 37 ‘Wilderness Areas of the World’ (Russell *et al.* 2003). It is inhabited by approximately 23 million people and corresponds to the largest populated semiarid area in the world located in a single country (Prado 2003). The vegetation of the Caatinga is strongly influenced by topography, human disturbance and, most importantly, a combination of average annual

rainfall and soil attributes (Sampaio 1995, Prado 2003). The Caatinga is classified as a BSh Köppen climate with a high evapotranspiration potential (1500-2000 mm annually) throughout the year and low pluviometric precipitation (300-1000 mm annually), which is usually concentrated over 3-5 months (Sampaio 1995). The soil of the Caatinga is highly saline (Dantas *et al.* 2006) caused by the high level of water evaporation from the soil and poor soil drainage, resulting in an accumulation of salt at the soil's surface (Sampaio 1995). Moreover, it is common to drill artesian wells in the Caatinga to alleviate water shortages and facilitate agricultural and livestock activities. This action greatly increases the soil's salinity from salts that were formerly present in groundwater, which strongly contributes to the secondary salinization of the Caatinga. These events, in combination with the rapid drying of the soil due to intense sunlight, contribute to extremely limited water availability throughout most of the year and create conditions that greatly affect seed germination (Barbosa *et al.* 2003) and seedling survival in this region.

The anthropization of areas of the Caatinga have commonly led to a loss of diversity and the establishment of exotic species such as *Prosopis juliflora*, which are introduced accidentally or intentionally, as an alternative source of protein for cattle feed during periods of water shortage.

Prosopis juliflora (Sw.) DC. (mesquite) is a native species of the North to South America. They occur in arid and semiarid areas of Venezuela, Colombia, and Ecuador and extend through Panama into Mexico (Pasiecznik 2001). *P. juliflora* is a species that is fast growing, highly aggressive, and able to cause substratum degradation in the arid and semiarid areas of north and northwest India (Singh 1995). *P. juliflora* has a broad ecological amplitude and is adapted to a very wide range of soils and site types from sand dunes to cracking clays. It is generally found in areas where water and poor soil fertility are the principal agents that limit plant growth and can survive and even thrive on some of the poorest land, which is unsuitable for any other tree species (El-Keblawy & Al-Rawai 2005). *P. juliflora* seems to prefer areas subjected to high temperatures and high evapotranspiration with wide variations in rainfall (El-Keblawy & Al-Rawai 2005, Pasiecznik 2001).

Seed collection and germination

Seeds of *Prosopis juliflora* (Sw.) D.C. (mean seed weight ± s.d. 37.70 ± 0.43 mg, n = 100) were collected in March 2011 from plants growing in the Caatinga ecosystem of the

Experimental Station at the IPA, Caruaru, PE. The median temperature and precipitation of this region are 23ºC and 671 ± 54 mm, respectively, which are irregularly distributed throughout the year (over 4-6 months). The rainy season occurs from April to August (Agritempo 2012).

The seeds were extracted from the fruits and immediately used in these experiments. Four replicates of 50 randomly selected seeds each were carried out for each treatment. The treatment factors were two osmotic salts, five osmotic potentials and four temperatures, which were applied in a randomized complete block design. In two separate experiments, drought and salt stress were induced by different osmotic potential levels and their effects were examined. Osmotic potential of -0.25, -0.50, -0.75 and -1.0 MPa for PEG 6000 and -0.5, -1.0, -1.5 and -2.0 MPa for NaCl were studied. An osmotic free medium was prepared to produce an osmotic potential of 0 MPa (control). The osmotic potentials of NaCl were calculated using the van't Hoff equation, $\Psi_s = -ciRT$ where Ψ_s is the osmotic potential in MPa, c is the concentration in mol L^{-1}, i is the dissociation constant of NaCl (*i.e.*, 1.8), R is the gas constant (*i.e.*, 0.0083 L atm^{-1} mol^{-1} K^{-1}) and T is the temperature in Kelvins. For PEG 6000, the osmotic potentials were calculated as described by Michel and Kaufman (1973). All osmotic potentials were confirmed ($t_6 = 91.76$, $r^2_a = 0.99$, $p \leq 0.001$) with a Wescor vapour pressure osmometer (model 5600, Wescor Biomedical Systems, Utah, USA).

The seeds were pretreated with H_2SO_4 for 5 minutes (Miranda *et al.* 2011), surface sterilized in a 1% solution of NaOCl for 10 minutes, and rinsed 5 times with sterile deionized water. Seeds were allowed to germinate in 110 mm x 110 mm x 35 mm germination boxes lined with a triple layer of filter paper and moistened with 20 mL of sterile solution plus Mycostatin solution 100 mg L^{-1}) (Bristol-Myers Squibb Pharmaceutics, New York, NY, USA) prior to incubation to control fungi. The germination boxes were covered with lids and kept in a growth chamber, where the temperature was maintained at 25, 30, 35 or 40ºC with a 12 h photoperiod provided by Sylvania cool-white fluorescent lamps, which have a light intensity of 40 μmol photons m^{-2} s^{-1}, and the pots were randomly moved every day to minimize positional effects. Germination was recorded daily for 20-d. Seeds were considered to have germinated upon emergence of the radicle. When no seeds germinated until 20-d, we considered germination to be completed. These data were used to determine the germinability, germination rate ($\bar{t}$), germination synchrony (Z), uncertainty (U) and germination index, as described in Ranal and Santana (2006) and Zhang *et al*. (2010). To investigate the effect of

darkness on germination and whether high salinities inhibited or damaged the seeds in the dark, another set of germination boxes was wrapped in aluminum foil to exclude light and placed in the same incubator. (Ranal & Santana 2006, Zhang *et al.* 2010)

To normalize the effect of each stress on germination, the sensitivity response was calculated as the osmotic sensitivity response (OSR) and salt sensitivity response (SSR). The OSR were calculated as $[(G_w - G_{PEG}) / G_w] \times 100$ and the SSR were calculated as $[(G_w - G_{NaCl}) / G_w] \times 100$; where G_W is germination in water, W_{PEG} is germination on PEG solution and G_{NaCl} is germination on NaCl soluctions.

Recovery of germination

To verify the resilience of seeds exposed to low osmotic potentials, we carried out an additional experiment to determine the NaCl and PEG concentrations that completely inhibit germination and cause irreversible damage to embryos. The methodology used was the same as described above. After 20-d, non-germinated seeds were delicately rinsed with deionized sterile water, gently surface-dried with sterile filter paper and transferred to germination boxes free of osmotic agents. Seeds were allowed to germinate for 20-d at 25ºC when we considered that germination had completed for that treatment. To ensure that the seeds used for the experiments were viable and maintained their viability after treatments, seed viability was determined by the ability to reduce 2,3,5-triphenyltetrazolium chloride to red colored formation (Brewer 1949).

Seed imbibition

To investigate the imbibition capacity of the seeds, four replicates of 50 seeds each were subjected to hydration at osmotic potentials of 0, -0.5 and -1 MPa with PEG and NaCl solutions. Seeds were allowed to imbibe the solutions for 120 h. During the first 12 h, the samples were weighed every hour and every 24 h thereafter. At each time point, the seeds were removed from the imbibition solution, weighed and then returned to a new solution with the same osmotic potential (Pompelli *et al.* 2010). The relative increase in the fresh weight of the germinating seeds was calculated using the formula: $[(W_f - W_i)/W_i] \times 100$, where W_i is the initial weight of seeds and W_f is the weight after *n* hours.

Statistical analysis

The collected data were analyzed with a mixed-model ANOVA, and means were compared using an SNK test with the statistical software package SigmaPlot 11.0 (Systat Software Inc., Chicago, USA). The correlations were made with the statistical software Statistica version 7.0 (StatSoft, Tulsa, OK, USA). The results were considered to be significant when $P \leq 0.05$.

Results

The variance analyses showed that there were significant differences between osmolytes, stress levels, temperatures and their interactions. Therefore, the effects of osmolytes (*i.e.*, PEG and NaCl) were separated for further analysis.

Final germination percentages decreased significantly with increases in both PEG (one-way ANOVA, $F_{4,75} = 111.21$, $P \leq 0.001$) and NaCl (one-way ANOVA, $F_{4,75} = 12.82$, $P \leq 0.001$). Germination of the seeds in all potentials promoted by NaCl or PEG indicated that the PEG treatments resulted in significantly ($p \leq 0.001$) less germination than the iso-osmotic NaCl solutions (Table 1). The germinability was inversely affected by the NaCl and PEG concentrations, *i.e.*, *P. juliflora* exhibited a reduction in germination with increases in NaCl or PEG concentrations. During NaCl treatments, no germination occurred at -2 MPa at 40ºC, and germination was very low in osmotic potentials below -1.50 MPa, especially at higher temperatures (Table 1). The PEG solutions inhibited the germination at osmotic potentials below -0.75 at 40ºC, and germination was greatly reduced at osmotic potentials below -0.5 MPa. Nevertheless, germination in the absence of NaCl/PEG or at low salinity was considerably higher. The final germination percentages of seeds soaked in PEG solutions was significantly affected by the osmotic potential ($R_2^a = 0.864$, $p \leq 0.001$) and temperature ($R_2^a = -0.444$, $p \leq 0.001$). Similar results were obtained for seeds soaked in NaCl as for the seeds soaked in PEG solutions; however, the correlations returned fewer negative values.

The seeds that were germinated in NaCl had the highest percentage of germination at a temperature of 35°C (Table 1) with a lower $\bar{t}$ (Table 2). However, at osmotic potentials below -1 MPa, the best temperature for germination was 30ºC because higher temperatures, together with more negative osmotic potentials promoted an acceleration in the metabolism of the seed and the death of the embryo; this finding was confirmed by the tetrazolium test (data not shown). However, for seeds germinated in PEG, the pattern was different because for osmotic potentials below to -0.5 MPa, the optimum temperature for germination was 25°C (Table 1-2).

The effects of temperature on the germination rate (Table 2), germination synchrony (Table 3) and uncertainty (Table 4) were not clear because the best temperature depended on the considered parameters.

For the germination rate, germination synchrony and uncertainty, the results showed that an increase in salt concentration promoted slower or more unsynchronized germination (Table 2-4). The $\bar{t}$ value increased with a decrease in the osmotic potential for both NaCl and PEG solutions. The germination of *P. juliflora* can be classified as fast because $\bar{t}$ was less than 5 days. However, at osmotic potentials below to -0.5 MPa with PEG or -1.5 MPa with NaCl, its germination was considered to be intermediate. Regardless of the temperature, germination always began on the second day, except in seeds allowed to germinate at osmotic potentials below -0.50 MPa or -1.5 MPa with PEG and NaCl, respectively, when germination began on the fourth day or later (Fig. 1).

All osmotic potentials, germinating seeds reached a water content of approximately 0.88 mg H_2O mg^{-1} fresh weight when treated with either PEG or NaCl. A positive relationship between the osmotic potential and the seed water content of germinating seeds was noted; however, seeds soaked in NaCl imbibed water faster than seeds soaked in PEG (Fig. 2). Seeds allowed to imbibe a solution at an osmotic potential of -0.5 MPa exhibited increases in fresh weight of 55% and 32% in NaCl and PEG solutions, respectively. In contrast, seeds allowed to imbibe a solution at an osmotic potential of -1 MPa exhibited increases in fresh weight of 53% and 26% (Fig. 2). It is worth to note that seed imbibe fresh water (0 MPa) exhibited increases in their fresh weight of at least 105%.

P. juliflora seeds completely recovered from treatment with -1 MPa PEG because after being rinsed with deionized water, these embryos exhibited a similar germinability (99.5 ± 0.5%) with osmotic agent free treatment within 3-4 days. In contrast, seeds that did not germinate in the presence of -2 MPa NaCl completely lost their germination capacity because the germination percentage during the recovery period was 0% (Table 5).

Compared with PEG treatments, the germination indices of *P. juliflora* seeds were typically greater for NaCl treatments, and seeds incubated in NaCl germinated at more negative osmotic potentials (Fig. 3). The relationship between the external osmotic potential and the germination index was linear, and NaCl had a smaller negative effect than PEG. The amplitude increased with temperature. However, in PEG, the gradient of the lines was significantly affected by increases temperature, with seeds at low osmotic potentials failing to

germinate. No significant difference in the relationship between the osmotic potential and the germination index was observed between the NaCl and PEG treatments at 25ºC and 30ºC, but strong differences were observed at other temperatures.

Seeds that were allowed to imbibe PEG solutions showed the highest sensitivity responses because germination was almost completely inhibited under moderate osmotic stress. In contrast, seeds that were allowed to imbibe NaCl solutions displayed the lowest sensitivity responses because germination was only slightly affected by the same stress. The range of variation in osmotic and salt sensitivity responses was between 0 and 100% and 0 and 4% at -1 MPa of PEG and NaCl, respectively. The osmotic salt sensitivity responses were found to be highly correlated ($p \leq 0.001$) (data not shown).

Discussion

Prosopis juliflora may be classified as an aphotoblastic species because its germination can proceed under both light and dark conditions. The data obtained in the present study corroborate with others studies of the *Prosopis* species (El-Keblawy & Al-Rawai 2005, Miranda *et al.* 2011, Villagra 1995, Perez & Moraes 1994). The study of the effect of light on *P. juliflora* seed germination was the secondary objective of the present study because many studies of seed germination in the presence of salt have been conducted under dark conditions. Thus, a previous experiment was carried out in which the aphotoblastic behavior of this species was determined because the percentage of germination in dark and light were never significantly among then (data not shown). Therefore, only data obtained in the light have been presented in this study because these data were easier to obtain and thus more reliable.

Peres and Tambelini (1995) described that the germination of *P. juliflora* as severely inhibited in the presence of NaCl. The results obtained in the present study disagree with those authors because the germinability of *P. juliflora* at 25ºC or 30ºC was not severely affected by osmotic potentials up to -2 MPa (Table 1). One possible explanation for such different results may be that the seeds utilized by Peres and Tambelini (1995) had previously been stored in sealed containers at temperatures between 5 and 10ºC for five years, which may have affected seed vigor and most likely their germinability (Escandon *et al.* 2013).

The results of the present study showed that the germination parameters are inversely proportional to the NaCl or PEG concentration at all temperatures tested. Therefore, the temperature that best promotes the germination of *P. juliflora* is strongly affected by its

interactions with the osmotic potential of the substrate where the germination occurs. Similar to other species (Barbosa *et al.* 2003, Meiado *et al.* 2010), *P. juliflora* is able to germinate at higher temperature ranges, such as those normally registered in the Caatinga ecosystem (Sampaio 1995). Although Meiado et al. (2010) did not test the combined effects of temperature and osmotic potencials, this authors found that the seeds of *Cereus jamacaru* had the highest germinability and lowest $\bar{t}$ when germinated at 30ºC, compared to other temperatures. Similar profiles were previously reported for *Prosopis juliflora* (El-Keblawy & Al-Rawai 2005), *P. argentina* and *P. alpataco* (Villagra 1995), *Dyckia encholirioides* (Pompelli *et al.* 2006) and some perennial grasses (Khan & Gulzar 2003).

Many researchers have reported that NaCl has a greater effect on germination and the early seedling stages than PEG (Mohammadkhani & Heidari 2008). However, in this study, we shown that PEG concentration was always more detrimental to the final germination percentage than the NaCl concentration. *P. juliflora* seeds treated with PEG germinated at osmotic potentials up to -0.5 MPa, and there was an abrupt decrease of germinability at osmotic potentials lower than -0.75 MPa. In contrast, seeds treated with NaCl did not exhibit a significant decrease in their germinability until -1.5 MPa. In other non-halophyte species, the osmotic potential that completely inhibits germination is generally greater than -1.5 MPa (Haigh & Barlow 1987, Hegarty 1977), while the threshold may be less than -5 MPa in halophyte species (Ungar 1982, Ungar 1978, Debez *et al.* 2004). The results of the present study on *P. juliflora* seed germination corroborate other studies on non-halophyte species, including *P. juliflora* (Zeng *et al.* 2010, Mohammadkhani & Heidari 2008, Zhang *et al.* 2010, Nassif & Perez 1997, Meiado *et al.* 2010, Peres & Tambelini 1995, Peres & Moraes 1991)

Na^+ is a small ion that can easily pass through cell membranes (Mansour & Salama 2004), and cells must expend energy to pump it out; otherwise, the water activity decreases and all metabolic pathways can be disturbed or disrupted, causing some misbalance in the energy production-consumption (Mohammadkhani & Heidari 2008). Na^+ can contribute to an increase in amylases, proteases or lipases activity (Ashraf & Foolad 2005). Moreover, the elongation process and the synthesis of cell wall carbohydrates are susceptible to water deficit (Sgherri *et al.* 1996) and are adversely affected by NaCl (Prisco & Gomes Filho 1978). Recent reports suggest that Na^+ may limit the mobilization of starchy endosperm reserves in several species (Bouaziz & Hicks 1990), reducing the germination percentage. However, Almansouri et al. (2001) showed that in seeds soaked in PEG and NaCl the content of starch

and soluble sugars was slightly lower than in control seeds, suggesting that a difference between the effects of NaCl and PEG on reserve mobilization is not the underlying cause of their contrasting effects on germination processes.

Although Na^+ is a major cation present in the soil, it is not considered to be an essential mineral for most plants. In saline soils such as the Caatinga's (Dantas *et al.* 2006), high concentrations of Na^+ disrupt the balance of other minerals such as K^+, thereby causing a reduction in cell turgor. A reduction in cell turgor leads to a drastic reduction in the rates of root and leaf elongation (Hegarty 1977, Verslues *et al.* 1998). This observation suggests that the salt acts primarily on water uptake (Hegarty 1977). Furthermore, the high intracellular concentration of Cl^- or Na^+ ions can inhibit some metabolic pathways (Campos *et al.* 2012), slowing germination and subsequent events, all that, *in totum*, leads to cell death. In contrast, seeds of salt-tolerant species tend to have lower osmotic potentials, allowing them to absorb water from the environment (Zhang *et al.* 2010). This decrease in osmotic potential can be achieved in one of two ways: exclusion of salt from the cytosol by vacuolar compartmentalization (Apse *et al.* 1999, Gao *et al.* 2003, Song *et al.* 2005), while maintaining an osmotic potential with organic solutes or by allowing Na^+ and Cl^- to enter the cells and using them as osmolytes while using mechanisms to mitigate the toxic effects of salt within the cell (Parida & Das 2005). These mechanisms generally involve the overexpression of the tonoplast Na^+/H^+ antiporter genes (Apse *et al.* 1999) or the proton pumps (ATPase) (Hahnenberger *et al.* 1996). Furthermore, in Arabidopsis plants, the expression of SOD2, an Na^+/H^+ antiporter on the plasma membrane, may restore the ability of the cells to export Na^+ and greatly increases their resistance to Na^+, improving seed germination and seedling growth (Gao *et al.* 2003).

Under PEG-induced water stress, a gradual decrease in osmotic potential causes a gradual decrease in germination, and this is correlated with the inhibition of storage protein degradation. Germination and the breakdown of storage proteins are likely both affected by low osmotic potentials (Khademi *et al.* 1991, Song *et al.* 2005). The lower germinability and increase in $\bar{t}$ of PEG-soaked seeds may be attributed to a reduction in oxygen availability at the surface of the seed due to the high viscosity of PEG 6000. Like the availability of water, the oxygen concentration (Verslues *et al.* 1998) can strongly affect the rate of seed germination because PEG acts as a barrier to gas exchange during seed imbibition (Verslues *et al.* 1998). Therefore, it is possible that the delay in seed germination of the PEG-soaked

seeds may have been due to a delay in water uptake caused by this osmolyte (Fig. 2). Moreover, the OSR2 and OSR4 QTLs were identified only when Arabidopsis seeds were incubated on a PEG solution (Vallejo *et al.* 2010). This results suggests that these OSR QTLs operate as a common molecular component of the signaling network that controls germination under low osmotic potentials generated by osmotic conditions and that they are relevant in the adaptation of plant populations to different ecological environments.

