UNIVERSIDADE FEDERAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA

MARA DANIELLE SILVA DO CARMO

ANÁLISE PROTEÔMICA DIFERENCIAL DE PALMA FORRAGEIRA SOB INFESTAÇÃO POR COCHONILHA-DE-ESCAMA E DÉFICIT HÍDRICO

Recife
2019

MARA DANIELLE SILVA DO CARMO

ANÁLISE PROTEÔMICA DIFERENCIAL DE PALMA FORRAGEIRA SOB INFESTAÇÃO POR COCHONILHA-DE-ESCAMA E DÉFICIT HÍDRICO

Tese apresentada ao Programa de Pós-graduação em Biotecnologia (RENORBIO), Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Biotecnologia.

Área de Concentração: Biotecnologia em Agropecuária

Orientador: Profº. Dr. Tercilio Calsa Junior

Coorientador: Dr. Amaro de Castro Lira Neto

Recife

2019

Catalogação na fonte
Elaine C Barroso
(CRB4 1728)

Carmo, Mara Danielle Silva do
Análise proteômica diferencial de palma forrageira sob infestação por cochonilha-de-escama e déficit hídrico / Mara Danielle Silva do Carmo – 2019.

128 f. : fig., tab.

Orientador: Tercílio Calsa Júnior
Coorientador: Amaro de Castro Lira Neto
Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Programa de Pós-Graduação em Biotecnologia, Recife, 2019.
Inclui referências.

1. Cactaceae 2. Estresse abiótico 3. Diaspis I. Calsa Júnior, Tercílio (orient.) II. Lira Neto, Amaro de Castro (coorient.) III. Título.

583.47 CDD (22.ed.) UFPE/CB – 2020- 066

MARA DANIELLE SILVA DO CARMO

ANÁLISE PROTEÔMICA DIFERENCIAL DE PALMA FORRAGEIRA SOB INFESTAÇÃO POR COCHONILHA-DE-ESCAMA E DÉFICIT HÍDRICO

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia, Área de Concentração Biotecnologia em Agropecuária, da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutora em Biotecnologia.

Aprovada em: 18/03/2019.

BANCA EXAMINADORA

_____

Profº. Dr. Tercilio Calsa Junior (Orientador)

Universidade Federal de Pernambuco

_____

Profª Drª. Katia Castanho Scortecci (Examinador Interno)

Universidade Federal do Rio Grande do Norte

_____

Profº. Dr. Hiram Marinho Falcão (Examinador Externo)

Universidade de Pernambuco

_____

Dr. João Pacífico Bezerra Neto (Examinado Externo)

Universidade Federal de Pernambuco

_____

Dr. Túlio Diego da Silva (Examinado Externo)

Centro de Tecnologias Estratégicas do Nordeste

AGRADECIMENTOS

Agradeço,

A Deus pela vida e força ao longo desta árdua jornada, que me proporcionou crescimento profissional e pessoal,

À minha família pelo desmedido apoio, compreensão e suporte nos momentos mais difíceis, mesmo à distância, em especial, minha mãe Maria José, pai Miguel, avó Josefa e irmãos Rachel e Samuel.

Ao professor Dr. Tercilio Calsa Junior pela recepção, oportunidade de experienciar este trabalho, confiança, encorajamento, exemplo profissional e contribuições científicas.

Aos colegas do LGPP: Fabiana, Romel, Marciana, Amanda, Adauto, Melquisedec, Sheyla, Elton, Geise, Rayssa, Lucas, José, Tainara, Rodrigo,

Pela companhia, trocas científicas, auxílio no desenvolvimento do trabalho desde a bancada, sendo um ânimo aliviando os momentos mais sobrecarregados com muitas risadas, ao lado dos quais foi possível se revigorar nessa jornada. Nunca esquecerei a contribuição de cada um.

Às instituições que possibilitaram a realização deste trabalho,

Universidade Estadual do Piauí (UESPI), Campus de Picos, PI, oportunizando minha qualificação docente,

Universidade Federal de Pernambuco (UFPE), Programa de Pós-graduação em Biotecnologia da Rede Nordeste de Biotecnologia, propiciando o desenvolvimento do projeto,

Instituto Agronômico de Pernambuco (IPA), por conceder infraestrutura e recursos para amparar a realização do trabalho, nas pessoas de Dr. Amaro de Castro, fornecendo assistência e recursos, Dr. José de Paula, incessantemente disponível e possibilitando toda a infraestrutura, Dr. Rodrigo Coitinho, auxiliando de forma essencial na área entomológica e fornecendo amostras, Dr. Antônio Felix, colaborando com amostras.

Aos laboratórios parceiros,

Laboratório de Fisiologia Vegetal, da UFPE, sob responsabilidade do professor Dr. Mauro Guida, com a colaboração dos discentes Laís, Mariana, Diego, Gabriela Frosi, que possibilitou análises bioquímicas e orientações nas análises.

Laboratório de Cultura de Tecidos Vegetais, da Universidade Federal Rural de Pernambuco (UFRPE), sob responsabilidade da professora Dra. Cláudia Ulisses, com a colaboração de Lindomar e Marciana, que possibilitou análises enzimáticas.

RESUMO

O efeito da seca tem se intensificado como um dos principais problemas na agricultura da região Nordeste do Brasil, causando perdas de até 80% na produção. A palma forrageira, *Opuntia* spp., pela sua capacidade de tolerar e superar adversidades ambientais associadas à menor disponibilidade hídrica, tem sido a alternativa para suprir a alimentação animal. Entretanto, apesar de responder a esta limitação da região semiárida, a cultura ainda sofre com pragas, sendo a cochonilha-de-escama (*Diaspis echinocacti*) a principal delas. Este trabalho objetivou a identificação de respostas bioquímica e proteômica de palma forrageira *Opuntia stricta* sob ataque de cochonilha-de-escama em diferentes regimes hídricos. Variedades de *O. stricta* de 1 ano de idade plantadas em casa-de-vegetação, foram submetidas aos tratamentos: irrigado não-infestado (HS); irrigado e infestado (HC); seca não-infestado (SS) e seca infestado (SC). Inicialmente foi estabelecida a infestação com *D. echinocacti* por contato, e posteriormente foi estabelecida a supressão da rega por 35 dias. Foram realizadas análises do teor de proteínas, aminoácidos, prolina, carboidratos, peróxido de hidrogênio, malondiadeído, superóxido dismutase, catalase e ascorbato peroxidase. Para análise proteômica foi realizada extração das proteínas, separação por eletroforese em gel bidimensional (2D-PAGE) e determinação e quantificação de proteínas diferencialmente acumuladas (DAPs) entre os tratamentos HS e HC, SS e SC, HS e SC. Seguiu-se à identificação das DAPs por espectrometria de massas associada à bancos de proteoma e *softwares* de bioinformática. As respostas bioquímicas avaliadas mostraram que o déficit hídrico afetou mais a planta que a praga, e ainda que a planta sob seca provavelmente apresenta menor capacidade da planta de combate à praga. Os processos biológicos evidenciados sob infestação foram os relacionados à defesa associada à via ácido jasmônico, organização celular, reconhecimento de elicitores pela membrana plasmática e eliminação de ROS. Estas proteínas podem ser utilizadas para pesquisas posteriores mais específicas que estruturem seu estabelecimento como biomarcadores da cochonilha-de-escama em palma forrageira, contribuindo para o rastreio de variedades tolerantes e resistentes para o manejo sustentável da cultura.

Palavras-chave: Cactaceae. 2D-PAGE. *Opuntia*. *Diaspis*. estresses biótico e abiótico.

ABSTRACT

The drought effect have been intensified as one of the main problems in agriculture in Brazil Northeast region, causing losses of up to 80% in production. Prickly pear, *Opuntia* spp., for its ability to tolerate and overcome environmental adversities associated with lower water availability, has been the alternative to supply animal feed. However, despite of responding to this limitation of the semiarid region, the crop still suffers with pests, with the cactus scale (*Diaspis echinocacti*) being the main one. This work aimed to identify biochemical and proteomic responses of prickly pear *Opuntia stricta* under cactus scale attack in different water regimes. One year old *O. stricta* varieties planted in greenhouse were submitted to treatments: non-infested irrigated (HS); irrigated and infested (HC); non-irrigated and non-infested (SS) and non-irrigated and infested (SC). Inittially the infestation with *D. echinocacti* was established naturally by contact, and then the suppression of irrigation was established for 35 days. The biochemical analyzes were: protein, amino acid, proline, carbohydrate, hydrogen peroxide, malondialdehyde, superoxide dismutase, catalase and ascorbate peroxidase levels. For proteomic analysis were performed protein extraction, separation by two-dimensional gel electrophoresis (2D-PAGE) and determination and quantification of differentially accumulated proteins (DAPs) between HS and HC, SS and SC, HS and SC treatments. Identification of DAPs was followed by mass spectrometry associated with proteome banks and bioinformatics software. The biochemical responses evaluated showed that the water deficit affected the plant more than the pest and also, the plant under drought probably has a lower capacity to pest control. The biological processes highlighted under infestation were related to defense associated to the jasmonic acid pathway, cellular organization, recognition of elicitors by the plasma membrane and, ROS scavenging. These proteins may be used for posterior specific researches, with their establishment as biomarkers to cactus scale infestation in prickly pear, contributing to screening of tolerant and resistant varieties for sustainable crop management.

Keywords: Cactaceae. 2D-PAGE. *Opuntia*. *Diaspis*. biotic and abiotic stresses.

LISTA DE ILUSTRAÇÕES

Revisão de Literatura

Artigo 1

Artigo 2

LISTA DE TABELAS

Artigo 1

Artigo 2

SUMÁRIO

1 INTRODUÇÃO

As regiões semiáridas e áridas ao redor do mundo apresentam-se sujeitas a padrões de precipitação irregulares, geralmente com escassez de água por longos períodos, e com isto manifestando características ambientais singulares, que refletem na constituição da sua fauna e flora (NIELSEN, U. N.; BALL, 2015). Estes ecossistemas, cobrindo cerca de 30% da superfície terrestre, abrigam 1/3 da população mundial, as quais em determinadas localidades dependem primariamente destes recursos, gerando uma demanda de práticas culturais específicas para promover a sustentabilidade e crescimento nestas áreas (MORTIMORE, 2009; NIELSEN, U. N.; BALL, 2015).

O cultivo de espécies adaptadas especificamente a tais condições é fundamental para garantir a manutenção e crescimento econômico nestas regiões (NUNES, 2011). A palma forrageira, pertencente ao gênero *Opuntia*, um dos mais reconhecidos na subfamília Opuntioideae, família Cactaceae, destaca-se como um dos grupos de angiospermas predominantes na fisionomia de regiões semiáridas (ANDERSON, 2001; DUBEUX JUNIOR; BEN SALEM; NEFZAOUI, 2017). Estas plantas possuem um agregado de características fisiológicas, morfológicas e anatômicas que as permitem tolerar condições de reduzida disponibilidade de água, como o metabolismo ácido das crassuláceas (CAM, *Crassulacean Acid Metabolism*), adaptação de folhas em espinhos, epiderme espessa e parênquima aquífero com mucilagem (FLORES-HERNÁNDEZ et al., 2004; HULTINE; CUSHMAN; WILLIAMS, 2019).

Nas últimas décadas suas áreas de plantio têm se expandido cada vez mais em regiões que sofrem com escassez de água, destacando-se de outras culturas por sua capacidade de cultivo mesmo durante longos períodos de sequeiro. Desta forma, a palma têm assegurado o suprimento forrageiro para várias espécies animais que amparam a população com o fornecimento de laticínios e cárneos para consumo de subsistência e comercialização (SANTOS et al., 2006; SÁENZ, 2013a).

Apesar do crescimento dos cultivos de *Opuntia* spp., estes tem sofrido redução na sua produtividade causada pelo ataque de pragas e patógenos (SCHEINVAR, 2001). Dentre estes estresses bióticos que atuam como limitantes da cultura, destaca-se o inseto-praga cochonilha-de-escama (*Diaspis echinocacti* Bouché,1833

Hemiptera: Diaspididae) que pertence a um grupo altamente adaptado à vida parasítica, por ser séssil à planta durante toda a vida e por secretar carapaça cerosa, que o protege dos predadores (MILLER; KOSZTARAB, 1979). A ausência de uma cultivar imune ao ataque da praga cochonilha-de-escama em campo tem implicado em redução da produtividade na região semiárida do Nordeste do Brasil (DIAS, M. S. C.; COSTA, A. C. F.; JESUS, 2017), evidenciando a necessidade de estudos para a compreensão das proteínas e vias moleculares induzidas e como estas inter-relacionam durante neste sistema planta-inseto para que possam auxiliar no manejo da praga.

Os insetos-praga, ao atacarem uma planta, manipulam seus processos celulares para promover a infestação, e a planta, diante da percepção da presença do inseto, sinaliza com a expressão de proteínas para ajustes de manutenção, que em condições normais não seriam induzidas (JAOUANNET et al., 2014). O conjunto de informações da expressão proteica (proteoma) de plantas sob condições de estresse biótico e abiótico é essencial como subsídio para compreensão das respostas endógenas da planta, além de fornecer embasamento na busca pela tolerância ao inseto, por exemplo, que pode variar sob diferentes condições ambientais (SERGEANT; RENAUT, 2010). Em se tratando da palma forrageira (*Opuntia* spp.), planta eficiente no que se refere ao aproveitamento de água, e com tendência de expansão do seu uso, são quase inexistentes as pesquisas mais direcionadas a respostas bioquímicas e moleculares durante o ataque de pragas, como a cochonilha-de-escama (PIMIENTA-BARRIOS; MUÑOZ-URIAS, 2001; OLIVEIRA et al., 2010). A maioria dos registros a respeito dos efeitos negativos do déficit hídrico e da infestação por cochonilha-de-escama em palma forrageira têm considerado principalmente observações visuais fenotípicas e de perda de produtividade (LIMA; GAMA, 2001; LIRA, 2017).

Neste contexto a principal contribuição deste trabalho refere-se à identificação de proteínas associadas às respostas moleculares da espécie de palma forrageira *Opuntia stricta* ao ataque do inseto cochonilha-de-escama *Diaspis echinocacti* sob condições hídricas irrigada e de seca, por meio de análise proteômica por eletroforese bidimensional. As proteínas selecionadas fornecerão informações importantes sobre os processos utilizados pelas plantas na defesa aos estresses submetidos, podendo viabilizar a indicação de biomarcadores auxiliares no melhoramento da cultura.

2 REVISÃO DE LITERATURA

2.1 Aspectos Gerais da Palma Forrageira

2.1.1 Taxonomia, Origem e Diversidade

As espécies pertencentes ao gênero *Opuntia* Mill., popularmente chamadas de palma forrageira (e no inglês *fedd palm*, *prickly pear, cactus pear*), constituem-se em cerca de 300 espécies, entre domesticadas e selvagens, incluídas em 11 subgêneros: *Opuntia, Consolea, Austrocylindropuntia, Brasiliopuntia, Corynopuntia, Cylindropuntia, Grusonia, Marenopuntia, Nopalea, Stenopuntia* e *Tephrocactus*. É um dos gêneros mais diversos e mais cultivados na família Cactaceae, devido à vasta utilização do cladódio (caule modificado) e dos frutos na medicina popular, alimentação humana e animal, se estendendo ainda à indústria de cosméticos, fármacos e subprodutos específicos (SCHEINVAR, 2001; SÁENZ, 2013a).

A classificação taxonômica das espécies do gênero é: Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Euphyllophyta; Spermatophyta; Magnoliophyta; Mesangiospermae; Eudicotyledons; Gunneridae; Pentapetalae; Caryophyllales; Cactineae; Família: Cactaceae; Subfamília: Opuntioideae (APG IV, 2016).

O gênero *Opuntia* tem como centro de origem o México (HOLLIS-BRAVO, 1978), onde sua domesticação ocorreu há cerca de 9.000 anos, a partir de espécies ancestrais dessa região, de forma a apresentar ultimamente pelo menos 126 diferentes níveis de domesticação entre as espécies (REYES-AGÜERO; RIVERA; FLORES, 2005). Atualmente espécies de palma forrageira encontram-se distribuídas na América do Sul, África e Europa (BARBERA, 2001). No México, ocupa cerca de 3 milhões de hectares (ha), incluindo mais de 70.000 ha cultivados. Na Itália, cerca de 3.000 ha são para produção comercial, 25.000 ha na Tunísia, 150.000 ha no Marrocos e mais de 1.000 ha em cada um dos países Chile, Argentina e África do Sul (INGLESE, 2010; ARBA et al., 2017).

No Brasil, sua introdução ocorreu no final do século XVIII, sendo inicialmente destinada para produção do corante carmim pelo inseto cochonilha-do-carmim (*Dactylopius coccus*), que se fixa na planta utilizando-a como substrato para sua alimentação (LOPES, 2007). Em seguida, a planta passou a ser usada como

ornamental, e somente no início do século XX, passa a ser utilizada como planta forrageira, o que se intensificou na década de 90, quando ocorreram secas prolongadas na região Nordeste (ALBUQUERQUE, 2000).

Por se tratar de uma cultura que se destaca especialmente para pequenos e médios produtores, não há relatos mais precisos e recentes da sua área ocupada por órgãos oficiais de agricultura. Os dados registrados até o momento, reportam que no Brasil a área ocupada pela palma forrageira é de 900.000 ha, tendo uma das maiores áreas de cultivo em relação a outros países, com cerca 600.000 ha destinados para produção do cladódio como forragem, predominantemente no Nordeste (LOPES, 2007). Com a ampliação de estudos revelando suas potencialidades, bem como pela sua capacidade de atender à demanda crescente de áreas sob seca, sua produção alcançou mais de 3 milhões e meio de toneladas, de acordo com o Censo Agropecuário de 2017 (IBGE, 2017), se constituindo essencialmente em uma alternativa forrageira de baixo custo, na maioria dos estados do Nordeste e norte de Minas Gerais (ROCHA, 2012).

Contudo, diferentemente de outros países como México e Estados Unidos com maior quantidade de variedades exploradas, no Brasil poucas são utilizadas, apesar da existência de mais de 1400 acessos em bancos de germoplasma. Desta quantidade, apenas 10 estão registradas junto ao órgão nacional brasileiro de registro de cultivares, Ministério da Agricultura, Pecuária e Abastecimento (MAPA), e têm seu potencial comercialmente explorado (TAYLOR; ZAPPI, 2004; MOURA et al., 2011; SILVA; ANDRADE, 2013; BRASIL, 2018).