The uptake of water by seeds can be considered to occurs in three sequential steps: imbibition, metabolism leading to initiation of radicle growth, and radicle emergence (Bewley 1997). A threshold level of hydration is required for subsequent radicle elongation (Bewley 1997). It can be hypothesized that in the presence of PEG, *i.e.*, an inert osmoticum that cannot enter the apoplast (Carpita *et al.* 1979), water is withdrawn not only from the cell but also from the cell wall. Therefore, the inhibition of germination is attributed solely to osmotic effects and not to ionic effects (Michel & Kaufmann 1973), which may have contributed to the decrease in germinability of seeds soaked in PEG (Fig. 1). The relative increase in the fresh weight of germinating seeds was invariably highest in the control and lowest in all PEG- and NaCl-treated seeds at all temperatures (see Fig. 2 for seeds soaked at 25ºC). This results confirms that these osmolytes had an inhibitory effect on water uptake by the seeds. Khademi *et al.* (1991) reported that low osmotic potentials delay water uptake, increase the length of the water uptake plateau, and subsequently delay or prevent germination. However, the inhibitory effect of PEG on germination might not be solely related to water uptake because the increase in fresh weight during the first hours of germination appeared to be similar to the controls (Fig. 2). Thus, the higher rate of germination observed in NaCl than in PEG could be explained by a more rapid water uptake in the NaCl solution (Fig. 2) (Song *et al.* 2005) and achievement of a moisture content that allowed germination, as previously reported in maize (Mohammadkhani & Heidari 2008) or durum wheat (Almansouri *et al.* 2001) during seed imbibition and germination. In other words, NaCl did not prevent water uptake by the germinating embryo and, in this case, injuries appeared after ion accumulation at a later stage in the germinating process (Almansouri *et al.* 2001).

When the osmotic potential was sufficiently low, *e.g.*, -0.75 MPa (for PEG solutions) or -1.5 MPa (for NaCl solutions), the seeds could contain sufficient water to start the germination process (Phase I and II) (Fig. 2) without passing to root cell growth (Phase III) (Fig. 3). The drop in the rate of water uptake by seeds when they were soaked in NaCl and

PEG solutions of increasing concentrations was most likely caused by the decrease in the osmotic potential gradient between the seeds and their surrounding media. The processes of elongation and cell wall synthesis are highly sensitive to water deficiency (Wenkert *et al.* 1978) and reductions in growth could occur due to decreases in the turgors of these cells. Very negative osmotic potentials, especially at the beginning of imbibition, influence the absorption of water, which begins the sequence of events that culminate in seed germination (Hegarty 1977). We hypothesized that the characteristics of stronger germination and higher $\bar{t}$ in drought conditions may be favorable traits in arid environments. However, in arid environments that are also saline, these traits are lethal.

Increased salinity leads to a reduction and/or delay in the germination of seeds of both halophytes and glycophytes (Ungar 1982, Khan & Gulzar 2003, El-Keblawy & Al-Rawai 2005). The results of the present study show that in *P. juliflora* seeds, $\bar{t}$ was more affected by osmotic stress than germinability in all tested osmotic solutions, temperatures and osmolytes (Table 2). *P. juliflora* seeds show an increased $\bar{t}$ at -0.5 MPa of PEG, while for NaCl the increase was only significant at osmotic potentials below -1 MPa. Water stress can reduce both the germinability and $\bar{t}$, and there is a wide variation of responses among species (Bewley 1997). Thus, species that are more tolerant of, or better adapted to, water stress have the ecological advantage of better seedling establishment in areas subjected to water deficit. These parameters can contribute substantially to an understanding of seed germination processes and seedling recruitment in the field, which are influenced by water and/or salt stress. The decrease in germination parameters (*e.g.*, germinability, germination rate, synchrony and uncertainty) after water or osmotic stress has been previously reported in some species including *P. juliflora* (Leatherwood *et al.* 2007, Nassif & Perez 1997, Zhang *et al.* 2010, Peres & Tambelini 1995).

The results of the present study show that 77% of the seeds treated with NaCl at an osmotic potential of -2 MPa did not germinate even after 20-d (Table 5), and the germinability of the non-germinating seeds was not recovered even after the transfer of the seeds to deionized water. Two processes mediated this reduction: osmotic effects due to a declining osmotic potential, creating water stress on the surface of the seeds, and ionic effects due to seed ion uptake and/or accumulation. When soaked in -0.88 MPa of NaCl at 12ºC, the Na^+ concentration of barley seeds was increased to 4 μg g^{-1} dry weight during the first 72 h of incubation (Zhang *et al.* 2010). This fact may have caused the damage of the seed membranes

(*i.e.*, perhaps they became more fluid), thereby preventing germination even after the alleviation of the stress. The specific cause for the lack of germination in the non-germinating seeds is unclear, although the water content of the seeds treated with NaCl was generally higher, suggesting that osmotic limitation of water influx is unlikely to be the cause (Zhang *et al.* 2010). It seems plausible that the membrane integrity may have been compromised or that salt accumulation may have caused a loss of tonoplast Na^+/H^+ antiporter function, thus preventing germination (Debez *et al.* 2004). The lack of the recovery in seeds treated with NaCl was previously reported in *P. juliflora* (Perez & Moraes 1994) similar to other cultivated species (Zhang *et al.* 2010). However several other studies showed that NaCl-treated seeds are still able to germinate after rinsing (Pujol *et al.* 2000, Ungar 1978, Ungar 1995, Ungar 1982); although most of these studies focused on halophyte species. Glycophyte seeds cannot remain viable for long periods under extremely high salinity/osmotic stress and germinate at a later time when the osmotic potential of the medium has been increased (Khan & Gulzar 2003, Ungar 1978).

When transferred to deionized water, the non-germinating seeds exhibited distinct behaviors depending of whether they been treated with PEG or NaCl. Seeds treated with PEG readily recovered their germinability (100%) (Table 5). This results indicates that *P. juliflora* seeds can remain non-germinated and viable on the soil surface; however, if the water stress is accompanied by salt stress, the viability of the seeds can be severely impaired. This may have ecological significance within highly saline environments, reflecting a physiological response that was under strong selective pressure during the evolution of these species (Ungar 1982). The suspension of germination of salt-adapted species under high salt conditions might represent an alternative strategy; it may be a method of escape or avoidance that facilitates successful completion of the life cycle in a high stress environment without damage (Ungar 1982, Song *et al.* 2005, Leatherwood *et al.* 2007). In the Caatinga, seeds normally germinate in rainy conditions (Barbosa *et al.* 2003), instead of dry conditions to avoid the increase in the of salt concentration of the soil (Dantas *et al.* 2006) caused by high levels of evaporation. This response might be favorable for germination of the species, as even during the rainy season in the Caatinga, the salt concentration at the soil interface may be rapidly alleviated (Günster 1994). This trait might be related to the high rain level in the Caatinga: despite being restricted to a short period over the year (only a few days), it can reach values of up to 900 mm in some areas of the ecosystem (Sampaio 1995) and keep the soil humid for long enough

to complete the germination process. Thus, for the successful establishment of plants in saline environments, seeds must remain viable at high salinity and germinate when salinity decreases (Ungar 1978, Ungar 1995). According to Flores and Briones (2001), the relationship between germination patterns and water availability highlights an important adaptation of species that germinate in arid and semiarid ecosystems, and these species would have an advantage in these environments (Pasiecznik 2001). Maybe this is one reason for the great reproductive success of *P. juliflora* in the Caatinga environment (Miranda *et al.* 2011, Perez & Moraes 1994).

Prosopis species survive and grow at salinity levels equal to that of sea water (Felker *et al.* 1981) and in soils with a pH of 10.5 (Singh 1995). *P. juliflora* has been found to tolerate salinity levels up to 18,000 mg NaCl L^{-1} with no reduction in growth or survival and still grows at 36,000 mg L^{-1} NaCl (Felker *et al.* 1981). However, El-Keblawy and Al-Rawai (2005) showed that *P. juliflora* has a depressive effect on the number, richness, evenness, density and frequency of associated native species because this species has a the potential to alter primary productivity, decomposition, hydrology, nutrient cycling, and natural disturbance regimes. The success of exotic plants in invading some communities has been attributed to the superiority of the exotic species over the native species in some measurable traits, such as reproductive and dispersal capabilities, seedling establishment and survivorship, genome size, phenotypic plasticity, growth-related characteristics, plant height, phenology, allelopathy and plant-soil relationships (Pasiecznik 2001). The present study adds one more factor: high germinability, even in soils with high levels of salinity or sails that are affected by long periods without rain. Furthermore, the mechanical dormancy exhibited by *P. juliflora* (Miranda *et al.* 2011) allows the lifespan of the seeds to be extended, allowing the formation of a persistent seed bank in the soil ant the distribution of the germination over time and space. This strategy can increase the likelihood that the species will find conditions for the establishment of their seedlings.

Conclusion

The results of the present study showed that the germinability, germination rate and germination requirements of *P. juliflora* differ significantly depending on the osmotic potential and temperature; thus, *P. juliflora* may be considered to be a halotolerant species. This trait ensures that even when conditions are not ideal, only a portion of the seeds will

germinate at one time, which is particularly important for species survival in arid environments that are characterized by spatial and temporal unpredictability in rainfall. Water stress can act positively in the establishment of this species because it promotes a delay in the germination rate and synchrony. In natural conditions, *P. juliflora* exhibited heterogeneity in germination; thus, germination is distributed over time and space, increasing the likelihood that a seedling will find conditions that are favorable for its establishment and development. Overall, the reduced seed germination due to reduced water availability is one of the special survival strategies used by *P. juliflora* in the semiarid Caatinga, because germination rate and synchrony are good indices for evaluating the occupation ability of a species in a given ecosystem. These findings could help both explain the rapid increase of the *P. juliflora* in the Caatinga and manage this species in this environment.

Acknowledgements

The authors would like to thank the National Council for Scientific and Technological Development, CNPq (Grants 473202/2008-5) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco, FACEPE (Grants APQ-0150-2.03/08) for financial support of this research.

References

Agritempo (2012) Sistema de Monitoramento Agrometeorológico, Version. Ministério da Agricultura, Pecuária e Abastecimento, São José dos Campos. Available from URL: www.agritempo.gov.br/agroclima/sumario?uf=BA.

Almansouri M., Kinet J. M. & Lutts S. (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* desf.). *Plant Soil,* **231:** 243-254.

Apse M. P., Aharon G. S., Snedden W. A. & Blumwald E. (1999) Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis. *Science,* **285:** 1256-1258.

Ashraf M. & Foolad M. R. (2005) Pre-sowing seed treatment: a shotgun approach to improve germination growth and crop yield under saline and none saline conditions. *Adv Agron,* **88:** 223-271.

Barbosa D. C. A., Tabarelli M. & Vicente A. (2003) Variation of seed dispersal spectrum of woody plants across a rainfall gradient in north-eastern Brazil. *J Arid Environ,* **53:** 197-210.

Bewley J. D. (1997) Seed germination and dormancy. *Plant Cell,* **9:** 1055-1066.

Bouaziz A. & Hicks D. R. (1990) Consumption of wheat seed reserves during germination and early growth as affected by soil water potential. *Plant Soil,* **128:** 161-165.

Boyer J. (1982) Plant productivity and environment. *Science,* **218:** 443-48.

Brewer H. E. (1949) Tetrazolium chloride as a test for damage in artificial cured peanuts. *Science,* **110:** 451-452.

Campos M. L. O., Hsie B. S., Granja J. A. A., Correia R. M., Silva S. R. S., Almeida-Cortez J. S. & Pompelli M. F. (2012) Photosynthesis and antioxidant activity mechanisms in *Jatropha curcas* L. under salt stress. *Braz J Plant Physiol,* **24:** 55-67.

Carpita N., Sabularse D., Montezinos D. & Delmer D. P. (1979) Determination of the pore size of cell walls of living plant cells. *Science,* **205:** 1144-1147.

Dantas J. A., Bezerra Neto E., Barreto L. P. & Santos M. V. F. (2006) Efeito da salinidade sobre o crescimento e composição mineral de seis clones de *Pennisetum*. *Rev Ciência Agr,* **37:** 97-101.

Debez A., Hamed K. B., Grignon C. & Abdelly C. (2004) Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritima*. *Plant Soil,* **262:** 179-189.

El-Keblawy A. & Al-Rawai A. (2005) Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *J Arid Environ,* **61:** 555-565.

Escandon J. M., Silva B. C. F., Silva S. R. S., Granja J. A., Alves M. C. J. L. & Pompelli M. F. (2013) Germination responses of *Jatropha curcas* L. seeds to storage and aging. *Ind Crops Prod, **in press,*** **doi: 10.1016/j.indcrop.2012.08.035**.

Felker P., Clark P. R., Laag A. E. & Pratt P. F. (1981) Salinity tolerance of the tree legumes: mesquite (*Prosopis glandulosa* var. torreyana, *P. velutina* and *P. articulata*), algarrobo (*P. chilensis*), kiawe (*P. pallida*) and tamarugo (*P. tamarugo*) grown in sand culture on nitrogen-free media. *Plant Soil,* **61:** 311-317.

Flores J. & Briones O. (2001) Plant life-form and germination in a Mexican inter-tropical desert: effects of soil water potential and temperature. *J Arid Environ,* **47:** 485-497.

Gao X., Ren Z., Zhao Y. & Zhang H. (2003) Overexpression of *SOD2* increases salt tolerance of Arabidopsis. *Plant Physiol,* **133:** 1873-1881.

Günster A. (1994) Seed bank dynamics - longevity, viability and predation of seeds of serotinous plants in the central Namib Desert. *J Arid Environ,* **28:** 195-205.

Hahnenberger K. M., Jia Z. & Young P. G. (1996) Functional expression of the *Schizosaccharomyces pombe* Na^+/H^+ antiporter gene, sod2, in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA,* **93:** 5031-5036.

Haigh A. M. & Barlow E. W. R. (1987) Germination and priming of tomato, carrot, onion, and sorghum seeds in a range of osmotica. *J Amer Soc Hort Sci,* **112:** 202-208.

Hegarty T. W. (1977) Seed activation and seed germination under moisture stress. *New Phytol,* **78:** 349-359.

Khademi M., Koranski D. S., Hannapel D. J., Knapp A. D. & Gladon R. J. (1991) Water stress and storage-protein degradation during germination of Impatiens seed. *J Amer Soc Hort Sci,* **116:** 302-306.

Khan M. A. & Gulzar S. (2003) Light, salinity, and temperature effects on the seed germination of perennial grasses. *Am J Bot,* **90:** 131-134.

Leatherwood W. R., Pharr D. M., Dean L. O. & Williamson J. D. (2007) Carbohydrate content and root growth in seeds geminated under salt stress. *J Amer Soc Hort Sci,* **132:** 876-882.

Mansour M. M. F. & Salama K. H. A. (2004) Cellular basis of salinity tolerance in plants. *Environ Exp Bot,* **52:** 113-122.

Meiado M. V., Albuquerque L. S. C., Rocha E. A., Rojas-Aréchiga M. & Leal I. R. (2010) Seed germination responses of *Cereus jamacaru* DC. ssp. jamacaru (Cactaceae) to environmental factors. *Plant Species Biol,* **25:** 120-128.

Michel B. E. & Kaufmann M. R. (1973) The osmotic potential of polyethylene glycol 6000. *Plant Physiol,* **51:** 914-916.

Miranda R. Q., Oliveira M. T. P., Correia R. M., Almeida-Cortez J. S. & Pompelli M. F. (2011) Germination of *Prosopis juliflora* (Sw) DC seeds after scarification treatments. *Plant Species Biol,* **26:** 186-192.

Mohammadkhani N. & Heidari R. (2008) Water stress induced by polyethylene glycol 6000 and sodium chloride in two maize cultivars. *Pak J Biol Sci,* **11:** 92-97.

Nassif S. M. L. & Perez S. C. J. G. A. (1997) Germinação de sementes de amendoim-do-campo (*Pterogyne nitens* Tul. - Fabaceae-Caesalpinoideae) submetidas a diferentes condições de estresse hídrico e salino. *Rev Bras Sem,* **19:** 142-149.

Parida A. K. & Das A. B. (2005) Salt tolerance and salinity effects on plants: a review. *Ecotox Environ Safe,* **60:** 324-349.

Pasiecznik N. M. (2001) *The Prosopis juliflora: Prosopis pallida complex: a monograph,* HDRA, Coventry, UK.

Peres S. C. J. G. A. & Moraes J. A. P. V. (1991) Influência do estresse hídrico e do pH no processo germinativo da algarobeira. *Pesqui Agropecu Bras,* **26:** 981-988.

Peres S. C. J. G. A. & Tambelini M. (1995) Efeito dos estresses salino e hídrico e do envelhecimento precoce na germinação de algarobeira. *Pesqui Agropecu Bras,* **30:** 1289-1295.

Perez S. C. J. G. A. & Moraes J. A. P. V. (1994) Estresse salino no processo germinativo de algarobeira e atenuação de seus efeitos pelo uso de reguladores de crescimento. *Pesqui Agropecu Bras,* **29:** 389-396.

Pompelli M. F., Fernandes D. & Guerra M. P. (2006) Germination of *Dyckia encholirioides* (Gaudichaud) Mez var. encholirioides under saline conditions. *Seed Sci & Technol,* **34:** 759-763.

Pompelli M. F., Ferreira D. T. R. G., Cavalcante P. P. G. S., Salvador T. L., Hsie B. S. & Endres L. (2010) Environmental influence on the physico-chemical and physiological properties of *Jatropha curcas* L. seeds. *Aust J Bot,* **58:** 421-427.

Prado D. E. (2003) As Caatingas da América do Sul. In Leal, I. R., Tabarelli, M. & Silva, J. M. C. (eds.) *Ecologia e conservação da Caatinga.* Editora Universitária da UFPE, Recife.

Prisco J. T. & Gomes Filho E. (1978) Effects of NaCl salinity *in vivo* and *in vitro* on the proteolytic activity of *Vigna sinensis* (L.) Savi cotyledons during germination. *Rev Bras Bot,* **1:** 83-88.

Pujol J. A., Calvo J. F. & Ramírez-Díaz L. (2000) Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Ann Bot,* **85:** 279-286.

Ranal M. A. & Santana D. G. (2006) How and why to measure the germination process? *Rev Bras Bot,* **29:** 1-11.

Russell A., Mittermeier C. G., Gil P. R. & Pilgrim J. (2003) *Wilderness - earth's last wild places,* The University of Chicago Press Chicago.

Sampaio E. V. S. B. (1995) Overview of the Brazilian Caatinga. In Bullock, S. H., Mooney, H. A. & Medina, E. (eds.) *Seasonally Dry Tropical Forests.* Cambridge University Press, Cambridge.

Sgherri C., Pinzino C. & Navari-Izzo F. (1996) Sunflower seedlings subjected to increasing stress by water deficit: changes in O_2^- production related to the composition of thylakoid membranes. *Physiol Plant,* **96:** 446-452.