Nacionalmente, espécies do gênero *Opuntia* têm sido conservadas nos seguintes bancos de germoplasma de centros de pesquisa e universidades públicas concentrados no Nordeste brasileiro (Quadro 1), com a finalidade de preservar a variabilidade de recursos genéticos:

Quadro 1 – Lista de bancos de germoplasma contendo espécies de palma forrageira (*Opuntia* spp.), conforme citado em literatura.

Banco de germoplasma de palma forrageira	**Local da citação**
Instituto Agronômico de Pernambuco (IPA), Estação Experimental de Arcoverde	SANTOS, D. C. *et al.*, 2013; SANTOS, D. C. *et al.*, 1999
Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Unidade Semiárido, Ceará, Banco Ativo de Germoplasma (BAG) de Forrageiras da Região Nordeste – Cactáceas	SANTOS, D. C. *et al.*, 2013; SANTOS, D. C. *et al.*, 1999b
Empresa de Pesquisa Agropecuária do Rio Grande do Norte (EMPARN)	PAIXÃO, 2012
Centro de Referência para Recuperação de Áreas Degradadas (CRAD) da Universidade Vale do São Francisco em Petrolina (PE)	ZAPPI *et al.*, 2011
Secretaria de Agricultura do Estado de Alagoas, Santana do Ipanema (AL)	AMORIM, 2011
Universidade Federal de Alagoas (UFAL)	ZAPPI *et al.*, 2011
Banco Ativo de Cactáceas da Universidade Estadual de Feira de Santana (BAGC-UEFS)	RAMOS *et al.*, 2008
Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig)	SISTEMA FAEMG, 2017

Fonte: Autor, 2019.

2.1.2 Aspectos Morfológicos, Anatômicos, Fisiológicos e Reprodutivos

A palma forrageira apresenta adaptações anatômicas, morfológicas e fisiológicas que permitem seu sucesso em ambientes com limitação de disponibilidade de água, como regiões áridas e semiáridas. Seu processo de fotossíntese é realizado através do Metabolismo Ácido das Crassuláceas (MAC ou CAM, do inglês *Crassulacean Acid Metabolism*), que leva à assimilação de gás carbônico (CO_2) no período da noite, seguido pela sua conversão em ácidos orgânicos (geralmente o malato) pela enzima fosfoenolpiruvato carboxilase (PEPC). Durante o dia, com intensa diminuição da abertura dos estômatos, cessa a assimilação de CO_2, os ácidos orgânicos gerados durante a noite são descarboxilados, liberando o CO_2, que é fixado pela enzima ribulose 1,5-bisfosfato carboxilase oxigenase (rubisco), exibindo atividade descarboxilase devido à maior concentração de CO_2. O fechamento dos estômatos neste período contribui para redução da perda de água por transpiração, permitindo a estas plantas maior eficiência no uso da água (CUSHMAN, 2001).

Em geral, plantas que apresentam metabolismo do tipo CAM têm várias características morfológicas e/ou anatômicas associadas que contribuem para o acúmulo e/ou redução da perda de água (CUSHMAN, 2001). Os cladódios de palma forrageira possuem na sua porção central um abundante parênquima aquífero e canais de mucilagem dispersos que resulta em alto teor de fibras. A mucilagem constitui aproximadamente 14% do peso seco dos cladódios e pode conter mais de 30% da água total do parênquima de reserva. A polimerização dos açúcares, a elasticidade das células parenquimatosas de reserva e a alta capacidade de retenção de água da mucilagem pode explicar a alta tolerância à seca e a capacidade de abrigar maiores reservas de água no tecido (SILVA; ACEVEDO; SILVA, 2001; NOBEL et al., 2002; OLVERA; ROMERO, 2006; VENTURA-AGUILAR et al., 2017).

A suculência da haste também decorre da presença de vacúolos de grandes células que servem tanto para o armazenamento de água, como de ácido málico durante a noite. Isto tende a reduzir o espaço aéreo interno e a área de superfície das células mesofílicas contendo cloroplastos expostos diretamente aos espaços aéreos intercelulares. Como consequência, a condutância interna e a absorção direta de CO_2 atmosférico são reduzidas, ao mesmo tempo em que aumentam a economia de carbono durante a fase de descarboxilação (NELSON; SAGE; SAGE, 2005).

Junto a estas características, a epiderme e a cutícula espessas contribuem para reduzir a perda de água, mas podem reduzir a digestibilidade e a ingestão da forragem, pois não são extensivamente digeridas como as células do mesófilo (OLVERA; ROMERO, 2006; VENTURA-AGUILAR et al., 2017).

Como a maioria das cactáceas, suas folhas são praticamente ausentes e possuem baixa densidade estomática (20 por mm^2), o que reduz a perda de água pela transpiração. Os brotos de crescimento tornam-se estruturas chamadas auréolas de onde se desenvolvem flores, frutos e espinhos (PIMIENTA-BARRIOS; LOZA; CASTILLO-ARANDA, 2003). Os espinhos são folhas modificadas, compostos de celulose, hemiceluloses, lignina, gordura e ceras, além disso, possuem uma camada de cutícula na superfície fornecendo proteção para evitar a predação, dessecação ou danos pela exposição prolongada à luz do sol (MALAININE et al., 2003).

As raízes são pivotantes com eixos primários que permitem fixar a planta, e que se estendem de forma superficial, possibilitando a captação rápida da precipitação pluvial. No entanto, em condições favoráveis de solo se ramificam e se desenvolvem

em camadas mais profundas de onde absorvem água e nutrientes atingindo uma elevada rede capilar (SANTOS et al., 1994; SILVA et al., 2015).

Quanto aos aspectos reprodutivos em *Opuntia* spp. que justificam seu sucesso ecológico e evolutivo, as espécies do gênero podem apresentar tanto modalidade sexual como assexual (BOWERS, 1996).

A reprodução sexuada é mais complexa, pois envolve vários órgãos, estágios e processos (broto, antese, polinização, geração do fruto, produção de sementes, germinação, desenvolvimento e crescimento de plântulas), os quais são demorados (espécie perene) e dependentes de condições ambientais adequadas até atingir a fase de indivíduo adulto (BARBERA; CARIMI; INGLESE, 1991; NERD; MIZRAHI, 1997; MANDUJANO et al., 1998; PIMIENTA-BARRIOS; CASTILLO, 2002; REYES-AGÜERO; AGUIRRE R.; VALIENTE-BANUET, 2006)

Seu sistema reprodutivo é bastante complexo, com maior frequência de flores hermafroditas, que podem realizar autofecundação ou fecundação cruzada, principalmente por abelhas, de acordo com as variedades da planta e condições ambientais. A fecundação cruzada é favorecida por fatores como a auto-incompatibilidade, a dicogamia (órgãos masculinos e femininos não amadurecem ao mesmo tempo em uma flor), a hercogamia (separação espacial entre as anteras e o estigma numa mesma flor) e a unissexualidade (ANDERSON, 2001; PIMIENTA-BARRIOS; CASTILLO, 2002; REYES-AGÜERO; AGUIRRE R.; VALIENTE-BANUET, 2006).

Pelo demorado ciclo de vida da planta, e etapas de desenvolvimento vulneráveis até seu pleno estabelecimento, a reprodução sexual não garante a multiplicação para fins comerciais. Assim, esta modalidade reprodutiva tem sido direcionada para programas de melhoramento genético (PASTORIZA, 2016). Já a propagação assexuada por meio de cladódios ou cultura de tecidos é historicamente mais utilizada no seu cultivo em maior escala (MONDRAGÓN-JACOBO; PIMIENTA-BARRIOS, 2001).

A reprodução assexual, pode ocorrer de forma natural, através da queda de frutos, ou até cladódios, perto da planta mãe, mas para fins de cultivo se dá por propagação vegetativa. O meristema presente nas aréolas (gemas axilares na palma forrageira, que ficam 2 mm abaixo da superfície da pele do cladódio), sob condições

favoráveis, sai da condição de dormência e se desenvolve em um broto ou flor ou raízes (NERD; MIZRAHI, 1997; MANDUJANO et al., 1998; HILLS, 2001).

A propagação vegetativa pode ser feita com um grupo de dois a três cladódios unidos entre si, separados da planta-mãe, plantados juntos, com o objetivo de formar brotos mais vigorosos e reduzir o tempo de frutificação; ou o cladódio pode ser utilizado individualmente ou ainda ser fracionado e, neste último caso, um único cladódio dá origem a dezenas de mudas (NULTSCH, 2000; MONDRAGÓN-JACOBO; PIMIENTA-BARRIOS, 2001; VILLALOBOS, 2001).

A associação entre reprodução sexual e reprodução assexuada (multiplicação clonal) pode reduzir os efeitos a longo prazo da endogamia e favorecer a diversidade genética e a instalação, geração, permanência e distribuição de populações naturais de diferentes espécies de cactos (MANDUJANO et al., 1998; LENZI; ORTH, 2012).

2.1.3 *Opuntia stricta* (Haw.)

Espécies de *Opuntia stricta* (Haw.) tem tido seu potencial avaliado para usos diversos, em detrimento de outras variedades dantes correntemente utilizadas, como para o combate à eutrofização de áreas superficiais por cianobactérias (NERY et al., 2019), alternativa alimentar para produção de gado de leite (SILVA et al., 2018), além de suas atividades citotóxicas, anti-inflamatórias e antioxidantes (IZUEGBUNA; OTUNOLA; GRAEME, 2019).