Singh G. (1995) An agroforestry practice for the development of salt lands using *Prosopis juliflora* and *Leptochloa fusca*. *Agrofor Syst,* **29:** 61-75.

Song J., Feng G., Tian C. & Zhang F. (2005) Strategies for adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed-germination stage. *Ann Bot,* **96:** 399-405.

Ungar I. A. (1978) Halophyte seed germination. *Bot Rev,* **44:** 233-264.

Ungar I. A. (1982) Germination ecology of halophytes. In Sen, D. N. & Rajpurohit, K. S. (eds.) *Contributions to the ecology of halophytes.* Dr. W. Junk Publishers, The Hague.

Ungar I. A. (1995) Seed germination and seed-bank ecology in halophytes. In Kigel, J. & Galili, G. (eds.) *Seed development and germination.* Marcel Dekker, New York.

Vallejo A. J., Yanovsky M. J. & Botto J. F. (2010) Germination variation in *Arabidopsis thaliana* accessions under moderate osmotic and salt stresses. *Ann Bot,* **106:** 833-842.

Verslues P. E., Ober E. S. & Sharp R. E. (1998) Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiol,* **116:** 1403-1412.

Villagra P. E. (1995) Temperature effects on germination of *Prosopis argentina* and *P. alpataco* (Fabaceae, Mimosoideae). *Seed Sci Technol,* **23:** 639-646.

Wenkert W., Lemon E. R. & Sinclair T. R. (1978) Leaf elongation and turgor pressure in field; Grown soybean. *Agron J,* **70:** 761-764.

Zeng Y. J., Wang Y. R. & Zhang J. M. (2010) Is reduced seed germination due to water limitation a special survival strategy used by xerophytes in arid dunes? *J Arid Environ,* **74:** 508-511.

Zhang H., Irving L. J., McGill C., Matthew C., Zhou D. & Kemp P. (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Ann Bot,* **106:** 1027-1035.

Figure 1

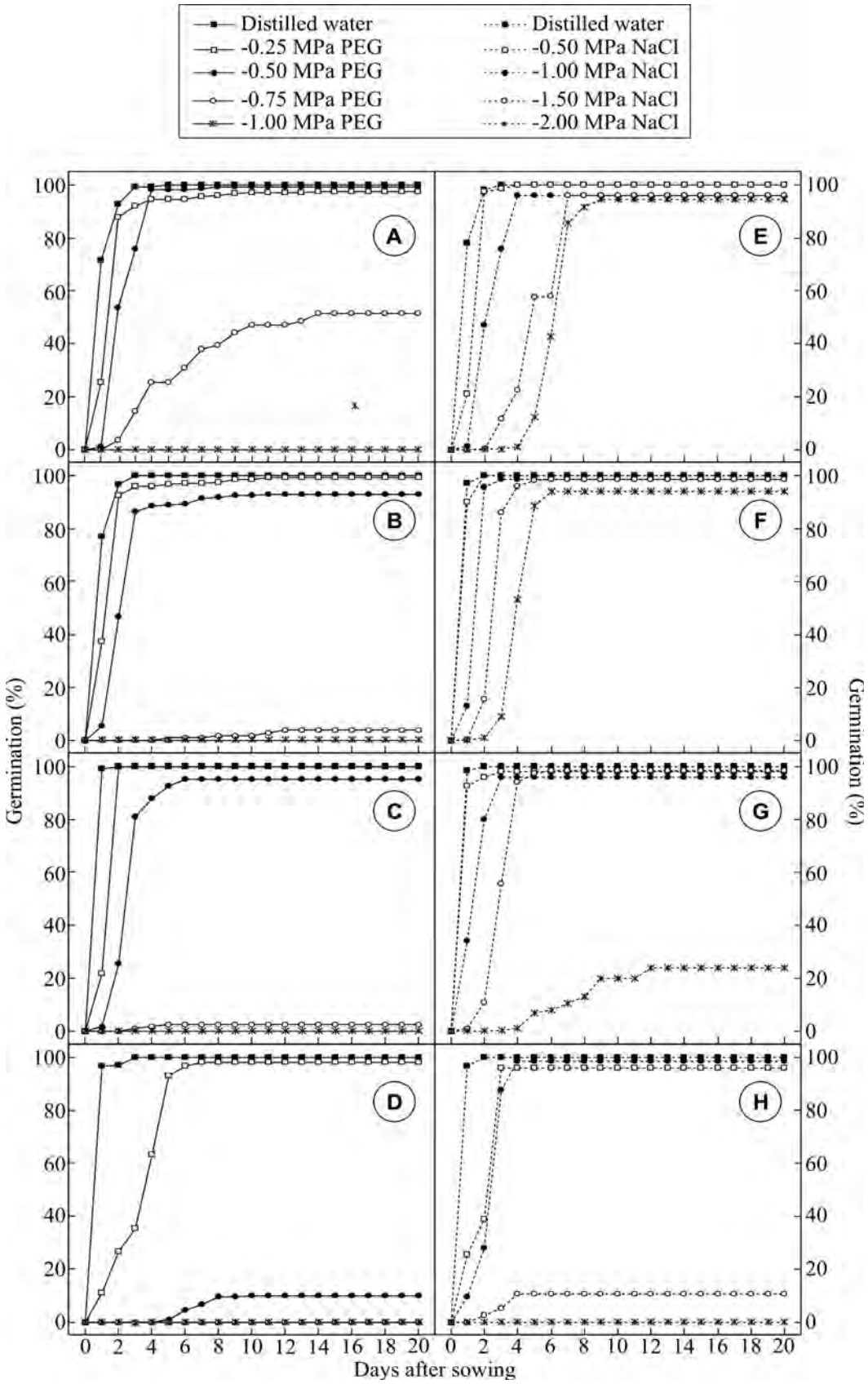

Distilled water
-0.25 MPa PEG
-0.50 MPa PEG
-0.75 MPa PEG
-1.00 MPa PEG
Distilled water
-0.50 MPa NaCl
-1.00 MPa NaCl
-1.50 MPa NaCl
-2.00 MPa NaCl
A
B
C
D
E
F
G
H
Germination (%)
Germination (%)
Days after sowing
0 2 4 6 8 10 12 14 16 18 20
0 20 40 60 80 100

Figure 2

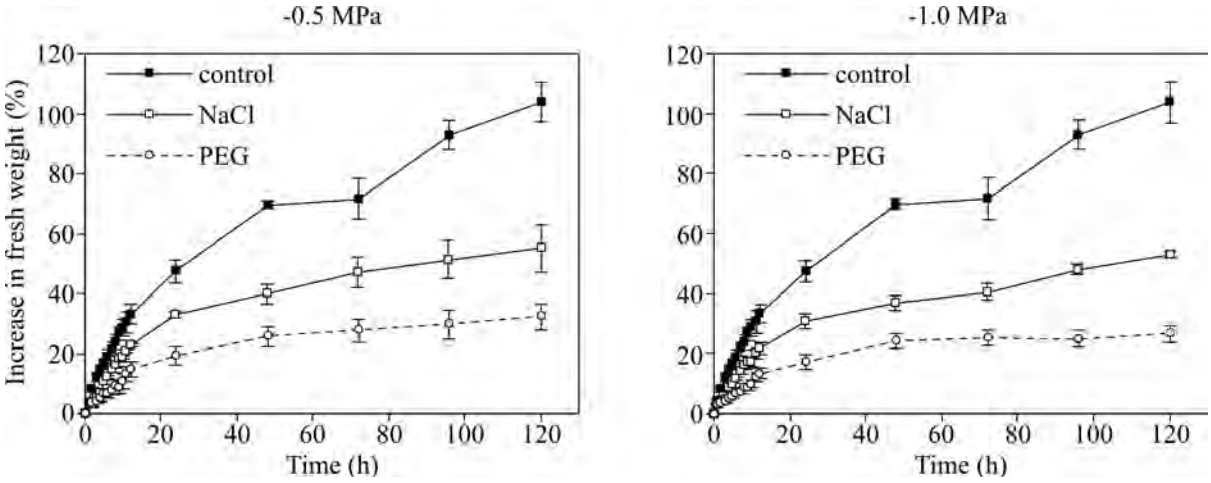
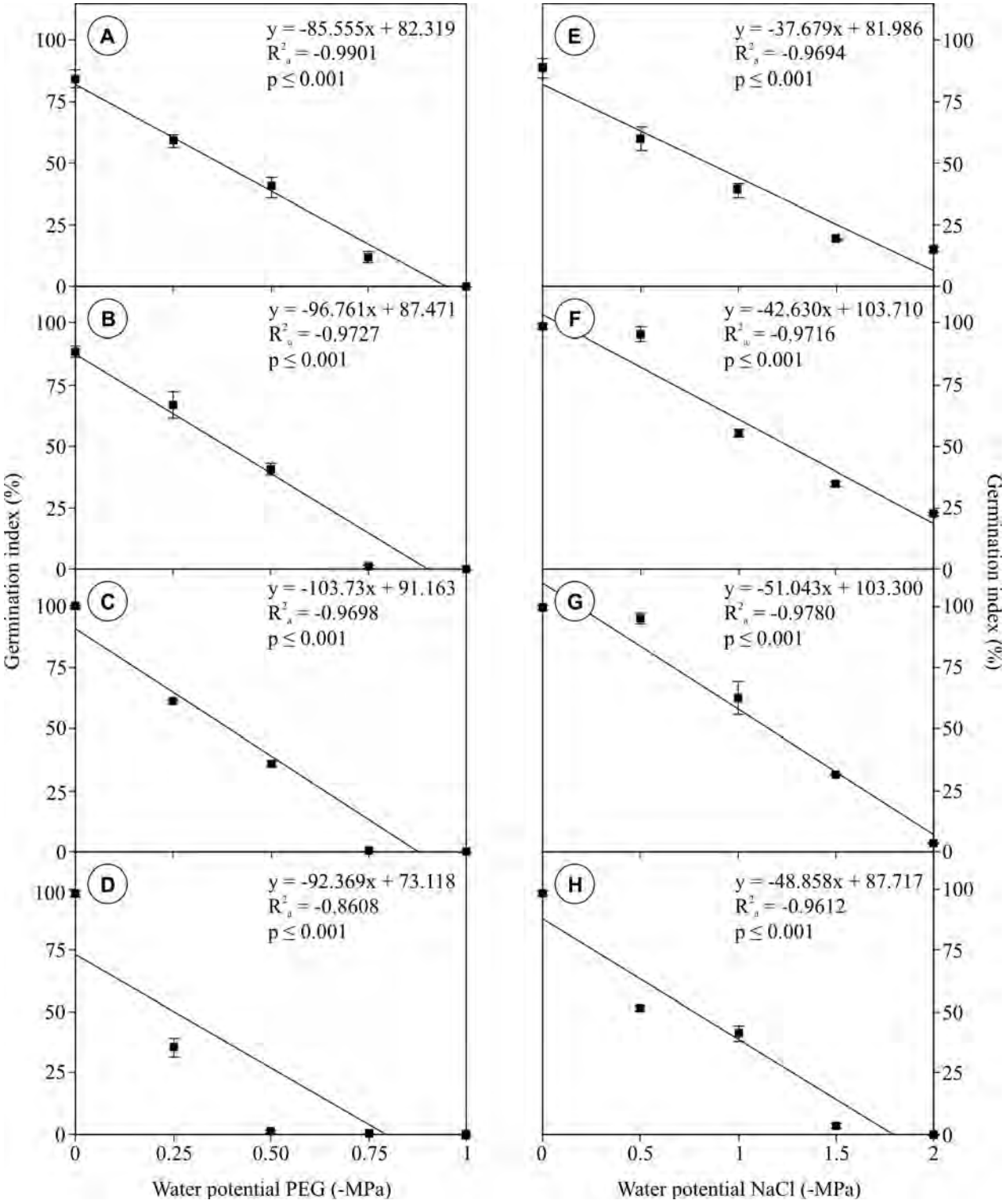

-0.5 MPa
-1.0 MPa
control
NaCl
PEG
Increase in fresh weight (%)
Time (h)
0
20
40
60
80
100
120

Figure 3


A
y = -85.555x + 82.319
R^2_a = -0.9901
p ≤ 0.001
B
y = -96.761x + 87.471
R^2_a = -0.9727
p ≤ 0.001
C
y = -103.73x + 91.163
R^2_a = -0.9698
p ≤ 0.001
D
y = -92.369x + 73.118
R^2_a = -0.8608
p ≤ 0.001
E
y = -37.679x + 81.986
R^2_a = -0.9694
p ≤ 0.001
F
y = -42.630x + 103.710
R^2_a = -0.9716
p ≤ 0.001
G
y = -51.043x + 103.300
R^2_a = -0.9780
p ≤ 0.001
H
y = -48.858x + 87.717
R^2_a = -0.9612
p ≤ 0.001
Germination index (%)
Germination index (%)
Water potential PEG (-MPa)
Water potential NaCl (-MPa)
0
25
50
75
100
0.25
0.50
0.75
1
0.5
1.5
2

Legend of Figures

Figure 1. Time course of germination (%) during the 20-d that seeds were in contact with distilled water (0 MPa) or solutions of PEG (A-D) or NaCl (E-H). The temperatures of incubation were 25ºC (A, E), 30ºC (B, F), 35ºC (C, G) or 40ºC (D, H). Each point represents the mean of four replicates.

Figure 2. Increase in fresh weight of P. juliflora seeds that were germinated in iso-osmotic solutions of NaCl and PEG. The osmotic potentials of solutions were 0 (control), -0.5 MPa (left panel) and -1.0 MPa (right panel) after 120 hours at 25ºC. The vertical bars indicate the standard errors of the means. n = 4.

Figure 3. The germination index plotted against the external water potential for P. juliflora seeds in contact with PEG (A-D) or NaCl (E-H) at 25ºC (A, E), 30ºC (B, F), 35ºC (C, G) and 40ºC (D, H). The symbols represent seeds germinated in NaCl or PEG as indicated. The vertical bars indicate the standard errors of the means. n = 4.

Table 1. Germinability (%) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35 or 40°C. The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman-Keuls test).

PEG 6000 solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa
-0.25	97.5 ± 2.5	Aa	99.0 ± 1.0	Aa	100.0 ± 0.0	Aa	98.0 ± 0.8	Ba
-0.50	99.0 ± 1.0	Aa	93.0 ± 3.3	Bb	95.0 ± 1.7	Bb	10.0 ± 2.2	Cc
-0.75	51.5 ± 4.3	Ba	4.0 ± 2.3	Cb	2.5 ± 0.5	Cb	0.0 ± 0.0	Dc
-1	0.0 ± 0.0	Ca	0.0 ± 0.0	Da	0.0 ± 0.0	Da	0.0 ± 0.0	Da
NaCl solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa
-0.50	100.0 ± 0.0	Aa	100.0 ± 0.0	Aa	98.0 ± 1.2	Aa	96.0 ± 0.8	Bb
-1	96.0 ± 0.8	Ba	98.5 ± 1.0	Aa	96.0 ± 1.4	Ba	98.5 ± 1.0	Aa
-1.50	95.8 ± 0.7	Bb	98.5 ± 1.5	Aa	98.5 ± 1.0	Aa	10.5 ± 1.0	Cc
-2	94.5 ± 1.3	Ba	94.0 ± 0.8	Ba	24.0 ± 2.2	Cb	0.0 ± 0.0	Dc

Table 2. The germination rate ($\bar{t}$, days) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35 or 40°C. The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different ($P \leq 0.05$, Newman-Keuls test).

PEG 6000 solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	1.38 ± 0.03	Aa	1.27 ± 0.02	Aa	1.01 ± 0.01	Aa	1.07 ± 0.02	Aa
-0.25	2.05 ± 0.14	ABa	1.84 ± 0.10	ABa	1.78 ± 0.01	ABa	3.68 ± 0.16	Bb
-0.50	2.72 ± 0.16	Ba	2.66 ± 0.17	Ba	2.96 ± 0.07	Ca	4.23 ± 1.39	Bb
-0.75	6.03 ± 0.42	Cb	9.00 ± 0.71	Cc	3.63 ± 0.38	Ca	---	
-1	---		---		---		---	
NaCl solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	1.24 ± 0.04	Aa	1.03 ± 0.01	Aa	1.02 ± 0.01	Aa	1.04 ± 0.01	Aa
-0.50	1.83 ± 0.05	Bb	1.10 ± 0.02	Aa	1.08 ± 0.01	Aa	2.33 ± 0.03	Bc
-1	2.70 ± 0.08	Cb	1.90 ± 0.03	Ba	1.82 ± 0.12	Ba	2.73 ± 0.09	Cb
-1.50	4.99 ± 0.02	Dd	2.99 ± 0.06	Ca	3.37 ± 0.01	Cb	4.39 ± 0.14	Dc
-2	6.52 ± 0.15	Eb	4.39 ± 0.03	Da	7.75 ± 0.34	Dc	---	

Table 3. The germination synchrony (*Z*) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35 or 40°C. The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different (P≤0.05, Newman-Keuls test). nd = not determined.

PEG 6000 solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	0.57 ± 0.04	Ab	0.63 ± 0.02	Ab	0.98 ± 0.01	Aa	0.93 ± 0.02	Aa
-0.25	0.47 ± 0.01	Bb	0.45 ± 0.01	Bb	0.65 ± 0.01	Ba	0.28 ± 0.05	Bc
-0.50	0.41 ± 0.04	Ba	0.42 ± 0.05	Ba	0.41 ± 0.05	Ca	0.15 ± 0.05	Cb
-0.75	0.17 ± 0.02	Cb	0.33 ± 0.10	Ca	0.00 ± 0.00	Dc	--- nd ---	
-1	--- nd ---		--- nd ---		--- nd ---		--- nd ---	
NaCl solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	0.65 ± 0.04	Ab	0.94 ± 0.02	Aa	0.97 ± 0.02	Aa	0.93 ± 0.02	Aa
-0.50	0.64 ± 0.07	Ab	0.82 ± 0.03	Ba	0.89 ± 0.01	Aa	0.44 ± 0.03	Bc
-1	0.39 ± 0.01	Bb	0.72 ± 0.02	Ca	0.43 ± 0.04	Bb	0.43 ± 0.06	Bb
-1.50	0.33 ± 0.01	Bb	0.54 ± 0.03	Da	0.37 ± 0.02	Bab	0.47 ± 0.05	Ba
-2	0.34 ± 0.03	Ba	0.37 ± 0.03	Ea	0.20 ± 0.05	Cb	--- nd ---	

Table 4. The uncertainty (*U*) of *Prosopis juliflora* seeds at different concentrations of PEG 6000 and NaCl at 25, 30, 35 or 40°C. The values represent the media (± SE) of four replicates of 50 seeds each. Means that are followed by different uppercase letters within columns or by different lowercase letters within rows for each attribute are significantly different (P≤0.05, Newman-Keuls test). nd = not determined.

PEG 6000 solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	1.06 ± 0.08	Ab	0.90 ± 0.05	Ab	0.07 ± 0.04	Aa	0.22 ± 0.04	Aa
-0.25	1.36 ± 0.10	Ab	1.33 ± 0.06	ABb	0.76 ± 0.01	Ba	2.08 ± 0.17	Bc
-0.50	1.41 ± 0.15	Aa	1.49 ± 0.17	Ba	1.60 ± 0.17	Cab	1.96 ± 0.33	Bb
-0.75	2.58 ± 0.12	Bc	1.00 ± 0.01	Ab	0.01 ± 0.01	Aa	--- nd ---	
-1	--- nd ---		--- nd ---		--- nd ---		--- nd ---	
NaCl solution (MPa)	25ºC		30ºC		35ºC		40ºC	
0	0.82 ± 0.08	Ac	0.18 ± 0.07	Aa	0.10 ± 0.06	Aa	0.22 ± 0.04	Aa
-0.50	0.86 ± 0.16	Ab	0.46 ± 0.07	ABa	0.32 ± 0.01	Aa	1.32 ± 0.08	Bc
-1	1.48 ± 0.04	Bb	0.72 ± 0.07	Ba	1.29 ± 0.12	Bb	1.50 ± 0.14	Bb
-1.50	1.66 ± 0.07	Bb	1.23 ± 0.12	Ca	1.63 ± 0.05	Cb	1.94 ± 0.04	Cb
-2	1.76 ± 0.12	Bab	1.56 ± 0.16	Da	2.07 ± 0.30	Db	--- nd ---	

Table 5. Percentage of germinated seeds (GS), non-germinating seeds (NGS) and seeds germinated after recovery (SGR) of *Prosopis juliflora* at different osmotic potentials of NaCl and PEG. The seeds were germinated at 35ºC. The values represent the media (± SE) of four replicates of 50 seeds each.

MPa, Osmolyte	GS	NGS	SGR
-0.75 MPa, PEG	2.5 ± 0.5	97.5 ± 0.5	99.1 ± 0.7
-1 MPa, PEG	0 ± 0	100.0 ± 0.0	99.5 ± 0.5
-1.5 MPa, NaCl	98.5 ± 1.5	1.5 ± 1.2	0 ± 0
-2.0 MPa, NaCl	23.0 ± 1.29	77.0 ± 1.3	0 ± 0

Capitulo III

Limitações fotossintéticas de *Prosopis juliflora* (Sw.) DC sob estresse hídrico

(Este manuscrito deverá ser enviado para a revista *Environmental and Experimental Botany* no período de Maio a Julho de 2013)

Limitações fotossintéticas de Prosopis juliflora (Sw.) DC sob estresse hídrico

Miranda, RQ; Pompelli, MF; Almeida-Cortez, JS

Resumo

Para identificar e analisar os principais mecanismos ecofisiológicos de tolerância à seca da espécie *Prosopis juliflora* (Sw.) DC., foram avaliadas as relações de P_N, C_i, CHR_f, compostos orgânicos e atividade enzimática, sob sete níveis de estresse hídrico. Os valores de P_N apresentaram um declínio logo após o segundo dia de experimento em todos os tratamentos. g_s ($r^2 = 0,8619$, $p<0,05$) e E ($r^2 = 0,8384$, $p<0,05$) apresentaram o mesmo padrão. Já a concentração interna de CO_2 (C_i/C_a) revelou dois comportamentos distintos, sendo nos tratamentos de 15 e 50% de capacidade de campo, uma relação positiva com P_N ($r^2 = 0,7186$, $p<0,05$), e nos tratamentos de supressão total de rega, um padrão inverso ($r^2 = -0,2129$, $p<0,05$). Sob condições ideais, g_s e P_N variam proporcionalmente, numa relação linear, mantendo C_i/C_a e a eficiência intrínseca do uso da água (EUA_i) constantes, no sentido de otimizarem as trocas gasosas. Contudo quando ocorre aumento no C_i, o decréscimo de P_N indica que esta limitação é devida a um decréscimo na eficiência de carboxilação acontece devido à influência de fatores não-estomáticos (*e.g.* limitação da capacidade mesofílica). Neste estudo, *P. juflilora* apresentou ambas limitações de P_N de acordo com a intensidade do estresse imposto. Quando sob 50% e 15% de capacidade de campo as plantas apresentaram alta eficiência no uso da água, e na capacidade de conter danos oxidativos em maiores proporções. Porém com o aumento da severidade do estresse, a eficiência de carboxilação (P_N/C_i) e EUA_i, em plantas jovens de *P. juflilora*, são substancialmente reduzidos, ameaçando o crescimento e a sobrevivência dessas plantas.

Palavras chaves: Caatinga; espécie exótica; algaroba; trocas gasosas; deficit hídrico.

Abstract

To identify and analyze the main adaptive strategies of *Prosopis juliflora* (Sw) DC to withstand drought conditions. We evaluated the relation of P_N, C_i, RWC_f, organic compounds e enzymes, under different levels of water stress. Net photosynthesis (P_N) declined right after the second day of experiment in all treatments. g_s ($r^2 = 0.8619$, $p<0.05$) e E ($r^2 = 0.8384$, $p<0.05$) followed the same pattern. However intracellular carbon (C_i/C_a) presented two different patterns, when plants were treated with 50% and 15% of total water capacity soil, a positive relation with P_N was observed ($r^2 = 0.7186$, $p<0.05$), and when plants were subjected to total suppression of water, a negative relation was revealed ($r^2 = -0.2129$, $p<0.05$). Under ideal conditions, g_s and P_N vary proportionally in a linear relationship, while keep C_i/C_a and intrinsic water use efficiency (WUE_i) constants to optimize gas exchanges, characterizing a pure stomatal limitations of P_N. However when C_i increase with P_N reduction, a decrease in carboxilation efficiency can be observed, and it's likely to be due to non-stomatal limitations of P_N, such as limitations in mesophilic capacity. In this study, *P. juflilora* presented both limitations of P_N. When under 7.90% (50% of soil water capacity) and 3.33% (15%) of soil moisture, plants of *P. juliflora* supported well the hydric stress, showing high water use efficieny, high capacity to counter oxidative damage. However when stress became more severe, carboxilation efficiency (P_N/C_i) and WUE_i of *P. juflilora* plants were substantially reduced, threating growth and survival of this species.

Keywords: Caatinga; exotic species; mesquite; gas exchange; water deficit.

1. Introdução

Em ecossistemas onde predominam ambientes áridos e semiáridos, as plantas estão geralmente sujeitas ao estresse hídrico sazonal, o qual limita o crescimento e o desenvolvimento (Bota et al. 2004; Elfadl e Luukkanen 2006; Boutraa 2010). Nestes ecossistemas, as árvores se distinguem em sua habilidade de superar essas condições xéricas, pois desenvolveram ao longo da evolução mecanismos complexos para enfrentar vários estresses ambientais (Shirke e Pathre 2004; Elfadl e Luukkanen 2006). Estes funcionam provocando e regulando mudanças fisiológicas e bioquímicas, as quais estão coletivamente relacionadas com os mecanismos de tolerância à seca (Souza et al. 2010a).

O principal mecanismo fisiológico que as plantas vasculares dispõem em situações de déficit hídrico é a capacidade de regular a condutância estomática (g_s) (Javot 2002; Souza et al. 2010a), pois esta controla a transpiração, reduzindo significativamente a perda de água pelas folhas (Javot 2002). Ademais, a forma como g_s relaciona-se com a taxa de assimilação de CO_2 (P_N) possui uma importância ecológica, pois quando g_s e P_N variam proporcionalmente, numa relação linear, é possível dizer que a concentração interna de CO_2 (C_i) e a eficiência intrínseca do uso da água (EUA_i) mantêm-se constantes, no sentido de otimizarem as trocas gasosas (Chaves et al. 2009; Souza et al. 2010b).

Contudo quando g_s atinge valores muito próximos de zero, espera-se que o C_i comece a diminuir proporcionalmente à g_s, sugerindo uma limitação estomática de P_N, na qual há um aumento da resistência à difusão de CO_2 para a câmara subestomática (Chaves et al. 2009). Entretanto quando ocorre aumento no C_i, o decréscimo de P_N indica que esta limitação é devido a um decréscimo na eficiência de carboxilação causada principalmente por influência de fatores não-estomáticos (*e.g.* limitação da capacidade mesofílica, limitação bioquímica no ciclo de Calvin) (Noormets et al. 2001; Carmo-Silva et al. 2012). Além disso já é bem documentado que sob condições de baixos níveis de g_s, há um aumento nas taxas de fotorrespiração, e, consequentemente, na produção de espécies reativas ao oxigênio (EROs) nos peroxissomos (Smirnoff 1993), os quais, em altos níveis, também podem ser considerados como um fator de limitação não-estomática de P_N (Imlay 2003).

Diversos estudos têm focado em análises de trocas gasosas e bioquímica de plantas de ambientes áridos e semiáridos (*e.g.* Souza et al. 2010b; Pompelli et al. 2010; Rodrigues et al. 2011; Campos et al. 2012), relatando o comportamento das plantas tanto sob condições naturais de estresse hídrico como sob condições artificiais em casa de vegetação. Nestes

estudos a escassez de água tem sido reportada como o principal estresse limitante da produtividade vegetal (Souza et al. 2010a; Rodrigues et al. 2011). Este trabalho estudou os principais mecanismos ecofisiológicos de tolerância à seca da espécie *Prosopis juliflora* (Sw.) DC., a qual tem sido introduzida e naturalizada em muitas áreas áridas e semiáridas no mundo (El-Keblawy e Al-Rawai 2006), e é conhecida por possuir crescimento rápido, e fixar nitrogênio (El-Keblawy e Al-Rawai 2006; Gallaher e Merlin 2010).

2. Material e métodos

2.1. Planta estudada e local de pesquisa

Prosopis juliflora (Sw.) DC. possui hábito arbustivo-arbóreo, raramente crescendo mais que 10 metros (Gallaher e Merlin 2010), além de apresentar produtividade máxima em áreas com água em abundância (Elfadl e Luukkanen 2006). *P. juliflora* é classificada como uma espécie "sempre verde" na Caatinga, pois mantem as folhas mesmo em períodos de estiagem. *P. juliflora* tem um sistema radicular do tipo pivotante e relativamente profundo podendo alcançar 20-25 m de profundidade (Pasiecznik et al. 2001), e suas folhas são compostas, bipinadas, apresentando filotaxia alterna. O comprimento da folha varia de 5 a 20 cm, e cada folha é composta por 1 a 5 pares de folíolos, e cada um com 6-29 pares de foliólulos (Pasiecznik et al. 2001).

Para este estudo, sementes de *P. juliflora* foram obtidas de frutos coletados na estação experimental do Instituto Agronômico de Pernambuco, localizada, a aproximadamente 140 km de Recife, em uma região semiárida de Caatinga em Caruaru (8°14′18″S, 35°55′20″W; 550 m de altitude).

2.2. Desenho experimental

Plântulas de *Prosopis juliflora* foram obtidas a partir da germinação de suas sementes em câmara de crescimento de plantas a 25ºC sob um fotoperíodo de 12 h claro/escuro segundo metodologia descrita em Miranda et al. (2011). Setenta plântulas resultantes, foram cultivadas em casa de vegetação (temperatura de 28 ± 2°C e umidade relativa do ar de 78.3 ± 8.9%) em sacos plásticos de 5 litros contendo 6 kg de uma mistura de solo e areia lavada (2:1). Passados 180 dias, as plântulas foram aleatoriamente divididas em seis tratamentos de estresse hídrico, sendo três 3 destes por diferenciação de rega de 100% (t_C), 50% (t1) e 15% (t2) da capacidade de campo, e 3 por suspensão total de rega por seis (t3), oito (t4) e dez (t5)

dias. Cada tratamento foi composto de dez repetições. O experimento teve inicio em 8 de fevereiro de 2012 e termino em 7 de março de 2012, e durante todo o experimento, a temperatura e a umidade relativa foram monitoradas diariamente (Fig. 1).

2.3. Conteúdo hídrico relativo do solo

A capacidade do solo de reter água foi mensurada da seguinte forma: cinco sacos extras contendo a mesma quantidade de substrato foram regados até saturar, e em seguida pesados (peso saturado, P_s). Depois os mesmos sacos foram deixados para secar a temperatura ambiente por sete dias, e em seguida pesados (peso seco, P_d). O conteúdo hídrico relativo do solo (CHR_s) a 100% foi determinado pela subtração do P_s pelo P_d, e expresso em litros (L) (Casaroli e Jong van Lier 2008). A quantidade de água necessária para impor cada tratamento foi estimada através do fracionamento do CHR_s a 100%. O CHR_s foi obtido apenas antes do estresse, durante o estresse máximo, e após reidratação completa em cada tratamento.

2.4. Conteúdo hídrico relativo foliar

O conteúdo hídrico relativo foliar (CHR_f) foi medido as 5h, horário local (GMT -3), no período *predawn*, a cada 2 dias durante todo o experimento. Dez foliólulos foram coletados e imediatamente acondicionados em potes plásticos a 4°C. Em seguida, os potes foram levados ao laboratório, onde o CHR_f foi determinado conforme metodologia descrita por Barrs e Weatherley (1962).

2.5. Trocas gasosas

As trocas gasosas foram mensuradas no horário de 8:00 as 10:00 (GMT -3) a cada 2 dias durante todo o experimento. Para tal, folíolos de uma folha totalmente expandida foram submetidos à análise com um analisador de gases por infravermelho (IRGA), modelo LCI-pro (ADC – UK), com uma radiação fotossinteticamente ativa ajustada para 1000 μmol fotóns m^{-2}, valor definido de acordo com curvas de luz previamente realizadas (dados não apresentados). As trocas gasosas forneceram valores de assimilação líquida de carbono (P_N), condutância estomática (g_s), taxa de transpiração (E), carbono intracelular (C_i) e temperatura foliar (T_L). Os valores de C_i foram apresentados em forma de fração C_i/C_a, onde C_a é a quantidade de carbono ambiente.

2.6. Análises bioquímicas

Para as análises bioquímicas foram utilizadas folhas totalmente expandidas de plantas controle e sob déficit hídrico coletadas, as 9h (GMT -3), três vezes por tratamento: antes do estresse, no estresse máximo e após a reidratação completa, a qual foi considerada quando a planta estressada obteve cinco medições de P_N sem diferença estatística do controle (Pompelli et al. 2010). Após a coleta, o material vegetal foi imediatamente congelado em nitrogênio liquido e armazenado a -20°C. Os teores de clorofilas *a, b*, totais e carotenoides totais (Chl*a*, Chl*b*, Chl*t* e Car), carboidratos solúveis totais (TSC), proteínas totais (TP), aminoácidos livres totais (TFAA), malondialdeído (MDA) e peróxido de hidrogênio (H_2O_2) foram determinados com espectrofotômetro ajustado ao comprimento de onda específico para cada composto de acordo com as metodologias de Lichtenthaler (1987), Dubois et al. (1956), Bradford (1976), Moore e Stein (1948), Cakmak e Horst (1991), e Alexieva et al. (2001) respectivamente. Para análise do teor de amido (S), o pallet da extração de TSC foi lavado 5x até apresentar coloração branca, e em seguida encubado em 14µl de Amiloglucosidade. A reação e leitura foi feita de acordo com Dubois et al. (1956).

2.7. Atividade da enzima superóxido dismutase

A atividade total da superóxido dismutase (SOD; EC 1.1.5.11) foi determinada pela habilidade da mesma de inibir a fotorredução do azul de nitrotetrazólio (NBT) segundo metodologia descrita por Giannopolitis e Ries (1977). Os tubos de ensaio, contendo essa mistura, foram colocados em uma caixa de 3,5 cm iluminada por lâmpadas fluorescentes de 40 w, as quais emitiram 130 µmol fótons m^{-2} s^{1} na superfície dos tubos. A reação iniciou com o acendimento das lâmpadas, e parou com o desligamento das mesmas. Os tubos resultantes foram submetidos a análise em espectrofotômetro ajustado a uma absorbância de 560 nm. Um tubo contendo a mistura, porém não irradiado, foi analisado a 560 nm e utilizado como controle, este foi subtraído de todas as outras amostras irradiadas. Uma unidade de SOD foi definida como o montante necessário para inibir 50% da taxa de redução do NBT.

2.8. Análise dos dados

2.8.1 Estimativas dos valores de EUA, EUA_i, P_N/C_i

Os parâmetros de eficiência do uso da água (EUA), eficiência intrínseca do uso da água (EUA_i) e de eficiência de carboxilação (P_N/C_i) foram obtidos através dos cálculos: P_N/E

(Campos et al. 2012), P_N/g_s (Souza et al. 2010b) e P_N/C_i (Shirke e Pathre 2004) respectivamente.

2.8.2 Correlação entre dados bioquímicos e enzimáticos, e trocas gasosas

Para identificar relações entre os parâmetros bioquímicos e as trocas gasosas, todos os dados foram submetidos a uma análise de correlação de Pearson ($\alpha = 0,05$). A análise utilizada foi selecionada de acordo com o perfil que os dados apresentaram quando tiveram suas normalidade e variância testadas (Brown e Forsythe 1974; Stephens 1979). Os dados usados para esta análise não foram filtrados em relação a *outliers*, pois, uma vez que os compostos bioquímicos e enzimáticos foram analisados em pontos discretos no fluxo temporal do experimento, as lacunas resultantes dificilmente seriam preenchidas com precisão.

2.8.3 Outras análises estatísticas

Todos os parâmetros de trocas gasosas foram analisados através de correlação de Pearson ($\alpha = 0,05$), e diferenças nas médias diárias de cada parâmetro em plantas controle e estressada foram analisadas com ANOVA Fatorial, seguida do teste Student Newman Keuls ($\alpha = 0,05$) (Zar 1996). As análises utilizadas foram selecionadas de acordo com o perfil que os dados apresentaram quando tiveram suas normalidade e variância testadas (Brown e Forsythe 1974; Stephens 1979).

3. Resultados

Os valores de CHR_s apresentaram declínio nos tratamentos t1, t2, t3, t4 e t5, alcançando, durante o máximo estresse, valores correspondentes a 46,9%, 18,8%, 7%, 8,8% e 7,1% dos valores controle respectivamente (detalhado em Tabela 1).

Os valores de P_N apresentaram um declínio logo após o segundo dia de experimento nos tratamentos t1, t2, t3, t4 e t5, atingindo, durante o máximo estresse, valores correspondentes a 57,7%, 39,9%, 0.3%, 0.2% e 22% das medidas obtidas no controle respectivamente (Fig. 2). g_s ($r^2 = 0,8619$, $p<0,05$) e E ($r^2 = 0,8384$, $p<0,05$) apresentaram o mesmo padrão que P_N (Fig. 3). Já a razão C_i/C_a revelou dois comportamentos distintos. Quando tratadas com 50% e 15% da capacidade de campo (t1 e t2), C_i/C_a apresentou uma relação positiva com P_N ($r^2 = 0,8911$, $p<0,05$). Entretanto, quando estas foram submetidas a tratamentos de supressão total de rega, C_i/C_a apresentou um padrão inverso ($r^2 = -0,1777$,

$p<0,05$). Além disso, neste último caso, puderam ser observados picos em C_i/C_a durante o máximo estresse, atingindo 790%, 309,6% e 212,4% dos valores do controle nos tratamentos t3, t4 e t5 respectivamente (Fig. 2).

Os resultados indicaram, nos tratamentos t3, t4 e t5, uma queda nos valores de EUA no quarto dia de estresse, seguida de uma pequena recuperação, estabilizando perto do ponto de compensação até a reidratação, quando voltaram a apresentar medidas equiparáveis ao controle (Fig. 4). No caso de EUA_i, no 4 dia, houve um pico, e no intervalo dos dias seguintes até a reidratação, puderam ser observados valores mais próximos a 0 (Fig. 4). Para os tratamentos t1 e t2, EUA apresentou uma queda pontual e depois oscilou em torno do controle (Fig. 4). Já EUA_i, para ambos os tratamentos, apresentou um aumento seguido de estabilização, e retorno a medidas próximas ao controle após reidratação (Fig. 5).