A variedade IPA 200016 utilizada neste trabalho trata-se de um clone da espécie *Opuntia stricta* (Haw.) Haw. subespécie *espatazae*, conhecida popularmente como Orelha de Elefante Mexicana (OEM). Foi introduzida do México no Brasil através do IPA e após avaliação da sua adaptação, foi registrada como cultivar junto ao Ministério da Agricultura e Pecuária (MAPA) (SANTOS et al., 2013).

Para multiplicação desta cultivar em escala comercial, no Brasil, tem sido utilizada a micropropagação partir do cultivo *in vitro*, através do qual tem revelado adequada viabilidade na aclimatação e estabelecimento em campo (SANTOS et al., 2013; SILVA; SOUZA, 2017). Dentre as suas características estão, além da adaptação às condições semiáridas, hábito de crescimento estendido, estrutura de planta estreita, de porte muito baixo, com cladódios largos, de muito curta longitude (pequena relação entre a longitude/largura), formato rômbico, espessura grossa e de coloração

verde escura, forte cerosidade, e contendo relativamente poucos espinhos, variando de 0 a 4 espinhos/aréola, facilitando o uso na demanda alimentar (SANTOS et al., 2013).

Apresentou produtividade média de 37 t de MS/ha/2 anos em população de 20.000 plantas/ha, considerada satisfatória em relação a outras variedades. Além dos aspectos agronômicos, uma das características que tem possibilitado uma ampla expansão desta variedade é a resistência à importante e devastadora praga cochonilha-do-carmim (*Dactylopius opuntiae*), o que tem possibilitado que instituições de pesquisa agronômica brasileiras promovam sua difusão por todos os estados do Nordeste brasileiro (VASCONCELOS et al., 2009; SANTOS et al., 2013).

Apesar de agregar características proveitosas como forrageira, possibilitando sua propagação nacionalmente, sua produtividade vem sendo ameaçada, pois esta variedade de palma, assim como as demais avaliadas até o momento, apresenta suscetibilidade à praga cochonilha-de-escama (*Diaspis echinocacti*) (VASCONCELOS et al., 2009; SANTOS et al., 2013).

2.1.4 Usos e Propriedades Nutricionais

Os relatos iniciais de uso da palma forrageira datam de no mínimo 9.000 anos na América Central, na medicina popular contra desinteria, diabetes, espasmos, inflamações e como cicatrizante e diurética. Com a expansão da distribuição geográfica da cultura e sua domesticação, seu uso tem se expandido, passando pela produção do altamente valioso corante carmim vermelho derivado da associação com o inseto cochonilha-do-carmim (*Dactylopius* spp.) até seu uso como alimento (SÁENZ, 2013a).

Avaliações bioquímicas têm revelado que espécies do gênero *Opuntia spp*. apresentam no fruto e cladódio valioso conteúdo nutricional, que varia conforme a espécie, variedade, idade e condições ambientais. No seu conteúdo destacam-se altos teores de minerais (especialmente cálcio, magnésio), vitaminas (A, beta-caroteno), antioxidantes (betacianinas) e compostos fenólicos, como flavonoides (SÁENZ, 2013b; DIÁZ et al., 2017; MELGAR et al., 2017).

Por serem ricos em tais compostos, estas espécies também têm suscitado interesse para avaliação do seu potencial para atividades biológicas, tendo comprovadas atividades antioxidante *in vivo,* antimicrobiana, antifúngica,

antidiabéticas, anti-inflamatória e antiulcerogênica (AHMED; AHMED-FARID, 2017; JAYA; KUMAR, 2017; MELGAR et al., 2017; SÁNCHEZ-HERRERA et al., 2017), assim servindo como matéria-prima para a indústria farmacêutica e de cosméticos.

A ampliação destas pesquisas revelando suas propriedades, tem contribuído para a expansão do consumo da fruta *in natura* e do cladódio como verdura. Já a indústria tem aproveitado a tendência contemporânea de cuidado da saúde aliado à ingestão dietética, produzindo novos alimentos derivados de *Opuntia* spp., como bebidas, sucos, geleias, *syrups*, além de extrair compostos funcionais para formular e enriquecer alimentos e cosméticos (SÁENZ, 2013a; DIÁZ et al., 2017; VENTURA-AGUILAR et al., 2017).

Outros usos da palma forrageira têm sido como cercas-vivas para casas, jardins e parcelas agrícolas; proteção do solo (HERNÁNDEZ, 2001; HOFFMAN, 2001; LEE et al., 2002; OSORIO-ESQUIVEL et al., 2011; DIÁZ et al., 2017; SÁNCHEZ-HERRERA et al., 2017), e ainda tem-se seguido pesquisas nos últimos anos sobre seu potencial como biocombustível (KULOYO et al., 2014; YANG et al., 2015; SANTOS et al., 2016).

No México, dentre as áreas de cultivo de Opuntia spp., 16% são usadas para produção como legume, 22% como forragem e 62% como fruto (REYES-AGÜERO; RIVERA; FLORES, 2005). No Brasil, atualmente o uso mais pronunciado é para produção de forragem, no Nordeste, onde predomina o clima semiárido (SÁENZ, 2013a). A região, bastante atingida pela escassez de alimento, tem a pecuária de pequeno porte como uma das principais atividades econômicas para a população menos favorecida (NUNES, 2011). Apresenta importante rebanho, principalmente de caprinos e ovinos, que representam, respectivamente, 91,3% e 57,2% do efetivo do país; enquanto o rebanho bovino é composto por 28 milhões de animais, que equivale a 14,3% do rebanho nacional (SILVA et al., 2010). A alimentação de todo esse rebanho requer produção de forragem ao longo de todo o ano, mas se torna difícil quando as precipitações são irregulares, se concentrando em 3-4 meses, limitando a lavoura forrageira (SAMPAIO, 2010).

A palma forrageira se consolidou como um suporte propício na dieta de rebanhos, por sua rusticidade, adaptação, palatabilidade e características nutricionais, além de contribuir diminuindo a necessidade da ingestão de água pelos animais, permitindo uma boa convivência com o semiárido (DUBEUX JUNIOR; BEN SALEM;

NEFZAOUI, 2017). Por ser rica em carboidratos não-fibrosos, serve como principal fonte de energia aos ruminantes, sendo no entanto necessário sua associação com alimentos mais fibrosos e proteicos, para evitar distúrbios digestivos e aumentar o consumo de matéria seca e proteína pelo animal, promovendo um balanceamento da sua dieta (LIRA et al., 2005; SANTOS et al., 2006). Para pequenos agricultores, também complementa os rendimentos com o cultivo adensado para venda subsequente no período de estiagem (OLIVEIRA et al., 2010).

2.2 Pragas na Palma Forrageira

Em todas as áreas produtoras, a palma forrageira está sujeita a danos por pragas ou doenças afetando a produção de frutas, bem como dos cladódios, partes consumíveis da planta na alimentação humana e animal. Entre os organismos que a atacam, os insetos desempenham um papel importante, em virtude da quantidade de espécies envolvidas e dos danos que podem causar (LONGO; RAPISARDA, 2001).

Há registros de uma grande variedade de insetos, cerca de 122 espécies vivendo em plantas do gênero *Opuntia* (MANN, 1969; ZIMMERMANN; MC FAYDEN; ERB, 1979; LIMA; GAMA, 2001; LONGO; RAPISARDA, 2001; VINICIO; JAIME, 2017; FLORES et al., 2018). Os insetos que mais causam danos na palma forrageira são as cochonilhas (superfamília Coccoidae) (AMOUROUX et al., 2017), cujas três famílias mais importantes são Diaspididae (419 gêneros), Pseudococcidae (272 gêneros) e Coccidae (170 gêneros), notáveis quanto ao número de gêneros e em termos de danos econômicos, não só para palma forrageira, mas também outras espécies hospedeiras perenes (CLAPS; ZAMUDIO; BRIZ, 2006; GERSON; ZOR, 2007; MORALES et al., 2016; CABALLERO; RAMOS-PORTILLA; KONDO, 2017). Além destas famílias, para palma forrageira, pode-se acrescentar a Dactylopiidae, que apesar de possuir apenas um gênero (*Dactylopius*), é um dos mais conhecidos e também de grande importância histórica e econômica, positivamente, pois os insetos produzem o corante carmim, e negativamente, por infestarem cultivos de palma forrageira, inviabilizando o uso da planta (WARUMBY; ARRUDA FILHO; CAVALCANTI, 2005).

No Nordeste brasileiro destacam-se como as espécies mais devastadoras para *Opuntia* spp. a cochonilha-do-carmim (*Dactylopius opuntiae*) e a cochonilha-de-escama ou escama blindada (*Diaspis echinocacti*) (LOPES, 2007; SILVA; ANDRADE,

2013). A introdução intencional da cochonilha-do-carmim no Brasil para produção do corante, juntamente com a introdução da palma forrageira como seu substrato alimentar, facilitou a disseminação do inseto, inicialmente tido como de valor comercial. Contudo, posteriormente o foco foi direcionado para o uso da planta para a produção de forragem, devido às agravantes condições de seca, e assim o inseto se configurou como praga, devido à sua rápida capacidade de se reproduzir e devastar a palma (WARUMBY; ARRUDA FILHO; CAVALCANTI, 2005).

Com o impacto de grandes perdas na produtividade causadas pelo inseto, pesquisas alcançaram o controle da cochonilha-do-carmim através do uso de variedades de palma forrageira com tolerância e resistência (LIRA et al., 2005; SILVA et al., 2009; VASCONCELOS et al., 2009; LOPES et al., 2010; SANTOS et al., 2013; SILVA; ANDRADE, 2013). A redução da cochonilha-do-carmim parece ter diminuído a competição por alimento entre insetos, fazendo com que atualmente, se dissemine a cochonilha-de-escama (MILLER; KOSZTARAB, 1979).