Os valores de P_N/C_i apresentaram um padrão similar ao encontrado em EUA_i (Fig. 4 e 5). No tratamento t1, CHR_f não mostrou diferença significativa em relação ao controle em nenhum ponto, já o t2 apresentou queda a partir do 6º dia de estresse, seguido de estabilização em torno de 72,89% variando de 80,25% a 57,38% neste período, e voltando a valores estatisticamente iguais ao controle com a reidratação (Fig. 5). Comportamentos parecidos foram observados nos tratamentos t3, t4 e t5, nos quais ocorreu uma queda a partir do 2º dia, atingindo valores de 36,17%, 34,48% e 35,77% em relação ao controle respectivamente (Fig. 5).

Dos dados de trocas gasosas analisados junto à bioquímica e enzima, CHR_f e *E* foram os parâmetros que se relacionaram com a maior porcentagem dos atributos bioquímicos e enzimáticos analisados. Os teores de Chl*b*, Car, TSC, TP, TFAA, S, MDA, H_2O_2 e SOD das plantas referentes aos tratamentos t3 t4 e t5 aumentaram de acordo com a diminuição do CHR_f e *E* (Fig. 6). Já Chl*a* e Chl*t* não apresentaram relação significativa com nenhum atributo das trocas gasosas (Fig. 6). No caso dos tratamentos t1 e t2, a variável que se destacou em termos de representatividade nas correlações com a bioquímica foi g_s, o qual diminuiu com o aumento de Chl*a*, Car, TSC, TP, S e H_2O_2 (Fig. 6). Enquanto Chl*b*, Chl*t*, TFAA, MDA e SOD não apresentaram relação significativa com nenhuma das variáveis fotossintéticas analisadas (Fig. 6). Além disso, MDA se correlacionou positivamente com H_2O_2 em todos os tratamentos ($r^2 = 0,9239$, $p<0,05$), porém quando o MDA foi correlacionado com o P_N, apenas nos tratamentos t3, t4 e t5 a correlação foi significativa ($r^2 = -0,4221$, $p<0,05$). O mesmo aconteceu quando P_N foi correlacionado com Chl*a*/Chl*b* ($r^2 = -0,3386$, $p<0,05$).

4. Discussão

A fim de entender as respostas que influenciam no uso de CO_2 e H_2O pela folha, e no crescimento inicial de plantas *Prosopis juliflora* sob diferentes regimes hídricos, será discutido as relações de P_N, C_i/C_a, compostos orgânicos e enzimas, descrevendo os principais mecanismos que a planta dispõe para evitar a desidratação total.

A escassez de água é considerada o principal fator ambiental limitante para a fotossíntese em plantas, especialmente em regiões áridas e semiáridas (Chaves et al. 2003), pois induz várias mudanças nos componentes fisiológicos, bioquímicos e moleculares da fotossíntese (Elfadl e Luukkanen 2006). Sob tais condições, CHR_s e CHR_f reduzem gradualmente, entretanto o decréscimo de CHR_s não possui uma relação linear com CHR_f , ou seja, o decréscimo de CHR_s não leva obrigatoriamente ao aumento do estresse hídrico na planta, pois este é mais dependente de respostas por parte de CHR_f (Chaves e Oliveira 2004; Prasad et al. 2008).

CHR_f varia diariamente de acordo com E, e é menor durante o dia do que a noite. Quando CHR_s é alto, CHR_f, em plantas C3 como *P. juliflora*, atinge seus valores máximos durante a noite, pois neste momento os estômatos estão fechados, e o potencial hídrico do solo e da planta tendem a se igualarem (Prasad et al. 2008). Os efeitos da escassez de água no CHR_f são sempre relativamente progressivos (Griffiths e Parry 2002), pois a absorção de água pelas células vegetais é mais lenta do que a perda (Chaves et al. 2003).

Quando CHR_f é baixo, P_N, g_s e P_N/C_i, se relacionam, geralmente, de forma diretamente proporcional numa relação linear, mantendo C_i/C_a e EUA_i constantes, com o objetivo de otimizar as trocas gasosas, diminuindo a perda de água pela transpiração. Esse tipo de comportamento indica a ocorrência de apenas limitação estomática da fotossíntese, a qual limita o fluxo de CO_2 apenas com o aumento da tolerância na difusão de CO_2 na planta (estomática e mesofílica) (Noormets et al. 2001; Chaves et al. 2003). Nestes casos, Mansfield e Davies (1985) sugerem que o decréscimo de g_s é induzido por uma maior produção e acúmulo de ácido abscísico (ABA) nas folhas, permitindo a manutenção de baixos valores de g_s, além da consequente conservação do turgor celular, resultante da redução da desidratação.

Quando as plantas foram submetidas a estresses leve e moderado (t1 e t2), as medidas de CHR_f estabilizaram em valores relativamente altos (em média 70%), e apresentaram uma redução de P_N menor que a de g_s e C_i/C_a, o que configura uma espécie bem adaptada à

deficiência hídrica, cujos valores de EUA_i e P_N/C_i, se mostraram mais alto que os do controle. Contudo, apesar dos valores não apresentarem relação linear, acredita-se que houve apenas limitação estomática. Esta conclusão foi reforçada pela recuperação rápida desta espécie após a reidratação (24h), devido, provavelmente, à ausência de danos oxidativos em proporções maiores, uma vez que os níveis de MDA e SOD não apresentaram nenhum padrão significativo em relação às variáveis influenciadas pelo estresse. Entretanto, os teores de H_2O_2, o qual é o produto da atividade da SOD (Weydert e Cullen 2010), foram significativamente reduzidos com o estresse, provavelmente devido ao aumento da atividade de outras enzimas do sistema antioxidativo (*e.g.* catalase, ascorbato peroxidase) (Weydert e Cullen 2010), o que sugere que, mesmo não tendo sido significativo, houve algum estresse oxidativo, o que já era, de certa forma, esperado, visto que, segundo Kar (2011), a regulação ou tolerância da difusão de CO_2 sempre favorece o aumento da fotorrespiração.

Durante a reidratação dos tratamentos t1 e t2, o aumento simultâneo de CHR_f e g_s sugere uma boa condutividade hidráulica do xilema, visto que a reidratação das células ocorreu rapidamente (Chaves et al. 2003). Esta pode ser mais uma característica adaptativa de *P. juflilora* a esse tipo de estresse. Dependendo da espécie e intensidade da desidratação, P_N pode atingir valores relativamente baixos sem significativo declínio da capacidade fotossintética do mesófilo (Chaves 1991).

Nos tratamentos t3, t4 e t5, com o aumento da severidade do estresse, foi observado uma forte desidratação dos tecidos, levando à deficiência metabólica. Sob tais condições, em algumas espécies, a inibição não estomática da fotossíntese ocorre, causando um temporário aumento em C_i/C_a, o que, consequentemente, causa fechamento estomático (Noormets et al. 2001). Embora o mecanismo que controla essa resposta ainda seja obscuro (Negi et al. 2008), a teoria mais aceita aponta para uma possível ligação com a síntese de malato, o qual é conhecido por regular os canais de anions da membrana plasmática das células guardas (Kim et al. 2010). Em casos como esse, Noormets et al. (2001) afirmam que o decréscimo de g_s pode ser o resultado e não a causa da redução de P_N. Vários estudos afirmam que este tipo limitação pode ser uma resposta a danos oxidativos aos cloroplastos devido ao acúmulo de espécies reativas de oxigênio (EROs) (Zhou et al. 2007), alterações bioquímicas no metabolismo da planta (Chaves et al. 2009), alteração da capacidade mesofílica (Bota et al. 2004), ou ao fechamento estomático inconsistente (Gunasekera e Berkowitz 1992).

Outra característica de plantas que demonstram tolerância ao deficit hídrico, é a

habilidade de efetivamente diminuir ou evitar danos causados por EROs, as quais podem causar danos ao DNA, ao fotossistema II (PSII), ou levar à peroxidação de membranas (Yazici et al. 2007), causando mudanças em sua fluidez (Chaves et al. 2009). A degradação de ácidos graxos devido à peroxidação produz não apenas íons de peroxido, mas também MDA (*e.g.* Pompelli et al. 2010; Arcoverde et al. 2011). O conteúdo de MDA, produto da peroxidação de lipídios, tem sido considerado como um bom indicador do estresse oxidativo (Pompelli et al. 2010; Arcoverde et al. 2011). Em plantas de *P. juliflora*, a relação inversamente proporcional dos teores de MDA, H_2O_2 e SOD com os teores de CHR_f e *E*, sugere um aumento significativo na atividade de EROs associada à atividade da enzima SOD.

O mecanismo de defesa contra as EROs, citado acima, geralmente varia de acordo com a intensidade do estresse, e consiste em manter altos níveis de enzimas antioxidantes como a SOD (Loggini et al. 1999; Kim et al. 2010), as quais possuem a capacidade de inativar as EROs (Jin et al. 2009). A regulação de enzimas do sistema oxidativos, por parte da planta, é um mecanismo importante para entender as respostas das plantas a estresses ambientais (Loggini et al. 1999), especialmente o estresse hídrico (Pompelli et al., 2010).

Ademais, Havaux (1992) apontou valores de CHR_f de 40%, como sendo o limite no qual o PSII não tem sua funcionalidade comprometida tanto no escuro como na luz. Teoria corroborada em *P. juliflora*, visto que a razão Chl*a*/Chl*b* diminuiu com o estresse, indicando possíveis danos ao PSII. A redução no teor de clorofilas pode contribuir, em geral, para a proteção do maquinário fotossintético contra os efeitos da radiação luminosa sob estresse hídrico, uma vez que minimiza a capacidade de coleta de luz e, consequentemente, a excitação do PSII (Zhou et al. 2007). Nestes casos, a recuperação de P_N, deve ser mais lenta, uma vez que a reparação dos danos sofridos sob estresse é mais lenta que a recuperação de g_s.

Em contra partida, a manutenção do CHR_f por vários dias sob CHR_s próximo de 0% sugere a ocorrência de ajustamento osmótico, que por sua vez mantêm o nível de turgescência através da diminuição do potencial osmótico foliar, resultante da maior concentração de solutos no citoplasma (*e.g.* carboidratos solúveis e aminoácidos) (Chaves e Oliveira 2004).

Vale ressaltar, que por muito tempo acreditou-se que a redução da fotossíntese sempre afeta negativamente o conteúdo de carbono na planta, prejudicando todo o metabolismo deste elemento (*e.g.* Imlay 2003), em última análise, promovendo a insuficiência do crescimento devido à falta de carbono (*e.g.* Arcoverde et al. 2011). Porém, a literatura vem convergindo para apoiar a conclusão de que, sob déficit hídrico, os compostos baseados em carbono na

maioria das vezes se acumulam nos órgãos resultando em um aumento de suas concentrações (Muller et al. 2011). Tal acumulação já foi relatada em diversas espécies, e em várias partes da planta para formar diferentes compostos (solúveis ou estruturais). Assim como em *P. juliflora*, a concentração de TSC aumenta, sob déficit hídrico, nas folhas de *Pinaceae* (Chaves et al. 2009), e *Vitis* sp. (Boyle et al. 1991). Essa acumulação acontece tanto devido a um rápido estresse hídrico (Turner et al. 1978), como a um lento crescimento sob deficit hídrico (Muller et al. 2011), e muitas vezes, como já foi dito anteriormente, está associada à manutenção do turgor celular (Chaves e Oliveira 2004).

A manutenção do turgor celular também tem um papel importante no crescimento celular (Alves e Setter 2004). A expansão da área foliar total é geralmente limitada sob estresse hídrico, e como consequência a expansão e o desenvolvimento da superfície transpiratória é drasticamente reduzida (Alves e Setter 2004). Esta sensibilidade é expressa em diminuição do tamanho das células, e redução no número de células produzidas pelos meristemas foliares (Cramer et al. 2007).

Sob estresse severo, a área foliar total pode diminuir através da redução ou parada total dos processos de brotamento de novas folhas (Cramer et al. 2007; Prasad et al. 2008). E com o estresse continuado, a senescência foliar pode ser acelerada através da morte dos tecidos (Cramer et al. 2007). A reidratação das plantas depois de um período de estresse relativamente curto (3 a 5 dias) não elimina completamente os efeitos da escassez de água no processo de senescência (Alves e Setter 2004). Em *P. juliflora*, a alta taxa de senescência foliar observada pode servir como um mecanismo de fuga da seca, uma vez que a redução da área foliar total ajuda a limitar a perda de água (Prasad et al. 2008).

Tais características fisiológicas para evitar a desidratação total podem influenciar significativamente na sobrevivência de *P. juliflora* em ambientes com baixa disponibilidade hídrica, permitindo superar longos períodos de estiagem.

5. Conclusão

As plantas jovens de *Prosopis juflilora* (Sw.) DC, mesmo sob relativa média ou baixa umidade do solo (t1 ou t2), suportaram o estresse apresentando alta eficiência no uso da água, e na capacidade de conter danos oxidativos em maiores proporções. Neste estudo, baseado nas respostas de P_N, C_i/C_a e g_s, podemos concluir que apenas sob total falta d'água (t3, t4 e t5), a eficiência de carboxilação (P_N/C_i), e a eficiência intrínseca do uso da água (EUA_i), em plantas

jovens de *P. juflilora*, são substancialmente reduzidos, ameaçando o crescimento e a sobrevivência dessas plantas.

6. Agradecimentos

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq (Recursos 473202/2008-5) e a Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco, FACEPE (Recursos APQ-0150-2.03/08) pelo financiamento deste trabalho.

7. Referências bibliográficas

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment 24, 1337–1344.

Alves, A.A.C., Setter, T.L., 2004. Response of cassava leaf area expansion to water deficit: cell proliferation, cell expansion and delayed development. Annals of botany 94, 605–613.

Arcoverde, G.B., Rodrigues, B.M., Pompelli, M.F., Santos, M.G., 2011. Water relations and some aspects of leaf metabolism of *Jatropha curcas* young plants under two water deficit levels and recovery. Brazilian Journal of Plant Physiology 23, 123–130.

Barrs, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences 15, 413–428.

Bota, J., Medrano, H., Flexas, J., 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? New Phytologist 162, 671–681.

Boutraa, T., 2010. Improvement of Water Use Efficiency in Irrigated Agriculture: A Review. Journal of Agronomy 9, 1–8.

Boyle, M.G., Boyer, J.S., Morgan, P.W., 1991. Stem Infusion of Liquid Culture Medium Prevents Reproductive Failure of Maize at Low Water Potential. 31, 1246–1252.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72, 248–254.

Brown, M.B., Forsythe, A.B., 1974. Robust Tests for the Equality of Variances. Journal of the American Statistical Association 69, 364–367.

Cakmak, I., Horst, W.J., 1991. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiologia Plantarum 83, 463–468.

Campos, M.L. de O., Hsie, B.S. de, Granja, J.A. de A., Correia, R.M., Almeida-Cortez, J.S. de, Pompelli, M.F., 2012. Photosynthesis and antioxidant activity in *Jatropha curcas* L. under salt stress. Brazilian Journal of Plant Physiology 24, 55–67.

Carmo-Silva, A.E., Gore, M.A., Andrade-Sanchez, P., French, A.N., Hunsaker, D.J., Salvucci, M.E., 2012. Decreased CO2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. Environmental and Experimental

Botany 83, 1–11.

Casaroli, D., Jong van Lier, Q. De, 2008. Critérios para determinação da capacidade de vaso. Revista Brasileira de Ciência do Solo 32, 59–66.

Chaves, M.M., 1991. Effects of Water Deficits on Carbon Assimilation. Journal of Experimental Botany 42, 1–16.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Annals of botany 103, 551–560.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought — from genes to the whole plant. Functional Plant Biology 30, 239–264.

Chaves, M.M., Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of experimental botany 55, 2365–2384.

Cramer, G.R., Ergül, A., Grimplet, J., Tillett, R.L., Tattersall, E.A.R., Bohlman, M.C., Vincent, D., Sonderegger, J., Evans, J., Osborne, C., Quilici, D., Schlauch, K.A., Schooley, D.A., Cushman, J.C., 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Functional & integrative genomics 7, 111–134.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28, 350–356.

El-Keblawy, A., Al-Rawai, A., 2006. Impacts of the invasive exotic *Prosopis juliflora* (Sw.) D.C. on the native flora and soils of the UAE. Plant Ecology 190, 23–35.

Elfadl, M.A., Luukkanen, O., 2006. Field studies on the ecological strategies of *Prosopis juliflora* in a dryland ecosystem. Journal of Arid Environments 66, 1–15.

Gallaher, T., Merlin, M., 2010. Biology and Impacts of Pacific Island Invasive Species. 6. *Prosopis pallida* and *Prosopis juliflora* (Algarroba, Mesquite, Kiawe) (Fabaceae). Pacific Science 64, 489–526.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant physiology 59, 309–314.

Griffiths, H., Parry, M.A.J., 2002. Plant responses to water stress. Annals of botany 89, 801–802.

Gunasekera, D., Berkowitz, G.A., 1992. Heterogenous stomatal closure in response to leaf water deficits is not a universal phenomenon. Plant physiology 98, 660–665.

Havaux, M., 1992. Stress Tolerance of Photosystem II in Vivo: Antagonistic Effects of Water, Heat, and Photoinhibition Stresses. Plant physiology 100, 424–432.

Imlay, J.A., 2003. Pathways of oxidative damage. Annual review of microbiology 57, 395–418.

Javot, H., 2002. The Role of Aquaporins in Root Water Uptake. Annals of Botany 90, 301–313.

Jin, X., Huang, Y., Zeng, F., Zhou, M., Zhang, G., 2009. Genotypic difference in response of peroxidase and superoxide dismutase isozymes and activities to salt stress in barley. Acta Physiologiae Plantarum 31, 1103–1109 LA – English.

Kar, R.K., 2011. Plant responses to water stress: role of reactive oxygen species. Plant signaling & behavior 6, 1741–1745.

Kim, T.-H., Böhmer, M., Hu, H., Nishimura, N., Schroeder, J.I., 2010. Guard cell signal transduction network: advances in understanding abscisic acid, CO2, and Ca2+ signaling. Annual review of plant biology 61, 561–591.

Lichtenthaler, H., 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in enzymology 148, 350–382.

Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F., 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant physiology 119, 1091–1100.

Mansfield, T.A., Davies, W.J., 1985. Mechanisms for leaf control of gas exchange. BioScience 35, 158–164.

Miranda, R.Q., Oliveira, M.T.P., Correia, R.M., Almeida-Cortez, J.S., Pompelli, M.F., 2011. Germination of *Prosopis juliflora* (Sw) DC seeds after scarification treatments. Plant Species Biology 26, 186–192.

Moore, S., Stein, W.H., 1948. Photometric ninhydrin method for use in the chromatography of amino acids. The Journal of biological chemistry 176, 367–388.

Muller, B., Pantin, F., Génard, M., Turc, O., Freixes, S., Piques, M., Gibon, Y., 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. Journal of Experimental Botany 62, 1715–1729.

Negi, J., Matsuda, O., Nagasawa, T., Oba, Y., Takahashi, H., Kawai-Yamada, M., Uchimiya, H., Hashimoto, M., Iba, K., 2008. CO2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature 452, 483–486.

Noormets, A., Sober, A., Pell, E.J., Dickson, R.E., Podila, G.K., Sober, J., Isebrands, J.G., Karnosky, D.F., 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO2 and/or O3. Plant, Cell and Environment 24, 327–336.

Pasiecznik, N.M., Felker, P., Harris, P.J.C., Harsh, L.N., Cruz, G., Tewari, J.C., Cadoret, K., Maldonado, L.J., 2001. The *Prosopis juliflora* - *Prosopis pallida* Complex. HDRA, Coventry, UK.

Pompelli, M.F., Barata-Luís, R., Vitorino, H.S., Gonçalves, E.R., Rolim, E. V., Santos, M.G., Almeida-Cortez, J.S., Ferreira, V.M., Lemos, E.E., Endres, L., 2010. Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. Biomass and Bioenergy 34, 1207–1215.

Prasad, P.V. V., Staggenborg, S.A., Ristic, Z., 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Ahuja, L.R., Reddy, V.R., Saseendran, S.A., Yu, Q. (Eds.), Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes. American Society of Agronomy, Madison, pp. 301–355.

Rodrigues, B.M., Arcoverde, G.B., Santos, M.G., 2011. Notas Científicas Water relations in physic nut according to climatic seasonality, in semiarid conditions. , 1112–1115.

Shirke, P.A., Pathre, U. V, 2004. Influence of leaf-to-air vapour pressure deficit (VPD) on the biochemistry and physiology of photosynthesis in *Prosopis juliflora*. Journal of Experimental Botany 55, 2111–2120.

Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125, 27–58.

Souza, B.D., Meiado, M. V., Rodrigues, B.M., Santos, M.G., 2010a. Water relations and chlorophyll fluorescence responses of two leguminous trees from the Caatinga to different watering regimes. Acta Physiologiae Plantarum 32, 235–244.

Souza, B.D., Rodrigues, B.M., Endres, L., Santos, M.G., 2010b. Ecophysiology parameters of four Brazilian Atlantic Forest species under shade and drought stress. Acta Physiologiae Plantarum 32, 729–737.

Stephens, M.A., 1979. Tests of fit for the logistic distribution based on the empirical distribution function. Biometrika 66, 591–595.

Turner, N., Begg, J., Tonnet, M., 1978. Osmotic Adjustment of Sorghum and Sunflower Crops in Response to Water Deficits and Its Influence on the Water Potential at Which Stomata Close. Australian Journal of Plant Physiology 5, 597–608.

Weydert, C.J., Cullen, J.J., 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nature protocols 5, 51–66.

Yazici, I., Türkan, I., Sekmen, A.H., Demiral, T., 2007. Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environmental and Experimental Botany 61, 49–57.

Zar, J.H., 1996. Biostatistical Analysis. Prentice Hall, Upper Saddle River.

Zhou, Y., Lam, H.M., Zhang, J., 2007. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. Journal of Experimental Botany 58, 1207–1217.

Fig. 1

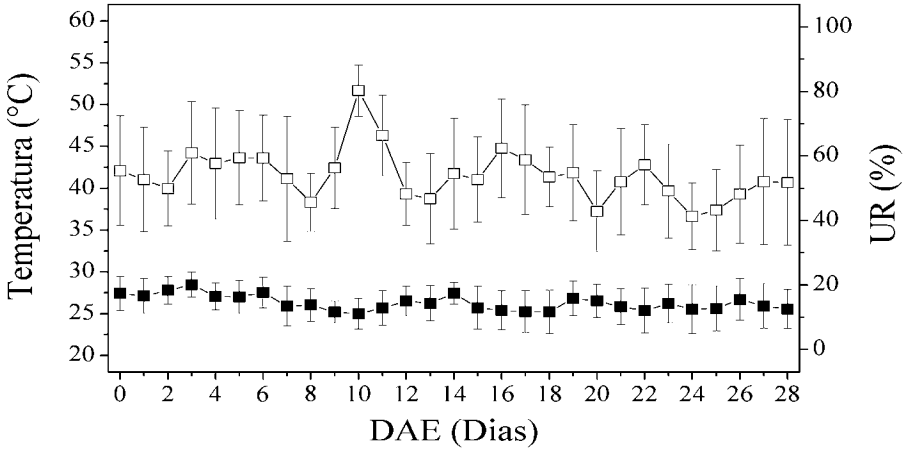

Temperatura (°C)
UR (%)
DAE (Dias)
60
55
50
45
40
35
30
25
20
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

Fig. 2

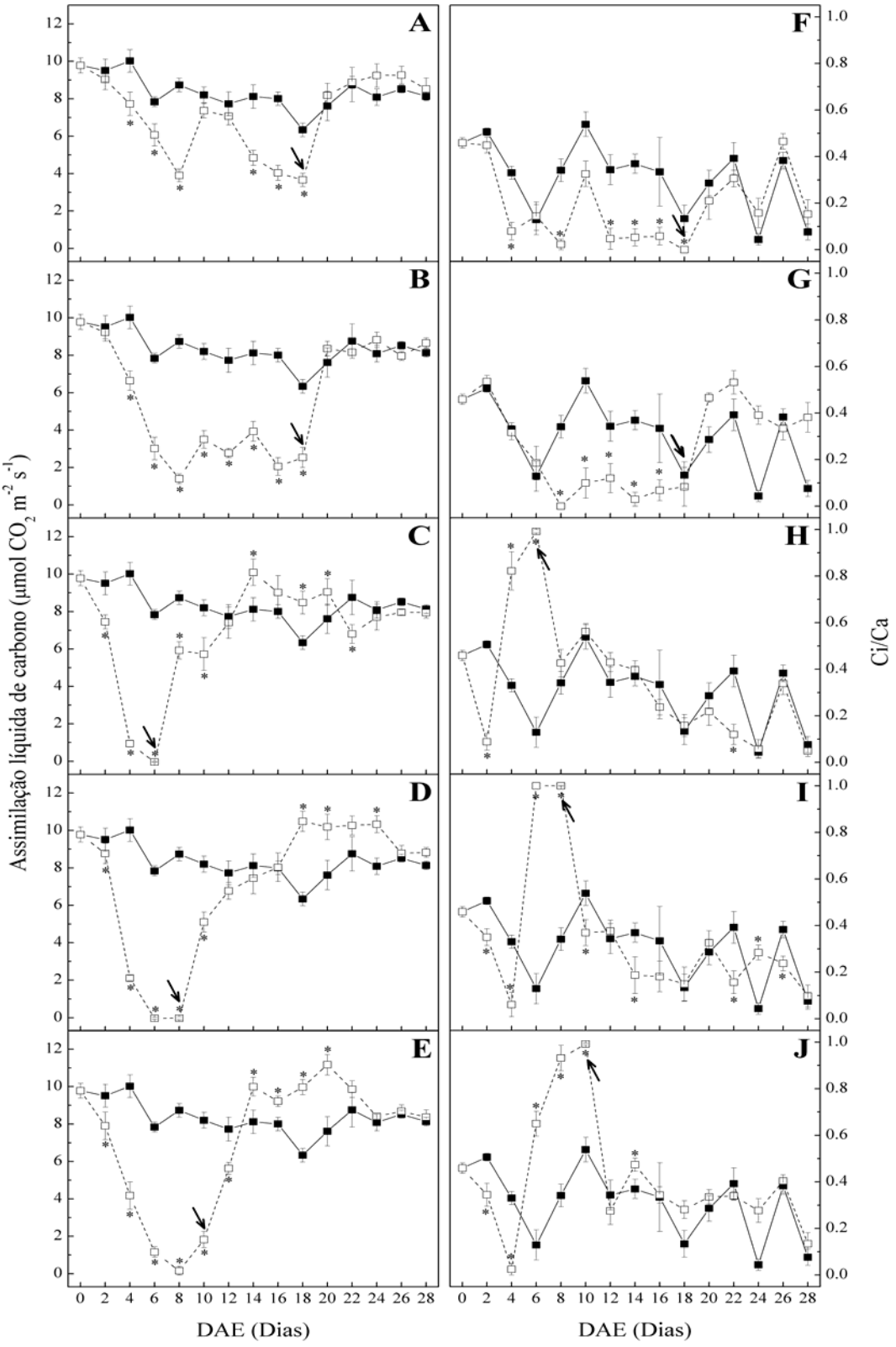

A
B
C
D
E
F
G
H
I
J
Assimilação líquida de carbono (µmol CO_2 m^{-2} s^{-1})
Ci/Ca
DAE (Dias)
DAE (Dias)

Fig. 3

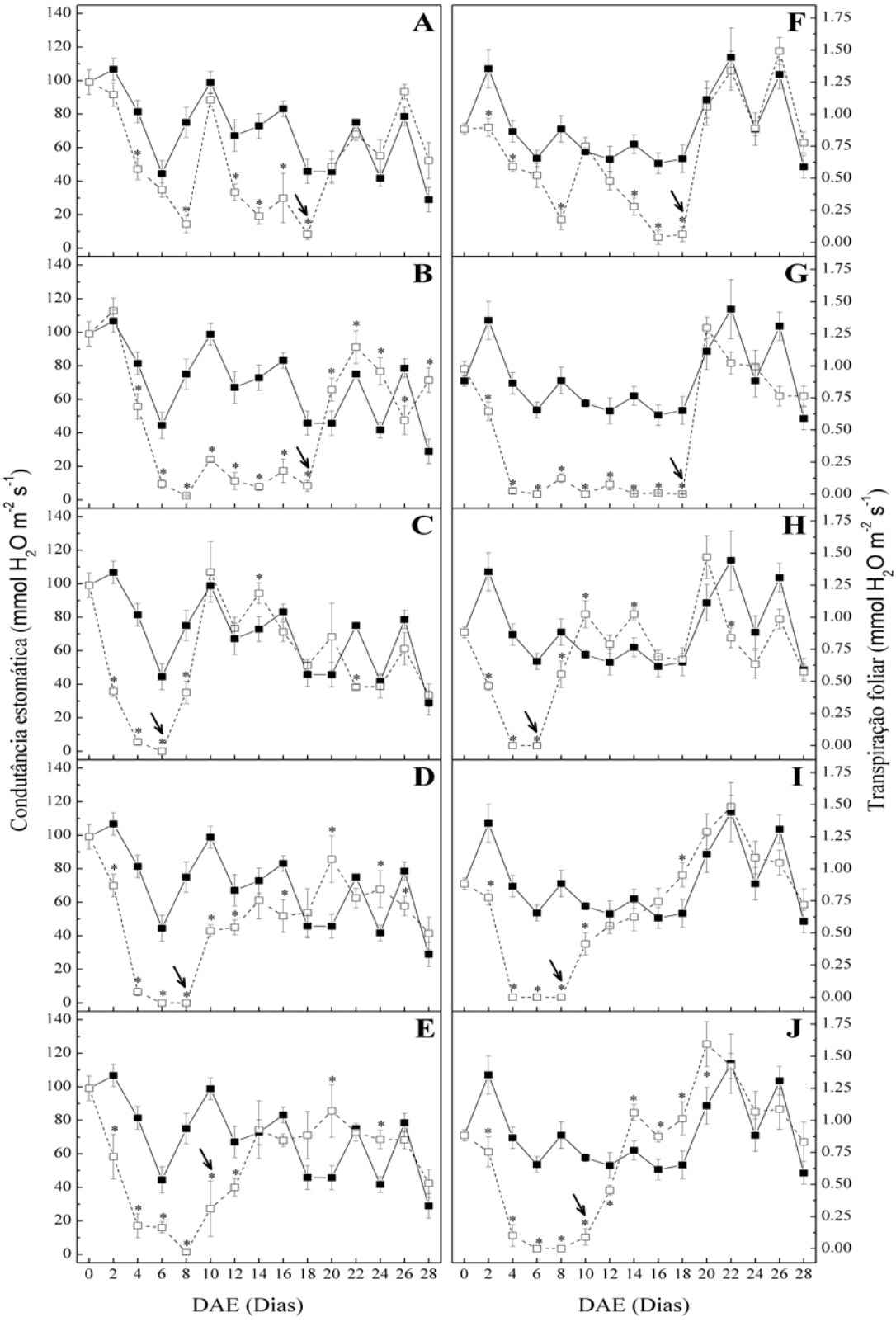

A
B
C
D
E
F
G
H
I
J
Condutância estomática (mmol H2O m-2 s-1)
Transpiração foliar (mmol H2O m-2 s-1)
DAE (Dias)
DAE (Dias)

Fig. 4

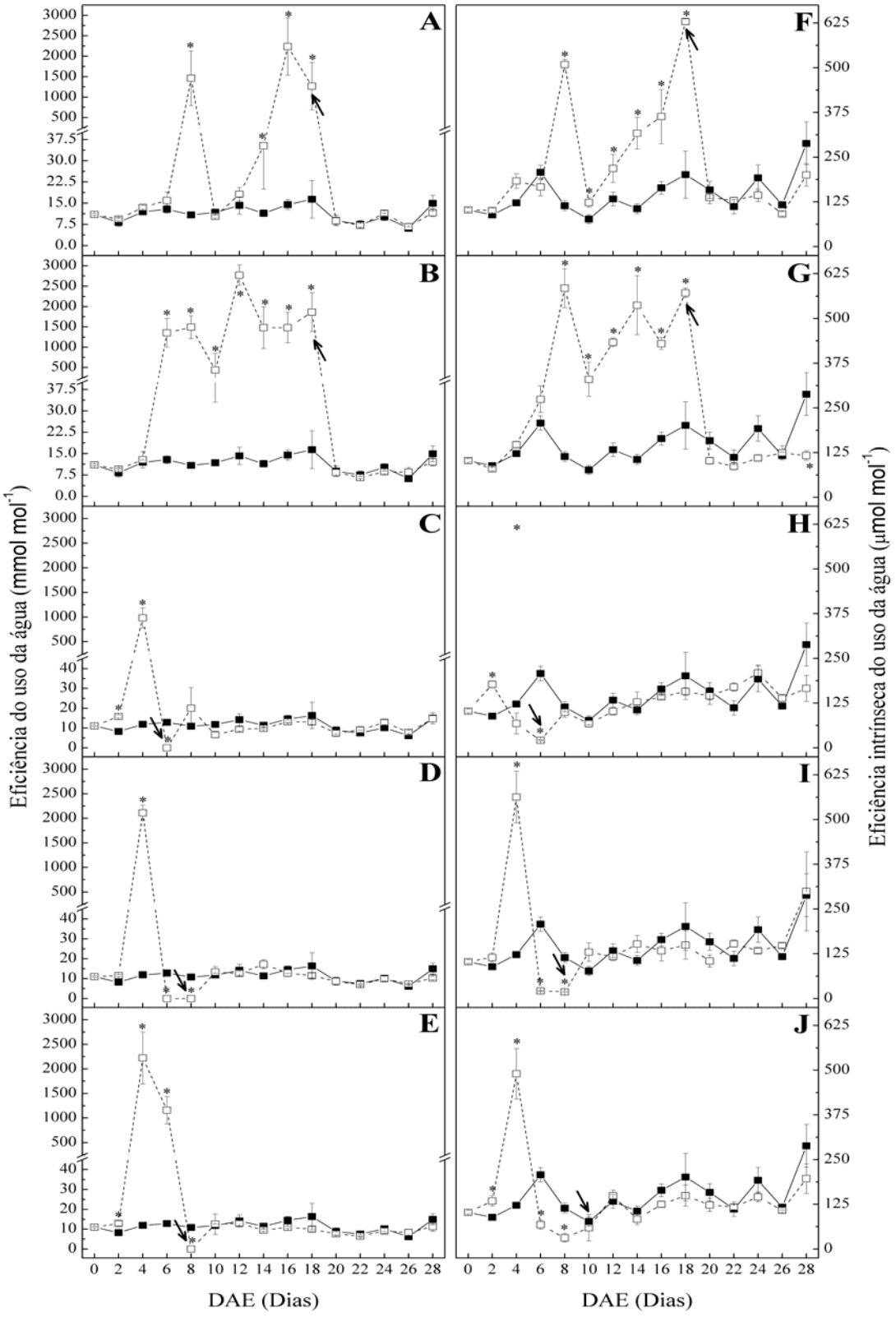

A
B
C
D
E
F
G
H
I
J
Eficiência do uso da água (mmol mol-1)
Eficiência intrínseca do uso da água (µmol mol-1)
DAE (Dias)
DAE (Dias)

Fig. 5

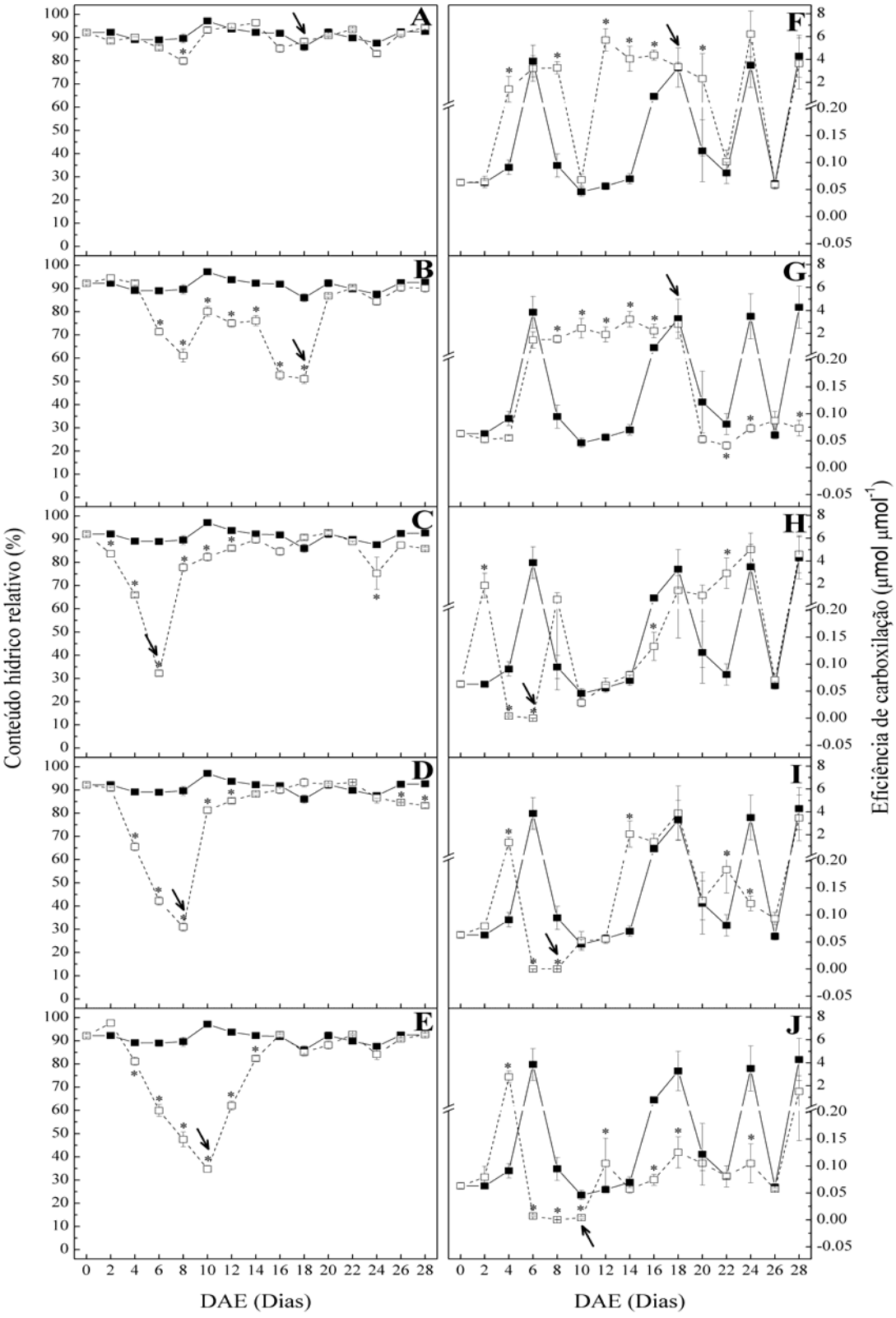
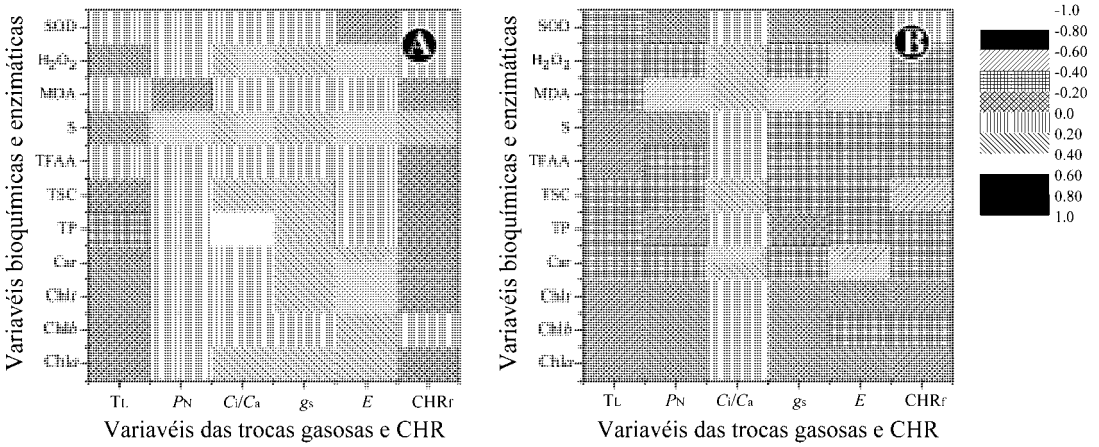