2.2.1 Aspectos Gerais da Cochonilhas-de-escama

A espécie *Diaspis echinocacti* (Bouché, 1833 Hemiptera, Diaspididae), conhecida como cochonilha-de-escama ou escama blindada (*cactus scale insect, armored scale insect*), pertence à família Diaspididae, a mais importante na superfamília Coccoidea, devido ao tamanho (mais de 400 gêneros com cerca de 2.650 espécies), abundância e importância econômica e ainda, por ocorrer virtualmente em qualquer lugar onde espécies hospedeiras de plantas vasculares são encontradas (BEARDSLEY; GONZALEZ, 1975). É uma das espécies que tem predominado como parasita na palma forrageira do Nordeste brasileiro (LIMA; GAMA, 2001; BORN et al., 2009) Possui a seguinte classificação taxonômica: Animalia; Bilateria; Protostomia; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Paraneoptera; Hemiptera; Sternorrhyncha; Coccoidea; Diaspididae; Diaspis (ITIS, 2018).

Assim como outros insetos que atacam a palma, *Diaspis echinocacti* é um inseto polífago em Cactaceae, com cerca de 40 gêneros hospedeiros na família. Além desta, há registros de sua presença em outras famílias como Crassullaceae, Fabaceae, Portulacaceae, Urticaceae, Zygophyllaceae, Aizoaceae, Asteraceae (GARCÍA MORALES et al., 2016).

Quanto à morfologia, estão entre os menores cocoídeos conhecidos, com apenas 1 a 3 milímetros de comprimento. As fêmeas adultas têm uma cobertura branca circular (1,4-2,2 mm de diâmetro), formada por secreção cerosa produzida pelo inseto ao longo dos estágios de desenvolvimento, com uma exúvia subcentral marrom-amarelada. A cobertura do macho é branca, alongada e oval (0,8-1,0 mm de comprimento) com três protuberâncias longitudinais (LONGO; RAPISARDA, 2001; MILLER; DAVIDSON, 2005).

A característica marcante, presença da cobertura cerosa sobre o corpo, garante proteção contra eventuais ataques do ambiente, como químicos e predadores. O aparelho bucal é do tipo picador-sugador, e permite que o inseto (fêmea) se fixe à planta. Tanto a presença de capa cerosa, como o aparelho bucal, representam especializações destes herbívoro e um alto grau de adaptação à vida parasítica (CLAPS; HARO, 2001).

Segundo Oetting (1984), à temperatura constante de 27ºC, o ciclo de vida se completa em 23 a 26 dias para as fêmeas e 1 a 2 dias a mais para os machos. Há ainda registro de ciclos durando em média 35 dias em condições mais amenas (ARRUDA FILHO; ARRUDA, 2002). Durante o amadurecimento até a fase adulta, os indivíduos passam por 3 estádios ninfais, ao longo dos quais aumenta o dimorfismo sexual. As fêmeas são móveis apenas no 1º ínstar, se fixando na planta através do aparelho bucal no 2º ínstar. Segundo Heriot (1934), há uma troca do estilete bucal para um mais longo no final do 2º ínstar. E o macho apesar de possuir asas, têm a duração da fase adulta de apenas 2 a 3 dias, o suficiente para fecundar a fêmea, não chegando a se alimentar da planta.

A reprodução sexual é realizada em várias gerações por ano. Além disso, as fêmeas podem realizar reprodução assexual por partenogênese gerando exclusivamente fêmeas (telitoquia), o que pode garantir que em um ano, uma fêmea chegue a deixar mais de $60x10^9$ indivíduos sobre a palma. Tais caracteres prolíficos contribuem para que se tornem pestes graves (ARRUDA, 1983; ARRUDA FILHO; ARRUDA, 2002). Geralmente a cochonilha-de-escama ocorre em baixas densidades, mas ocasionalmente pode se converter em praga sob determinadas condições, como em estufas ou em plantações especializadas, podendo cobrir completamente a planta com fêmeas adultas (SANTOS; ARRUDA; ARRUDA, 1991; LIRA, 2017).

Os danos causados pelos insetos de escama blindadas são devido à sucção da seiva, injeção de toxinas e indiretamente pela transmissão de vírus e microrganismos que enfraquecem a planta, levando à sua redução do vigor e queda em altos níveis de infestação, afetando a produtividade. Além disso, os insetos danificam seriamente os frutos, causando uma depreciação estética, devido a manchas cloróticas nas áreas em que se encontram as escama, reduzindo a qualidade comercial (LONGO; RAPISARDA, 2001; AMOUROUX et al., 2017).

O controle normalmente é realizado por meio de manejo integrado, associando práticas culturais, mecânicas, químicas e biológicas. Dentre as espécies utilizadas para controle biológico estão os entomófagos predadores (Coleoptera: Coccinellidae) ou parasitóides (Hymenoptera: Aphelinidae e Encyrtidae) (LIMA; GAMA, 2001; LONGO; RAPISARDA, 2001; GERSON; ZOR, 2007; BORN et al., 2009). No entanto, o controle por meio da resistência ou tolerância, natural ou induzida, é umas das formas mais sustentáveis de contenção de pragas e doenças. Tal medida evita o controle químico, o que é oportuno para a segurança alimentar, bem como para a redução de custos de produção, com vantagem para produtores e consumidores (LONGO; RAPISARDA, 2001).

Pouco é conhecido sobre a tolerância à cochonilha-de-escama em variedades de plantas hospedeiras de palma forrageira, pois os testes de avaliação fenotípica realizados até o momento, ainda não têm revelado resistência em acessos já utilizados ou nos oriundos de bancos de germoplasma (LIMA, 2013; LIRA, 2017).

2.3 Plantas e Estresses Ambientais

As plantas, por serem sésseis, não tem a possibilidade de escapar fisicamente de intempéries do ambiente onde estão inseridas, e estas se constituem em estresse (biótico ou abiótico) por afetar as condições celulares ótimas, o crescimento e a reprodução (MADLUNG; COMAI, 2004). O impacto destes estresses é uma redução de até cerca de 40% na produtividade anual (PANDEY et al., 2017). Embora os estresses afetem o desenvolvimento das plantas, estas têm a capacidade de adaptar o seu metabolismo, dependendo de um sistema de sinalização fino e eficiente, para que gerar transporte de sinais e respostas (ONAGA; WYDRA, 2017).

Historicamente numerosos estresses bióticos têm sido registrados devastando produções alimentares e levado milhões de pessoas à morte ou migração para outros países. O estresse causado por insetos é um dos que têm afetado e limitado a produtividade agrícola globalmente, devido à tendência de plantios de monocultivos comerciais associada à ocorrência de novos biótipos de insetos (SANGHERA et al., 2011; GHOSH et al., 2017).

No ambiente natural, no entanto, estes estresses não ocorrem isoladamente. Devido às mudanças climáticas, com o aquecimento global, as combinações entre estresses, como bióticos e abióticos, são cada vez maiores. A interação entre diferentes condições estressantes, nem sempre tem efeito aditivo ou sinérgico sobre as plantas, mas podem apresentar efeitos neutros ou mesmo melhorar a performance da planta, de acordo com as combinações. Estas complexas respostas que variam para sistemas específicos tornam necessária a análise individualizada das respostas simultâneas e/ou cruzadas sob variadas condições (PANDEY et al., 2017).

Em relação à interação planta-inseto, as espécies envolvidas coevoluíram sob variações sazonais do ambiente, logo, apresentam sistema flexível de regulação metabólica (FOYER et al., 2016). Durante a herbivoria, por exemplo, conforme as condições de temperatura, o ciclo de vida de insetos da família Diaspididae pode variar de 1 a 6 gerações por ano, além disso as condições ambientais podem favorecer ou não o parasitismo, e da mesma maneira as plantas podem responder diferentemente ao inseto, conforme sua performance perante as variações abióticas a que seja submetida (OETTING, 1984; ARRUDA FILHO; ARRUDA, 2002; HUBERTY; DENNO, 2004).

2.3.1 Interação Planta-Inseto

Plantas têm interagido com insetos e artrópodes por vários milhões de anos, tanto de forma benéfica e mutualista, por exemplo realizando polinização ou dispersão de sementes, assim como de forma danosa, em que insetos herbívoros se constituem em pragas (GATEHOUSE; FERRY; ROMAAN, 2002). Durante a interação planta-praga, as espécies envolvidas coevoluem, desenvolvendo estratégias para evitar as defesas um do outro, em geral sendo favorecida, a espécie com maior capacidade de

variabilidade genética ao longo do tempo (WAR et al., 2012; STAHL; HILFIKER; REYMOND, 2018).

Duas amplas categorias de insetos fitófagos externos são reconhecidas conforme a guilda de alimentação: os mastigadores e os sugadores, que geram diferentes tipos de dano e cuja extensão do dano pode variar conforme o estágio de desenvolvimento da planta e do inseto. Os mastigadores, como lagartas e besouros, geram prejuízo mais visível na planta, consumindo partes de tecidos de raízes, caules, folhas, flores e frutos, já os sugadores, como tripes, cigarrinhas, afídeos, cochonilhas, causam mínima destruição direta do tecido, pois usam estruturas especializadas da boca, o estilete, para localizar, penetrar e drenar tecidos específicos como a seiva dos elementos do floema dos tecidos vasculares das plantas (BUCHANAN; GRUISSEM; JONES, 2015).

Infestações pesadas de insetos sugadores tem como efeito direto a redução crônica de fotossintatos, resultando em severa redução do potencial de crescimento da planta, queda na produção de biomassa e longevidade da folha, e como efeito indireto a transmissão de vírus e microrganismos para o tecido vascular (BUCHANAN; GRUISSEM; JONES, 2015).

Para descrever a interação entre planta e insetos, pode-se categorizar as seguintes respostas: antibiose, antixenose ou não-preferência e tolerância. A antibiose afeta negativamente a biologia do inseto, através de estruturas morfológicas ou químicas presentes na planta, levando à retardo no crescimento e diminuição da longevidade e fecundidade, de forma suave à letal. A antixenose, também diz respeito à indução de efeitos adversos no comportamento do inseto, ou ainda à ausência de fitoquímicos que estimulem a alimentação do inseto, no entanto considera isto em relação à outras plantas, de modo que o inseto apresenta reduzida sua preferência a uma planta hospedeira em relação a outras plantas suscetíveis. Já a tolerância consiste na redução dos efeitos negativos do herbívoro sobre a planta, depois que a herbivoria já começou, ao mesmo tempo em que mantém populações de insetos semelhantes àquelas de plantas suscetíveis (SMITH, 2005).

Em relação aos mecanismos que as plantas usam na interação com herbívoros, estes podem ser constitutivos, expressos de forma contínua, independente da ação de herbívoros, ou induzidos, que se expressam após injúrias. Ambas, em vários níveis, desde o morfológico, com estruturas especializadas como cerdas, tricomas,

espinhos, depósitos cuticulares, espessamento da epiderme e parede celular, abundância de cristais e fibras na folha, tornando a alimentação do inseto mais difícil, até o nível bioquímico e molecular, reconhecendo moléculas estranhas ou sinais de moléculas próprias modificadas e ativando resposta imune para minimizar os danos e reduzir a palatabilidade (WAR et al., 2012).

Além das estratégias mais diretas, que afetam a sobrevivência e sucesso reprodutivo dos herbívoros (inibidores de proteinases do sistema digestório de insetos, compostos fenólicos, enzimas oxidativas etc), as plantas também utilizam meios indiretos, através da emissão de compostos orgânicos voláteis, que atraem inimigos naturais dos insetos (HOWE; JANDER, 2008; WAR et al., 2012; BUCHANAN; GRUISSEM; JONES, 2015).

A incompatibilidade genética entre planta e inseto, ou resistência da planta, ocorre quando a planta é inadequada como hospedeira para o inseto por não oferecer condições para a sua sobrevivência, ou por possuir barreiras estruturais e/ou compostos tóxicos pré-formados, ou pelo reconhecimento do ataque com manifestação de mecanismos de defesa de modo a manter a invasão localizada, sendo que apenas esta última depende de respostas de defesa induzidas (BUCHANAN; GRUISSEM; JONES, 2015). Estas respostas dependem de uma percepção precisa da presença do agressor, seguida por uma cascata de transdução de sinais que culmina em uma reprogramação transcricional e síntese de compostos de defesa específicos (STAHL; HILFIKER; REYMOND, 2018). Já nas interações compatíveis, ocorre a suscetibilidade da planta hospedeira em níveis que podem variar principalmente conforme o genótipo. Mesmo diante da suscetibilidade, mecanismos de adaptação e defesas das plantas são ativados, embora em um nível insuficiente para se proteger completamente contra o ataque de insetos, assim, mesmo a análise das respostas destas plantas suscetíveis se torna fundamental, especialmente quando se desconhece outros materiais vegetais com resistência ou tolerância (BUCHANAN; GRUISSEM; JONES, 2015).

A primeira camada do sistema imune da planta é a imunidade induzida por padrão (PTI, do inglês *pattern-triggered immunity*), que consiste no reconhecimento e atenuação de moléculas padrão por receptores imunes presentes na membrana plasmática ou no interior das células das plantas, os receptores de reconhecimento de padrão (PRR, do inglês *pattern recognition receptors*). Estes receptores

reconhecem moléculas elicitoras conservadas presentes na secreção oral dos insetos, saliva, glândulas reprodutoras, ovos ou corpo inteiro, os padrões moleculares associados aos herbívoros (HAMP, do inglês *herbivory-associated molecular pattern*), ou moléculas específicas das próprias células da planta hospedeira, resultantes de dano pelo inseto, os padrões moleculares associados a danos (DAMPs, *damage-associated molecular patterns*), desencadeando uma cascata de transdução de sinais ativando a resposta à defesa (BUCHANAN; GRUISSEM; JONES, 2015; LI et al., 2016; STAHL; HILFIKER; REYMOND, 2018).

Herbívoros adaptados são capazes de secretar um arsenal de proteínas efetoras que podem suprimir a PTI e conduzir a invasão, resultando em suscetibilidade desencadeada por efetores (ETS – do inglês *effector-triggered susceptibility*). Neste processo, sob pressão de seleção ao longo de gerações, as plantas podem apresentar proteínas de resistência (R) que reconhecem estes efetores, resultando na segunda camada de defesa da planta, a imunidade desencadeada por efetor (ETI, do inglês *effector-triggered immunity*). A ETI é um sistema mais amplificado e rápido que a PTI, e geralmente se desenvolve na resposta hipersensível (HR), com a ativação de respostas de defesa no local de invasão de patógenos ou insetos, levando a célula hospedeira infectada à apoptose. A morte celular programada ao mesmo tempo em que impede o crescimento e reprodução do inseto/patógeno, desintoxica e limita o espalhamento de enzimas prejudiciais produzidas pelos invasores. A ETI também pode levar à produção sistêmica de proteínas e metabólitos de defesa, a chamada resistência sistêmica adquirida (SAR, do inglês *systemic acquired resistance*) (BUCHANAN; GRUISSEM; JONES, 2015; STAHL; HILFIKER; REYMOND, 2018).

Ao longo do tempo, os insetos são capazes de desenvolver novos efetores para manipular os processos de defesa das plantas, e estas por sua vez desenvolvem novas proteínas R de defesa, o que sugere uma constante e indefinida “corrida armamentista” na interação planta-praga (WAR et al., 2012; BUCHANAN; GRUISSEM; JONES, 2015; STAHL; HILFIKER; REYMOND, 2018)

Como mecanismo geral em diversas espécies de plantas, após o reconhecimento de HAMPs, DAMPs e/ou efetores do inseto, há uma mudança na estrutura do receptor PRR e concomitante ativação da cascata de sinalização citoplasmática pelo domínio intracelular do receptor, seguindo-se com despolarização da membrana, ativação do influxo de íons de cálcio (Ca^{2+}) para o interior das células,

o que modifica seu pH e regula a sinalização inicial de eventos que ocorrem dentro de segundos a minutos, a produção de espécies reativas de oxigênio (ROS) e ativação de proteínas quinases ativadas por mitógeno (MAPKs, *mitogen activated protein kinases*) (STAHL; HILFIKER; REYMOND, 2018). A amplificação desses sinais ocorre por meio de hormônios reguladores, como ácido salicílico (SA), ácido jasmônico (JA) e o etileno (ET), cuja especificação e ação varia conforme a espécie de inseto, resultando na ativação de fatores de transcrição e de genes de defesa, proteínas-PR (*pathogenesis-related proteins*), fitoalexinas, metabólitos secundários com ação tóxica inseticida ou anti-nutricional, lignificação de tecidos, deposição de calose e outros reforços da parede celular (GRANT; LAMB, 2006).

O entendimento e a manipulação de componentes chave envolvidos em mecanismos de ataque e defesa de herbívoros, como HAMPs e efetores, e de como a planta responde, por meio da identificação de PRRs, e de genes e proteínas de resistência e metabólitos, pode levar ao desenvolvimento de novas estratégias para o manejo de pragas nos vegetais, através da prospecção de biomarcadores. Estes biomarcadores podem ser fontes de genes de resistência, e ser utilizados para transformação genética com a inserção de genes envolvidos na resistência, que se expressem constitutivamente ou de forma induzida após infestação média, assim promovendo uma produção agrícola mais sustentável (WAR et al., 2012).

2.3.2 Estresse por Déficit Hídrico

Plantas experimentam estresse hídrico pela diminuição do suprimento de água para suas raízes ou quando a taxa de transpiração se torna intensa (LISAR et al., 2012). Com uma menor captação de água pela planta a partir do solo, há redução do potencial hídrico e turgor celular, isto leva ao aumento da concentração de solutos no citosol e na matriz extracelular, dentre eles osmólitos como prolina, de ácido abscísico (ABA). A prolina é conhecida como um bom indicador de deficiência hídrica em plantas, que induz uma cascata de eventos que levam a planta à produção de outros compostos osmoticamente ativos, para diminuir o potencial osmótico celular e levar à entrada de água na célula. Além disso, a prolina também pode atuar na limpeza de ROS (BHASKARA; YANG; VERSLUES, 2015; RANA; RAM; NEHRA, 2017).

Estas alterações refletem em várias outras mudanças fisiológicas, como o fechamento estomatal para reduzir a perda de água por transpiração ao mesmo tempo em que limita as trocas gasosas e consequentemente reduz a assimilação de carbono (LISAR et al., 2012).