A
B
C
D
E
F
G
H
I
J
Conteúdo hídrico relativo (%)
Eficiência de carboxilação (µmol µmol-1)
DAE (Dias)
DAE (Dias)

Fig. 6


A
B
SOD
H_2O_2
MDA
S
TFAA
TSC
TP
Car
Chlt
Chla
Chlb
T_L
P_N
C_i/C_a
g_s
E
CHRf
Variavéis bioquímicas e enzimáticas
Variavéis das trocas gasosas e CHR
-1.0
-0.80
-0.60
-0.40
-0.20
0.0
0.20
0.40
0.60
0.80
1.0

Legenda das figuras

Fig. 1. Curso temporal da temperatura atmosférica (■) e umidade relativa do ar (□) de 8 de fevereiro a 7 março em Recife, Brasil, 2012.

Fig. 2. Efeito do estresse hídrico em P_N (A-E) e C_i (F-J) em plantas de *Prosopis juliflora* (Sw.) DC. submetidas a tratamentos por diferenciação de rega de 50% (A, F) e 15% (B, G) da capacidade de campo, e por suspensão total de rega por 6 (C, H), 8 (D, I) e 10 (E, J) dias. As setas indicam o dia em que as plantas foram reidratadas, e os asteriscos indicam diferenças significativas entre as médias diárias dos valores de cada parâmetro de plantas estressadas (□) e plantas controle (■) ($p \leq 0.05$, Newman-Keuls) Todos os dados estão expressos pela média ± erro padrão, n = 10.

Fig. 3. Efeito do estresse hídrico em g_s e E em plantas de *Prosopis juliflora* (Sw.) DC. submetidas a tratamentos por diferenciação de rega de 50% (A, F) e 15% (B, G) da capacidade de campo, e por suspensão total de rega por 6 (C, H), 8 (D, I) e 10 (E, J) dias. As setas indicam o dia em que as plantas foram reidratadas, e os asteriscos indicam diferenças significativas entre as médias diárias dos valores de cada parâmetro de plantas estressadas (□) e plantas controle (■) ($p \leq 0.05$, Newman-Keuls) Todos os dados estão expressos pela média ± erro padrão, n = 10.

Fig. 4. Efeito do estresse hídrico em EUA e EUA_i em plantas de *Prosopis juliflora* (Sw.) DC. submetidas a tratamentos por diferenciação de rega de 50% (A, F) e 15% (B, G) da capacidade de campo, e por suspensão total de rega por 6 (C, H), 8 (D, I) e 10 (E, J) dias. As setas indicam o dia em que as plantas foram reidratadas, e os asteriscos indicam diferenças significativas entre as médias diárias dos valores de cada parâmetro de plantas estressadas (□) e plantas controle (■) ($p \leq 0.05$, Newman-Keuls) Todos os dados estão expressos pela média ± erro padrão, n = 10.

Fig. 5. Efeito do estresse hídrico em CHR_f e P_N/C_i em plantas de *Prosopis juliflora* (Sw.) DC. submetidas a tratamentos por diferenciação de rega de 50% (A, F) e 15% (B, G) da capacidade de campo, e por suspensão total de rega por 6 (C, H), 8 (D, I) e 10 (E, J) dias. As

setas indicam o dia em que as plantas foram reidratadas, e os asteriscos indicam diferenças significativas entre as médias diárias dos valores de cada parâmetro de plantas estressadas (□) e plantas controle (■) ($p \leq 0.05$, Newman-Keuls) Todos os dados estão expressos pela média ± erro padrão, n = 10.

Fig. 6. Matriz da relação das medidas de temperatura foliar (T_L), assimilação líquida de carbono (P_N), carbono intracelular (C_i/C_a), condutância estomática (g_s), taxa de transpiração (E) e conteúdo hídrico relativo foliar (CHR_f) versus teores de clorofilas *a, b*, totais e carotenoides totais (Chl*a*, Chl*b*, Chl*t* e Car), carboidratos solúveis totais (TSC), proteínas totais (TP), aminoácidos livres totais (TFAA), amido (S), malondialdeído (MDA), peróxido de hidrogênio (H_2O_2) e atividade da enzima superóxido dismutase (SOD). Todas as variáveis foram mensuradas antes, durante, e após estresses hídricos diferenciados em *Prosopis juliflora* (Sw.) DC. Sendo (A) referente aos tratamentos de 50% e 15% da capacidade de campo, e (B) os de 6, 8 e 10 sem rega. Valores abaixo de -0,20 e acima de 0,20 são significativos ($p \leq 0.05$, r-Pearson).

Tabela 1. Conteúdo hídrico relativo médio do solo mensurado antes, durante, e após estresses hídricos diferenciados em *Prosopis juliflora* (Sw.) DC. Sendo t1 e t2 os tratamentos de 50% e 15% da capacidade de campo, e t3, t4 e t5 os de 6, 8 e 10 sem rega respectivamente.

DAE (Dias Após Estresse)	Controle (t_C)	Estresse (Tratamento)
0	17,02±0,39%	-
6	17,36±0,35%	1,23±0,06% (t3)
8	17,15±0,14%	1,51±0,09% (t4)
10	17,15±0,53%	1,23±0,16% (t5)
18	16,81±0,40%	3,33±0,18% (t1)
-	-	7,90±0,16% (t2)
26*	16,98±0,46%	17,92±0,25% (t3)
-	-	17,13±0,14% (t4)
-	-	16,91±0,23% (t5)
28*	17,74±0,25%	17,43±0,82% (t1)
-	-	17,58±0,15% (t2)

*Dia no qual as plantas foram totalmente reidratadas.

7. Conclusões

Os resultados do presente estudo mostraram que a escarificação com H_2SO_4 é mais eficiente na promoção da germinação de sementes de *Prosopis juliflora* (Sw.) DC do que a escarificação mecânica, uma vez que esta causa danos aos cotilédones, o que leva a má formação de mudas, diminuindo significativamente a porcentagem de sobrevivência das plântulas regeneradas. Entretanto, embora o H_2SO_4 promova a germinação, ele pode afetar da mesma forma o desenvolvimento do embrião de acordo com o tempo de exposição ao ácido. Para as sementes das quais os endocarpos foram removidos, 5 minutos na presença de H_2SO_4 é o suficiente para promover a germinação enquanto nas sementes ainda acopladas aos endocarpos a taxa de germinação aumenta proporcionalmente ao aumento do tempo de exposição ao ácido.

Além disso, foi observado neste trabalho que a taxa de germinação, e a velocidade de germinação de sementes de *P. juliflora* diferem significativamente dependendo do potencial osmótico e temperatura do substrato. À medida que o potencial osmótico decresce a taxa, a velocidade e a sincronia de germinação diminuem. Este comportamento foi observado para todas as sementes tratadas com PEG 6000 ou NaCl sob qualquer uma das temperaturas avaliadas, exceto o tratamento controle, no qual a germinabilidade não variou com a mudança de temperatura. Contudo os efeitos do PEG 6000 foram mais severos que o do NaCl, pois a porcentagem de germinação reduziu pela metade ou menos quando o potencial osmótico baixou de -0,5 MPa para -0,75 MPa, chegando a valores nulos no potencial osmótico de -1,0 MPa, enquanto as sementes tratadas com NaCl atingiram 94,5% de germinabilidade sob -2,0 MPa a 25°C. Além disso, nos tratamentos de menores potenciais osmóticos a temperatura influenciou significativamente de modo que quanto maior a temperatura, menores foram a germinabilidade, a sincronia e o tempo médio de germinação, chegando a não haver germinação no tratamento de 40 °C -0,75 MPa.

Em habitats áridos caracterizados pela imprevisibilidade espacial e temporal das chuvas, a germinação geralmente acontece quando o ambiente se apresenta mais favorável para a sobrevivência e crescimento da plântula. Consequentemente, velocidade e sincronia na germinação são características adaptativas. No presente estudo, *P. juliflor*a germinou rapidamente e sincronicamente quando tratadas com H_2SO_4, sob temperaturas mais amenas e em potenciais menos baixos. Contudo *P. juliflor*a apresentou tolerância a uma ampla gama de potenciais osmóticos e temperatura, podendo ser considerada uma espécie halotolerante. Esta

característica assegura que, mesmo quando as condições não são ideais, uma parte das sementes irá germinar, o que é particularmente importante para a sobrevivência da espécie em ambientes áridos. Pois em condições naturais, uma germinação heterogênea, distribuída ao longo do tempo, aumenta a probabilidade de uma muda encontrar condições favoráveis para o seu desenvolvimento. Condições que foram analisadas no terceiro capitulo desta dissertação, e mostraram que mesmo sob 7,90% (50% da hidratação completa do solo) e 3,33% (15%) de umidade do solo, as plantas de *P. juliflora* suportaram muito bem o estresse apresentando alta eficiência no uso da água, e na capacidade de conter danos oxidativos em maiores proporções. Neste estudo, baseado nas respostas de P_N, C_i/C_a e g_s, concluímos que apenas sob total falta d'água (1,32±0,05%), a eficiência de carboxilação (P_N/C_i), e a eficiência intrínseca do uso da água (EUA_i), em plantas jovens de *P. juflilora*, são substancialmente reduzidas, ameaçando o crescimento e a sobrevivência dessas plantas.

A colonização de *P. juliflora* vem causando à Caatinga uma série de mudanças em sua dinâmica ecológica. Por essa razão, estudos avançados sobre a ecofisiologia dessa espécie vem sendo desenvolvidos em nosso laboratório. Acreditamos que nos próximos anos seremos capazes de desenvolver metodologias baseadas em ecofisiologia, as quais poderão ser combinadas com metodologias baseadas em dinâmicas ecológicas.

8. Resumo

Este estudo teve como objetivo elucidar as principais estratégias adaptativas de *Prosopis juliflora (Sw) DC* a condições de estresse hídrico e osmótico, afim de entender ecofisiologicamente porque essa espécie se tornou uma invasora tão agressiva do ecossistema Caatinga. Para tal, foi avaliada a germinação de *P. juliflora* sob pré-tratamentos de escarificação mecânica e quimíca, e sob tratamentos de diferentes potenciais osmóticos do subtrato com NaCl e PEG 6000. Além disso foram analisadas as relações de P_N, C_i, CHR_f, compostos orgânicos e enzimas, sob diferenciação de rega. Os resultados do presente estudo mostraram que a escarificação com H_2SO_4 é mais eficiente na promoção da germinação. Para sementes sem endocarpo, 5 minutos na presença de H_2SO_4 é o suficiente para promover a germinação enquanto em sementes com endocarpo a taxa de germinação aumenta proporcionalmente ao aumento do tempo de exposição ao ácido. Ademais, foi observado neste trabalho que a taxa de germinação, e a velocidade de germinação de sementes de *P. juliflora* diferem significativamente dependendo do potencial osmótico e temperatura do substrato. À medida que o potencial osmótico decresce a taxa, a velocidade e a sincronia de germinação diminuem. Este comportamento foi observado para todas as sementes tratadas com PEG 6000 ou NaCl sob qualquer uma das temperaturas avaliadas. Os resultados também mostraram que mesmo sob apenas 7,9% (50% da hidratação completa do solo) ou 3,33% (15%) de umidade do solo, as plantas de *P. juliflora* suportaram muito bem o estresse apresentando alta eficiência no uso da água, e na capacidade de conter danos oxidativos em maiores proporções. Neste estudo, baseado nas respostas de P_N, C_i/C_a e g_s, concluímos que apenas sob total falta d'água (1,32±0,05%), a eficiência de carboxilação (P_N/C_i), e a eficiência intrínseca do uso da água (EUA_i), em plantas jovens de *P. juflilora*, são substancialmente reduzidos, ameaçando o crescimento e a sobrevivência dessas plantas.

Palavras chaves: Caatinga; espécie exótica; algaroba; trocas gasosas; deficit hídrico.

9. Abstract

The goal of this study is describe the main adaptive strategies of *Prosopis juliflora* (Sw) DC to withstand drought and osmotic stress conditions. Moreover, we aim to understand why this species has become an invader so aggressive in Caatinga ecosystem. To reach our objectives, we evaluated the germination of *P. juliflora* after mechanical and chemical scarification treatments, and under different osmotic potentials, which were imposed using NaCl e PEG 6000. In addition the relation of P_N, C_i, CHR_f, organic compounds e enzymes, under different water regimes. The results showed that scarification with H_2SO_4 is the most efficient to promote germination. When seeds are without endocarp, 5 minutes under acid are sufficient to provoke germination, while when seeds are within endocarp, germination rate increases proportionally with time under acid. In this study, we also observed that germinability and speed of germination of *P. juliflora* differ significantly with different temperatures and osmotic potentials. As osmotic potential decreases, germinability and synchrony of germination decrease too. This behavior was observed for all seeds treated with PEG 6000 or NaCl under any of the temperatures. In the third chapter, results presented that even under 7.9% (50% of soil water capacity) or 3.33% (15%) of soil moisture, plants of *P. juliflora* supported well the hydric stress, showing high water use efficieny, high capacity to counter oxidative damage. In this study, based on P_N, C_i/C_a and g_s responses, we have concluded that only under total water suppression conditions (1.32±0.05%), carboxilation efficiency (P_N/C_i), intrinsic water use efficiency (EUA_i) of *P. juflilora* plants were substantially reduced, threating growth and survival of this species.

Keywords: Caatinga; exotic species; mesquite; gas exchange; water deficit.

10. Anexos

Plant Species Biology

Edited By: Masashi Ohara

Impact Factor: 0.792

ISI Journal Citation Reports © Ranking: 2011: 108/134 (Ecology); 129/190 (Plant Sciences)

Online ISSN: 1442-1984

Author Guidelines

Aims and Scope

Plant Species Biology is published three times a year by The Society for the Study of Species Biology. *Plant Species Biology* publishes research manuscripts in the fields of population biology, pollination biology, evolutionary ecology, biosystematics, co-evolution, and any other related fields in biology. In addition to full length papers, the journal also includes short research papers as notes and comments. Invited articles may be accepted or occasion at the request of the Editorial Board. Manuscripts should contain new results of empirical and/or theoretical investigations concerning facts, processes, mechanisms or concepts of evolutionary as well as biological phenomena. Papers that are purely descriptive are not suitable for this journal. The journal is introducing 'Life history monographs of Japanese plant species'. The journal is dedicated to minimizing the time between submission, review and publication and to providing a high quality forum for original research in Plant Species Biology.

Acceptance

The acceptance criteria for all papers are the quality and originality of the research and its significance to our readership. Except where otherwise stated, manuscripts are peer reviewed by two anonymous reviewers and the Editor. The Editorial Board

reserves the right to refuse any material for publication and advises that authors should retain copies of submitted manuscripts and correspondence as material cannot be returned. Final acceptance or rejection rests with the Editorial Board.

Early View
Plant Species Biology is covered by Wiley-Blackwell Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Articles are therefore available as soon as they are ready, rather than having to wait for the next scheduled print issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article. More information about DOIs can be found at http://www.doi.org/faq.html (http://www.doi.org/faq.html)

Submission of manuscripts

All articles submitted to the Journal must comply with these instructions. Failure to do so will result in return of the manuscript and possible delay in publication. Manuscripts should be written so that they are intelligible to the professional reader who is not a specialist in the particular field.

Where contributions are judged as acceptable for publication on the basis of scientific content, the Editor or the Publisher reserve the right to modify typescripts to eliminate ambiguity and repetition and improve communication between author and reader. If extensive alterations are required, the manuscript will be returned to the author for revision.

Pre-submission English-language editing
Authors for whom English is a second language may choose to have their manuscript professionally edited before submission. A list of independent suppliers of editing services can be found at
http://authorservices.wiley.com/bauthor/english_language.asp
(http://authorservices.wiley.com/bauthor/english_language.asp)

All services should be paid for and arranged by the author, and the use of these services does not guarantee acceptance or preference for publication.

Online Submission

Authors should submit their articles to Plant Species Biology online to facilitate even quicker and more efficient processing. Please log directly onto the site: http://mc.manuscriptcentral.com/psb (http://mc.manuscriptcentral.com/psb) and upload your manuscript following the instructions given on screen. In case you encounter any difficulties while submitting your manuscript online, please contact Masashi Ohara, Editor-in-Chief: ohara@ees.hokudai.ac.jp (mailto:ohara@ees.hokudai.ac.jp).

Copyright

Authors publishing in the Journal will be asked to sign a Copyright Transfer Agreement form. In signing the form it is assumed that authors have obtained permission to use any copyrighted or previously published material. All authors must read and agree to the conditions outlined in the form, and must sign the form or agree that the corresponding author can sign on their behalf. Articles cannot be published until a signed form has been received.

Preparation of the manuscript

The manuscript must be doubled-spaced, on one side only of A4 paper. The top, bottom and side margins should be 30 mm. All pages should be numbered consecutively in the top right-hand corner, beginning with the title page. Indent new paragraphs. Turn the hyphenation option off, including only those hyphens that are essential to the meaning.

Covering letter

Papers are accepted for publication in the Journal on the understanding that the content has not been published or submitted for publication elsewhere. This must be stated in the covering letter. Authors should declare any financial support or relationships that may pose conflict of interest.