Plantas CAM obrigatórias, apesar de apresentarem fisiologia diferenciada na qual já mantêm os estômatos fechados durante o dia, quando sob déficit hídrico severo podem manter os estômatos fechados também durante a noite, restringindo a respiração noturna e o crescimento e alterando as fontes de carbono de amido para sacarose para aclimatação à seca, para reforçar suprimentos para a atividade noturna de PEPC (CEUSTERS; BORLAND; PROFT, 2009; HERRERA, 2009). A existência de tecidos capazes de armazenar água (parênquima aquífero ou hidrênquima) promovem atraso na desidratação por meio do movimento de água do hidrênquima para o mesófilo, como um mecanismo de manutenção da capacidade fotossintética sob déficit hídrico (HERRERA; FERNÁNDEZ; TAISMA, 2000; HERRERA, 2009).

Em relação à fotossíntese, sob seca esta é inibida devido a um desbalanço entre a captura de luz e sua utilização, resultando em um desbalanço entre elétrons gerados e utilizados, com a consequente geração aumentada de espécies reativas de oxigênio (ROS) (LISAR et al., 2012).

Para contrabalancear estes efeitos adversos, as plantas apresentam respostas adaptativas, variáveis entre e intra espécies, as quais envolvem desde a percepção de sinais e sua transdução por osmosenssores, quinases e mensageiros secundários; controle transcricional por meio de fatores de transcrição específicos e aumento na expressão de genes para função antioxidante e produção de proteínas de estresse, além da indução do sistema antioxidativo e acúmulo solutos compatíveis (LISAR et al., 2012).

2.3.3 Estresse oxidativo em decorrência de estresses biótico e abiótico

As mudanças em nível molecular nas plantas em decorrência de estresses bióticos e abióticos são intermediadas por metabólitos secundários, como as espécies reativas de oxigênio (ROS), resultando em alterações fisiológicas (FRAIRE-VELAZQUEZ; RODRIGUEZ-GUERRA; SANCHEZ-CALDERO, 2011; KERCHEV et al., 2012).

O déficit hídrico, um dos mais relevantes estresses abióticos em plantas, pode provocar acúmulo de ROS, devido ao desbalanço no fluxo de elétrons, com a redução da fotossíntese; ao aumento de processos como fotorrespiração, que geram ROS no peroxissomo e ainda pela produção pela membrana plasmática e pelo apoplasto em resposta a sinais endógenos e exógenos a partir de estímulos ambientais (CARVALHO, 2008; YOU; CHAN, 2015). Já em relação ao estresse biótico por insetos, a presença de elicitores de herbívoros pode ou não desencadear surtos de espécies reativas de oxigênio (ROS) em plantas pouco tempo após sua percepção ou mesmo dias depois, demonstrando dependência da interação específica planta-inseto (KERCHEV et al., 2012).

Espécies reativas de oxigênio (ROS, do inglês *reactive oxygen species*) são geradas inevitavelmente durante eventos metabólicos dependentes de oxigênio (O_2), os quais ocorrem, principalmente, nas mitocôndrias, cloroplastos e peroxissomos, pela redução parcial de O_2. Durante o transporte de elétrons (e^-), muitos destes e^- são perdidos, e então excitam o elétron externo de O_2, o que altera a distribuição dos seus elétrons formando oxigênio simples, $^1O_2$, ou são captados pelo O_2 sucessivamente, produzindo $O_2^{\cdot-}$, H_2O_2 e $OH^{\cdot}$ (BHATTACHARJEE, 2010; KARUPPANAPANDIAN et al., 2011). Estas espécies moleculares são consideradas “ativas” porque não necessitam da entrada de energia para reagir com outras moléculas (RESENDE; SALGADO; CHAVES, 2003).

Dentre os relevantes sistemas alterados pelas ROS estão os relacionados à fisiologia do cloroplasto e seus componentes, com efeito significante na transcrição através da sinalização retrógrada, que decorre da mudança nos níveis de açúcares, como o amido, e JA, devido à diminuição das taxas de assimilação de metabólitos ricos em nitrogênio e carbono (SINGH et al., 2011; SCHWARZLÄNDER et al., 2012; DE CLERCQ et al., 2013). Também afetam sistemas relacionados ao crescimento, pela danificação dos meristemas ou ainda pelo desvio de energia para a defesa. Mais diretamente, ROS interagem alterando macromoléculas, como bases nitrogenadas, levando à inativação e modificações ou mutação do DNA; proteínas, desnaturando e inibindo enzimas; danificam moléculas de carboidratos e retiram átomos de hidrogênio de grupos metileno de ácidos graxos poli-insaturados (BLOKHINA; VIROLAINEN; FAGERSTEDT, 2003; BARREIROS; DAVID, 2006).

A oxidação não-enzimática de ácidos graxos poli-insaturados componentes das membranas de fosfolipídios (peroxidação lipídica) é o sintoma mais óbvio de estresse oxidativo em plantas. Dentre os produtos finais das modificações oxidativas dos lipídios, o malondialdeído (MDA) causa danos nas membranas alterando suas propriedades intrínsecas, como fluidez, transporte de íons, perda de atividade enzimática e ligação cruzada com proteínas, eventualmente resultando na morte celular (LABBUDA, 2013). Avaliação de mudanças no nível de MDA é uma das principais formas de mensurar o estresse oxidativo em plantas, como observado em trigo, onde níveis de peroxidação lipídica mais baixos tem sido associado à tolerância ao déficit hídrico (SINGH; GUPTA; KAUR, 2012).

Em condições normais, ROS são produzidas e logo detoxificadas, sendo mantida a homeostase redox por enzimas e antioxidantes, no entanto, ao se acumularem em resposta a estresses ambientais, geram estresse oxidativo, causando toxicidade às células vegetais (KOTCHONI; GACHOMO, 2006). Sistemas protetivos não específicos, como o sistema antioxidante, contribuem significativamente para a planta tolerar as ações complexas dos fatores estressantes (HUSEYNOVA; ALIYEVA; ALIYEV, 2014), com sua resposta dependendo da espécie, variedade, estágio de desenvolvimento da planta e da intensidade e duração do estresse imposto (APEL; HIRT, 2004).

Vários estudos mostram sua relação mais direta com a tolerância a diversos tipos de estresses ambientais (LASCANO et al., 2001; ARIAS-MORENO et al., 2017). Sob déficit hídrico, observou-se em crassuláceas, níveis elevados de ROS aos 12 dias, mas reduzidos com o aumento de antioxidantes após 20 dias (HABIBI; HAJIBOLAND, 2012). Já em avaliações de plantas tolerantes e resistentes à herbívoros, observou-se expressão em maior nível e de forma constitutiva de genes de antioxidantes, em comparação com plantas suscetíveis sob infestação (SMITH et al., 2010; PEREZ; BROWN, 2014; SYTYKIEWICZ et al., 2014).

Embora atuem de forma destrutiva quando acumuladas, há evidências de que ROS podem agir na sinalização da resposta imune basal à estresse biótico, que aciona respostas induzidas por hormônios vegetais, como JA e SA, com o subsequente remodelamento da expressão gênica e a indução de proteínas R associadas com resistência sistêmica adquirida (SAR), além de regular a morte celular programada para limitar a infestação (FOYER, 2005; KWAK; NGUYEN;

SCHROEDER, 2006; KERCHEV et al., 2012; SANTAMARIA et al., 2013; FOYER et al., 2016; BLOCK et al., 2017).

2.3.4 Alterações no metabolismo primário e estresses biótico e abiótico

A atuação do metabolismo redox, além de intermediar a defesa induzida durante as respostas de plantas sob estresses bióticos, como tolerância à insetos sugadores, também tem sido demonstrada interagindo com o metabolismo primário (KOCH et al., 2016).

A associação entre tolerância à inseto e maior capacidade antioxidante tem sido relacionada à capacidade de recuperação das funções foliares, com rebalanceamento da função dos cloroplastos, indução de vias de captação de metabólitos centrais, como o nitrogênio, recuperação de meristemas e outras funções de crescimento de plantas que requerem superação da reprogramação celular causada pelo inseto (WILSON; STERNBERG; HURLEY, 2011). Assim, torna-se relevante avaliar conjuntamente os efeitos da herbivoria nos variados sistemas metabólicos passíveis de serem afetados, desde antioxidantes até os que se referem ao metabolismo primário, uma vez que carboidratos e aminoácidos são a principal fonte de energia tanto para a planta como para os insetos herbívoros e precursores para muitos metabólitos de defesa de plantas (ZHOU et al., 2015).

Durante a herbivoria, com o direcionamento do metabolismo basal para a produção de defesas físicas e químicas, ocorrem várias mudanças no metabolismo primário, tais como, aumento ou supressão da eficiência fotossintética, remobilização de recursos de carbono e nitrogênio e alteração da taxa de crescimento. A compreensão da causa de tais mudanças pode ser complexa, uma vez que pode ser reflexo da indução de resposta de defesa ou resultante da manipulação pelo inseto em seu próprio benefício (ZHOU et al., 2015).