Style

Style should conform to the *Council of Biology Editors Style Manual* 5th edn (CBE Publications, 1994). Please follow the format in the current issue of the Journal. The Journal uses US spelling and authors should therefore follow the latest edition of the *Merriam-Webster's Collegiate Dictionary*.

All measurements must be given in SI units as outlined in the latest edition of *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors* (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long, technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation. Upon its first use in the title, abstract and text, the common name of a species should be followed by the scientific name (genus, species and authority) in parentheses. Common names of organisms, if used, must be accompanied by the correct scientific name on every mention. At the first mention of a chemical substance, give the generic name only. Trade names should not be used.

Parts of the manuscript

Manuscripts should be presented in the following order: (i) title page, (ii) abstract and keywords, (iii) text, (iv)acknowledgments, (v) references, (vi) tables (each table complete with title and footnotes), (vii) figures.

Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter. Author is required to put a line number in the manuscripts except (vi) tables and (vii) figures in order to Editor and Reviewer find a line for attention.

(i) Title page

The title page should contain (i) the title of the paper, (ii) the full names of all authors and (iii) the addresses of the institutions at which the work was carried out together with (iv) the full postal and email address, plus facsimile and telephone numbers, of the author to whom correspondence about the manuscript, proofs and requests for offprints should be sent. Plus membership number in case that Papers in which The Society for the Study of Species Biology Member is a Corresponding Author.

The title should be short (less than 50 words), informative and contain the major keywords. A short running title (less than 50 characters, including spaces) should also be provided.

(ii) Abstract and keywords

Articles must have a brief but informative abstract, not exceeding 250 words for a full regular article and review article, and less than 150 words for notes and comments. The abstract should state the purpose; basic procedures, main findings and principal conclusions of the study, and it should not contain abbreviations or references. Five key words should be supplied below the abstract for the purposes of indexing.

(iii) Text

Authors should use subheadings to divide the sections of their manuscript for Original Article: Introduction, Materials and methods, Results, Discussion, Acknowledgments, and References.

(iv) Acknowledgments

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. The contribution of colleagues or institutions should also be acknowledged.

(v) References

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. EndNote reference styles can be searched for here: http://www.endnote.com/support/enstyles.asp (http://www.endnote.com/support/enstyles.asp)
Reference Manager reference styles can be searched for here: http://www.refman.com/support/rmstyles.asp (http://www.refman.com/support/rmstyles.asp)

The Harvard (author, date) system of referencing is used. In the text give the author's name followed by the year in parentheses: Sago (2000). If there are two authors use 'and': Baskin and Baskin (1998); but if cited within parentheses use '&': (Baskin & Baskin 1998). When reference is made to a work by three or more authors, the first name followed by *et al.* should be used: Powles *et al.* (1998).

In the list, references should be listed in alphabetical order. Cite the names of all authors.

Personal communications, unpublished data and publications from informal meetings

are not to be listed in the reference list but should be listed in full in the text (e.g. Smith A, 2000, unpublished data).

References should be listed in the following form.

Standard Journals

Boecklen W. J., Mopper S & Price P. C. (1991) Size and shape analysis of mineral resources in arroyo willow and their relation to sawfly densities. *Ecological Research* **6**: 317-331.

Standard Journal article using DOI articles published online in advance without volume, issue, or page number (More information about DOIs: http://www.doi.org/faq.html (http://www.doi.org/faq.html))

Beckmann C & Shine R (2009) Impact of Invasive Cane Toads on Australian Birds. *Conserv. Biol.* Published online: 8 June 2009; DOI: 10.1111/j.1523-1739.2009.01261.x

Books

George C. J. & McKinley D. (1974) *Urban Ecology*. McGraw-Hill, New York.

Chapter in a book

Endler J. A. (1991) Interactions between predators and prey. In: Krebs J. R. & Davies N. B. (eds). *Behavioural Ecology*. Blackwell Scientific Publications, Oxford, pp.169-196.

Electronic material
Goudet J. (2001) *FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices, Version 2.9.3*. [homepage on the Internet]. University of Lausanne, Lausanne. [cited 9 March 2004]. Available from URL: http://www.unil.ch/lizea/softwares/fstat.html/ (http://www.unil.ch/lizea/softwares/fstat.html/)

(vi) Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Tables should be numbered consecutively in Arabic numerals. Column headings should be brief, with units of measurement in parentheses; all abbreviations should be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for *P*-values. The table and its legend/footnotes should be understandable without reference to the text.

(vii) Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be cited in consecutive order in the text. Each figure must be restricted to the minimum needed to clarify the text. Figure legends must be brief, self-sufficient explanations of the illustrations. The legends should be placed at the end of the manuscript text.

Submit all figures with high resolution (at least 600 d.p.i. for line drawings and 300 d.p.i. for halftone illustrations and photographs). The figures should be sized to match the column (80 mm) or the full text width (171 mm). Figure legends should be included in the size above. The publisher reserves the right to reduce or enlarge illustrations.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and unites of measurement.

Electronic Artwork

Artwork should be supplied in electronic form. Vector graphics (e.g. line artwork) should be saved in Encapsulated Postscript Format (EPS), and bitmap files (e.g. half-tones) in Tagged Image File Format (TIFF). Ideally, vector graphics that have been saved in metafile (.WMF) or pict (.PCT) format should be embedded within the body of the text file.

Color figures

Color illustrations (including color photographs) will be accepted and should be submitted as good quality. In print, however, they will appear in color only if the author agrees to make a contribution (¥64 000 for the first three color figures and ¥32 000 for each extra color figure) to printing costs. Otherwise, the figures will be printed in black and white.

Color on Web Services

In the event that an author is not able to cover the costs of reproducing color figures in color in the printed version of the journal, Plant Species Biology offers authors the opportunity to reproduce color figures in color for free in the online version of the article (but they will still appear in black and white in the print version). If an author wishes to take advantage of this free color-on-the-web service, they should liaise with the Publisher to ensure that the appropriate documentation is completed. Please note that, in such cases, it is the authors' responsibility to prepare figures to be illustrated enough to convey the necessary information even after they are converted into black and white.

Author Services for tracking production processes

If possible, authors should visit the Wiley-Blackwell website for authors which details further information on the preparation and submission of articles.

Author Services enables authors to track their article, once it has been accepted, through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated emails at key stages of production so they do not need to contact the production editor to check on progress. Visit http://authorservices.wiley.com/default.asp (http://authorservices.wiley.com/default.asp) for more details on online production tracking and for a wealth of resources, including FAQs and tips on article preparation,

submission and more.

Proofs, offprints and page charges

Proofs

The corresponding author will receive an email alert notifying them when their proofs are ready for collection from a website that hosts the proofs. A working email must therefore be provided for the corresponding author. The proof is downloaded as a PDF (portable document format) file. Acrobat Reader will be required in order to read this file, which can be downloaded (free of charge) from the following website:

http://www.adobe.com/products/acrobat/readstep2.html (http://www.adobe.com/products/acrobat/readstep2.html)

This will enable the file to be opened, read on screen and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Proofs will be faxed if no email address is available. If absent, authors should arrange for a colleague to access their email, retrieve the PDF proof and check and return them to the Publisher on their behalf. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately.

Offprints

A minimum of 50 offprints will be provided upon request, at the author's expense. These paper offprints may be ordered online. Please visit http://offprint.cosprinters.com/ (http://offprint.cosprinters.com/), fill in the necessary details and ensure that you type information in all of the required fields. If you have queries about offprints please email offprint@cosprinters.com (mailto:offprint@cosprinters.com)

Page charges

It is Journal policy that a page charge fee is levied on articles appearing in the journal with the following conditions:

Papers in which The Society for the Study of Species Biology MEMBER is a Corresponding Author:

Regular articles are limited to 10 printed pages and **Notes and comments** to 4 printed pages. For manuscripts exceeding these limits a page charge of ¥5 000/US$50 per each addition page is levied.

NON-MEMBER:

Regular articles are limited to 8 printed pages and **Notes and comments** to 4 printed pages. For manuscripts exceeding these limits a page charge of ¥10 000/US$100 per each addition page is levied.

This procedure notwithstanding, no paper will be rejected or given extraordinary treatment on any basis other than its scientific merit. Any articles received by Wiley-Blackwell with page charges will not be published until the form has been returned.

Membership inThe Society for the Study of Species Biology is open to anyone who has interested in plant species. Please contact Masashi Ohara, Editor-in-Chief: ohara@ees.hokudai.ac.jp (mailto:ohara@ees.hokudai.ac.jp)

OnlineOpen

OnlineOpen is a service from Wiley-Blackwell that offers authors whose papers are accepted for publication the opportunity to pay for their article to become open access (i.e. free for all to view and download) via the Wiley InterScience Web site. Each OnlineOpen article will be subject to a one-off fee of $3,000 (payable only in US Dollars) to be met by or on behalf of the Author in advance of publication. Upon online publication, the article (both full-text and PDF versions) will be available to all for viewing and download free of charge. The print version of the article will also be branded as OnlineOpen and will draw attention to the fact that the paper can be downloaded for free via the Wiley InterScience service.

Any authors wishing to have their paper OnlineOpen will be required after acceptance of the manuscript to complete the OnlineOpen Order Form at:

http://www.blackwellpublishing.com/pdf/TPJ_OOF.pdf
(http://www.blackwellpublishing.com/pdf/TPJ_OOF.pdf)

(Please note this form is for use with OnlineOpen material ONLY.)

This form should be faxed or emailed to the Editorial Office as soon as possible after notification of acceptance (preferably within 24 hours to avoid any delays in processing).

**IMPORTANT: Please do not inform the Editorial Office or Editors prior to acceptance whether or not you intend to take up the Online Open option. **

Effective from 1 January 2010

Guide for authors

Submit your paper

Track your paper

Order journal

View articles

Abstracting and indexing

Editorial board

Browse journals > Environmental and Experimental Botany > Guide for authors

Guide for Authors

Introduction

Environmental and Experimental Botany (EEB) publishes research papers on the physical, chemical, biological, molecular mechanisms and processes involved in the responses of plants to their environment.

In addition to research papers, the journal includes review articles. Submission is in agreement with the Editors-in-Chief.

The Journal also publishes special issues which are built by invited guest editors and are related to the main themes of EEB.

The areas covered by the Journal include:

(1) Responses of plants to heavy metals and pollutants

(2) Plant/water interactions (salinity, drought, flooding)

(3) Responses of plants to radiations ranging from UV-B to infrared

(4) Plant/atmosphere relations (ozone, CO_2 , temperature)

(5) Ecophysiology of Northern plants under global change and environmental stress

Each submitted manuscript related to these areas should be based on an explicitly elaborated mechanistic hypothesis.
The following types of manuscripts are not suitable for EEB: purely descriptive studies, use of unicellular or micro-organisms as models, agricultural investigations, phytopathological studies, ecological reports, studies of plant biology without considering environmental factors.

Types of issues
Alongside the standard issues, the journal publishes two other types of issues: *Special issues* and *Virtual special issues*. Special issues focus on a specific research topic and are organised by a guest editor. Virtual special issues bring together content already published on ScienceDirect. Articles appearing in virtual special issues have been selected by the Journal editor, highlighting topics that are of particular interest to the readers. The virtual issues are hosted on Elsevier.com, linking directly to the original articles on ScienceDirect.

Types of paper

1. Original full papers (Regular Papers) 2. Review articles on Environmental Issues in Plant Science.

Original papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

Reviews should be related to an area covered by the journal. These are written on invitation or after initial contact with the Editor-in-Chief.

Before You Begin

Ethics in publishing

For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/ethicalguidelines.

Policy and ethics

The work described in your article must have been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* http://www.wma.net/en/30publications/10policies/b3/index.html; *EU Directive 2010/63/EU for animal experiments* http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; *Uniform Requirements for manuscripts submitted to Biomedical journals* http://www.icmje.org. This must be stated at an appropriate point in the article.

Conflict of interest

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/postingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service CrossCheck http://www.elsevier.com/editors/plagdetect.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.
After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright see http://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

Retained author rights

As an author you (or your employer or institution) retain certain rights; for details you are referred to: http://www.elsevier.com/authorsrights.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

Open access

This journal does not ordinarily have publication charges; however, authors can now opt to make their articles available to all (including non-subscribers) via the ScienceDirect platform, for which a fee of $3000 applies (for further information on open access see http://www.elsevier.com/about/open-access/open-access-options). Please note that you can only make this choice after receiving notification that your article has been accepted for publication, to avoid any perception of conflict of interest. The fee excludes taxes and other potential costs such as color charges. In some cases, institutions and funding bodies have entered into agreement with Elsevier to meet these fees on behalf of their authors. Details of these agreements are available at http://www.elsevier.com/fundingbodies. Authors of accepted articles, who wish to take advantage of this option, should complete and submit the order form (available at http://www.elsevier.com/locate/openaccessform.pdf). Whatever access option you choose, you retain many rights as an author, including the right to post a revised personal version of your article on your own website. More information can be found here: http://www.elsevier.com/authorsrights.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier's WebShop http://webshop.elsevier.com/languageediting/ or visit our customer support site http://support.elsevier.com for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Submit your article
Please submit your article via http://ees.elsevier.com/eeb/

Referees
Please submit, with the manuscript, the names, addresses and e-mail addresses of 5 potential referees. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

Preparation

Use of wordprocessing software

It is important that the file be saved in the native format of the wordprocessor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the wordprocessor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your wordprocessor.

Article structure

1. Manuscripts should be written in English. Authors whose native language is not English are strongly advised to have their manuscripts checked by an English-speaking colleague prior to submission.

Subdivision - numbered sections
Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction
State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods
Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results
Results should be clear and concise.

Discussion
This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on.

Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

• **Title.** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
• **Author names and affiliations.** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
• **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that phone numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
• **Present/permanent address.** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Nomenclature and units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Authors and Editor(s) are, by general agreement, obliged to accept the rules governing biological nomenclature, as laid down in the *International Code of Botanical Nomenclature*, the *International Code of Nomenclature of Bacteria*, and the *International Code of Zoological Nomenclature*.

All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names when the English term is first used, with the exception of common domestic animals.

All biocides and other organic compounds must be identified by their Geneva names when first used in the text. Active ingredients of all formulations should be likewise identified.

For chemical nomenclature, the conventions of the International Union of Pure and Applied Chemistry and the official recommendations of the *IUPAC-IUB Combined Commission on Biochemical Nomenclature* should be followed.

Database linking

Elsevier encourages authors to connect articles with external databases, giving their readers one-click access to

relevant databases that help to build a better understanding of the described research. Please refer to relevant database identifiers using the following format in your article: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN). See http://www.elsevier.com/databaselinking for more information and a full list of supported databases.

Math Formulae

Present simple formulae in the line of normal text where possible. In principle, variables are to be presented in italics.
Number consecutively any equations that have to be displayed separate from the text (if referred to explicitly in the text).
Subscripts and superscripts should be clear.
Greek letters and other non-Roman or handwritten symbols should be explained in the margin where they are first used. Take special care to show clearly the difference between zero (0) and the letter O, and between one (1) and the letter l.
Give the meaning of all symbols immediately after the equation in which they are first used. For simple fractions use the solidus (/) instead of a horizontal line.
Equations should be numbered serially at the right-hand side in parentheses. In general only equations explicitly referred to in the text need be numbered.
The use of fractional powers instead of root signs is recommended. Also powers of e are often more conveniently denoted by exp.

Levels of statistical significance which can be mentioned without further explanation are: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

In chemical formulae, valence of ions should be given as, e.g., Ca^{2+}, not as Ca^{++}. Isotope numbers should precede the symbols, e.g., ^{18}O.

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the printed version.
- Submit each illustration as a separate file.

A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.
Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF, EPS or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables

and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References

Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference

Note: Authors are strongly encouraged to check the accuracy of each reference against its original source.

1. All publications cited in the text should be presented in a list of references following the text of the manuscript. The manuscript should be carefully checked to ensure that the spelling of author's names and dates are exactly the same in the text as in the reference list.

2. In the text refer to the author's name (without initial) and year of publication, followed - if necessary - by a short reference to appropriate pages. Examples: "Since Peterson (1988) has shown that..." "This is in agreement with results obtained later (Kramer,1989, pp. 12-16)"

3. If reference is made in the text to a publication written by more than two authors the name of the first author should be used followed by "et al.". This indication, however, should never be used in the list of references. In this list names of first author and co-authors should be mentioned.

4. References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on authors' names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publication dates -- publications of the same author with one co-author -- publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 1994a, 1994b, etc.

5. Use the following system for arranging your references, please note the proper position of the punctuation:

a. *For periodicals* Chettri, M.K., Sawidis, T., Zachariadis, G.A., Stratis, J.A., 1997. Uptake of heavy metals by living and dead Cladonia thalli. Environ. Exp. Bot.37, 39-42.

b. *For edited symposia, special issues, etc., published in a periodical*
Rice, K., 1992. Theory and conceptual issues. In: Gall, G.A.E., Staton, M. (Eds.), Integrating Conservation Biology and Agricultural Production. Agriculture, Ecosystems and Environment 42, 9-26.

c. *For books* Gaugh, Jr., H.G., 1992. Statistical Analysis of Regional Field Trials. Elsevier, Amsterdam.

d. *For multi-author books* DeLacy, I.H., Cooper, M., Lawrence, P.K., 1990. Pattern analysis over years of regional variety trials; relationship among sites. In: Kang, M.S. (Ed.), Genotype by Environment Interaction and Plant Breeding. Louisiana State University, Baton Rouge, LA, pp. 189-213.

6. Abbreviate the titles of periodicals mentioned in the list of references according to the Bibliographic Guide for Editors and Authors (The Chemical Abstracts Service, The Ohio State University, Columbus, OH 43210, USA).

7. In the case of publications in any language other than English, the original title is to be retained. However, the titles of publications in non-Roman alphabets should be transliterated, and a notation such as "(in Russian)" or "(in Greek, with English abstract)" should be added.

8. Work accepted for publication but not yet published should be referred to as "in press".

9. References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

Reference style
Text: All citations in the text should refer to:
1. *Single author*: the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors*: both authors' names and the year of publication;
3. *Three or more authors*: first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ...'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source

Abbreviate the titles of periodicals mentioned in the list of references according to the Bibliographic Guide for Editors and Authors (The Chemical Abstracts Service, The Ohio State University, Columbus, OH 43210, USA).

Video data

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary data

Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address
- Phone numbers

All necessary files have been uploaded, and contain:

- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations

- Manuscript has been 'spell-checked' and 'grammar-checked'
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

After Acceptance

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. Example of a correctly given DOI (in URL format; here an article in the journal *Physics Letters B*):
http://dx.doi.org/10.1016/j.physletb.2010.09.059
When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

Proofs

One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or, a link will be provided in the e-mail so that authors can download the files themselves. Elsevier now provides authors with PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 (or higher) available free from http://get.adobe.com/reader. Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site: http://www.adobe.com/products/reader/tech-specs.html.
If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately – please let us have all your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication:

please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail (the PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use). For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's WebShop (http://webshop.elsevier.com//myarticleservices/offprints). Authors requiring printed copies of multiple articles may use Elsevier WebShop's 'Create Your Own Book' service to collate multiple articles within a single cover (http://webshop.elsevier.com//myarticleservices/offprints/myarticlesservices/booklets).

Author Inquiries

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. For detailed instructions on the preparation of electronic artwork, please visit http://www.elsevier.com/artworkinstructions. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs at http://www.elsevier.com/authorFAQ and/or contact Customer Support via http://support.elsevier.com.