Em relação à fotossíntese, há duas amplas e opostas hipóteses (ZHOU et al., 2015). Uma de que a atividade fotossintética é promovida (como fonte de carbono para produzir compostos de defesa e para compensar área foliar perdida pelo ataque do inseto ou pela manipulação do inseto para obter mais recursos), observando-se exemplo do aumento da fixação de CO_2, em plantas de *Nicotiana attenuata* resistentes e tolerantes na interação com *Tupiocoris notatus* (HALITSCHKE; HAMILTON;

KESSLER, 2011); e em trigo e cevada resistentes ao pulgão *Diuraphis noxia*, com expressão de genes relacionados à fotossíntese aumentada (BOTHA et al., 2006; GUTSCHE et al., 2009). A outra hipótese é de que ela é inibida (para direcionar os custos energéticos para a defesa; como consequência da senescência ou abscisão foliar; ou para tornar a disponibilidade de carboidratos menor para insetos). O que se observa em geral é que esta tende a reduzir mais do que seria esperado após danos dos insetos, ou mesmo sem dano efetivo do inseto, como por exemplo, através da percepção de ovoposição ou de compostos voláteis emitidos por outras plantas infestadas, o que demonstra ser esta uma resposta regulada da planta, e não apenas um efeito da herbivoria. Mesmo quando há atividade fotossintética reduzida, a planta ainda enfrenta alta demanda energética e de carbono para produção de compostos de defesa induzidos, e embora não seja um padrão, um dos meios na busca por energia é por realocação de carbono de outros tecidos, ou pelo catabolismo de macromoléculas, como carboidratos (sacarose, amido) e proteínas (CALDANA et al., 2011).

Há que se acrescentar neste contexto, que a via de sinalização do ácido jasmônico, hormônio induzido sob estresse biótico, é necessária para supressão da fotossíntese e do crescimento, mesmo não causando redução imediata na fixação de carbono, o que também pode indicar uma resposta hormonal regulada da planta para o metabolismo fotossintético (WASTERNACK; HAUSE, 2013).

Em relação às fontes de nitrogênio, estas podem ser diminuídas nas interações planta-inseto, de forma a atuarem como limitantes do crescimento do inseto em termos nutricionais (ZIEGLER; FACCHINI, 2008; ZHOU et al., 2015). Algumas espécies de plantas dispõem de compostos ricos em nitrogênio na seiva na forma de compostos pré-manufaturados, e não como aminoácidos livres, como forma de evitar a utilização pelo inseto para melhoria da sua nutrição (CHIOZZA; NEAL, 2010; ZHOU et al., 2015; FLORENCIO-ORTIZ et al., 2018).

Outra resposta da planta à herbivoria é a elicitação de defesas da planta para longe do local atacado, para reduzir o valor nutritivo do tecido da planta (LU et al., 2015) ou para promover crescimento posterior ao ataque como estratégia de tolerância, ou ainda redirecionar estes metabólitos para serem exsudados e atrair micróbios ou insetos que colaborem na defesa (defesa indireta) (ZHOU et al., 2015).

A resposta da planta a insetos depende tanto de fatores intrínsecos (ou endógenos), como de extrínsecos, como o tipo de aparato usado para se alimentar, a especificidade da dieta do inseto (generalistas ou especialistas), a extensão e o tempo de duração do dano e a composição da secreção dos insetos (ZHOU et al., 2015).

Insetos generalistas, que se hospedam em plantas de mais de uma família, tendem a possuir estratégias de defesa de faixa ampla, apresentado baixa tolerância a toxinas especializadas de plantas (BALLHORN; KAUTZ; LIEBEREI, 2010). *Diaspis echinocacti* tem como hospedeiro preferencial a família Cactaceae (GARCÍA MORALES et al., 2016), o que pressupõe alto grau de especialização por meio de toxinas durante o ataque, além do fato de possuir o corpo coberto por capa cerosa protetora e ser séssil, não buscando outras plantas ou outras regiões da própria planta após se estabelecer inicialmente para se alimentar; quando este é o caso, estudos relatam que para a planta parece ser mais vantajoso investir na manutenção do metabolismo primário, para manter-se viva o maior tempo possível durante o ataque (tolerância) (ZHOU et al., 2015).

Por possuir um hábito alimentar picador-sugador por meio de estilete, e por comparação com espécies semelhantes, como *Planococcus citri*, da mesma superfamília (SANTA-CECÍLIA; PRADO; OLIVEIRA, 2013), acredita-se que a cochonilha-de-escama acessa a seiva do floema para se alimentar (HERIOT, 1934; BEARDSLEY; GONZALEZ, 1975; CLAPS; HARO, 2001). Este hábito alimentar, ocasiona danos específicos através de elicitores ativos da saliva de insetos sugadores, assim a sua identificação também auxilia no entendimento das alterações no metabolismo primário na interação entre planta e inseto (STAHL; HILFIKER; REYMOND, 2018).

No contexto da resposta à déficit hídrico, uma das respostas moleculares mais comuns relativas ao metabolismo primário é o ajuste osmótico das células, com o acúmulo de aminoácidos, açúcares, ácidos orgânicos e íons no citosol. Além da maior expressão de algumas proteínas específicas para proteção da estrutura celular, como proteínas de choque térmico e proteínas Lea (late embryogenesis abundant) (NEPOMUCENO et al., 2001).

Quanto à fotossíntese, sob estresse severo e desidratação das células, esta é inibida, com consequente diminuição na síntese, consumo e exportação de fotoassimilados (TAIZ et al., 2017).

O teor relativo de água (TRA) é um dos parâmetros para representar o *status* de hidratação da planta em relação à sua capacidade máxima. De acordo com Mullan & Pietragalla (2012) o TRA integra o potencial da água na folha (ψ; outra estimativa útil do estado da água da planta) com o efeito de ajuste osmótico (um poderoso mecanismo de conservação da hidratação celular), podendo então fornecer uma medida do grau de estresse hídrico foliar sob seca e sob calor, uma vez que a capacidade de minimizar o estresse mantendo a turgidez da folha é vantajosa fisiologicamente e pode variar conforme o genótipo.

De acordo com Hsiao (1973), quanto ao TRA, estresse hídrico leve implica na diminuição do TRA em até 8 ou 10 pontos percentuais abaixo dos valores correspondentes em plantas bem irrigadas sob demanda evaporativa moderada; estresse moderado refere-se a uma redução maior que 10 e menor que 20 pontos percentuais; estresse grave se for reduzido em mais de 20 pontos percentuais e a dessecação para casos em que mais de metade da água do tecido é removida.

Em plantas CAM, mesmo sendo mais tolerantes à seca, sob déficit hídrico e salinidade de períodos variando de 20 dias a 4 meses, tem se observado redução no TRA, a depender da variedade, entre 60% a 40% (LOIK; NOBEL, 1991; PIMIENTA-BARRIOS et al., 2005; FRANCO-SALAZAR; VÉLIZ, 2007; HABIBI; HAJIBOLAND, 2011; SCALISI et al., 2016).

A capacidade de manutenção do status hídrico, permite processos dependentes do turgor, como crescimento e atividade estomática, refletindo maior assimilação de CO_2, além de proteger e manter o complexo fotossistêmico (MULLAN; PIETRAGALLA, 2012).

De acordo com modelos para mudanças climáticas, a probabilidade de as plantas encontrarem ambientes cada vez mais severos é crescente, e pode ser mais alta que o antecipado (RIZHSKY et al., 2004; MITTLER, 2006; SUZUKI et al., 2014). A tendência de aumento na temperatura, dentre os vários aspectos afetados, também reflete em maior demanda evaporativa, agravando o desbalanço hídrico. Com isto, as plantas enfrentarão cada vez mais condições de estresses associados, tanto entre abióticos (seca e calor, por exemplo), como entre bióticos e bióticos (pragas e seca, por exemplo) (ZANDALINASA et al., 2018).

Em relação à interação planta-praga, observa-se de forma geral o aumento do número de gerações do inseto quando há aumento da temperatura (DE LUCIA et al.,

2012); já em relação ao déficit hídrico há resultados que apoiam tanto a indução como a inibição de populações de insetos, conforme as espécies envolvidas, evidenciando que alterações no metabolismo da planta, principal fonte de nutrientes para o inseto, alteram seu comportamento (HUBERTY; DENNO, 2004). Copolovici et al., (2014), por exemplo, demonstraram maior atração de herbívoros em *Alnus glutinosa* irrigada, em função de maior elicitação de compostos orgânicos voláteis sob seca.

Uma das hipóteses que tem sido aceita, para o aumento do desempenho de insetos que se alimentam do floema em plantas sob seca, é o aumento da concentração de aminoácidos que se tornam benéficos à dieta dos insetos, mas ao mesmo tempo a necessidade de osmorregulação do próprio metabolismo do inseto à seca, para lidar com uma dieta osmoticamente desafiadora, pode ser prejudicial para seu desempenho (VICKERS, 2011).

O fato de as plantas terem coevoluído com uma enorme variedade de fatores biótico e abióticos com variação diária e sazonal, as permite responder a estes diversos estresses de forma finamente regulada, no entanto a forma como os múltiplos estresses são regulados é pouco entendida. Como algumas respostas a diferentes estresses usam sinais e vias comuns, incluindo cascatas de sinalização de ácido abscísico, etileno, ácido jasmônico e ácido salicílico que podem atuar antagonicamente ou sinergisticamente, estas levam ao fenômeno de tolerância cruzada, de forma que exposição prévia a um tipo de estresse pode ativar respostas que facilitam a tolerância à vários tipos de estresse (FOYER et al., 2016; ZANDALINASA et al., 2018).

A especificidade e variação da resposta de diferentes sistemas de interação planta-inseto, conforme as condições a que estão submetidos, impede que sejam feitas generalizações, exigindo estudos específicos.

2.4 Análise Proteômica

As plantas se aclimatam diante de condições que perturbam sua fisiologia através da transdução de sinais, adotando mudanças sistêmicas que influenciam seu genoma, transcriptoma, proteoma e metaboloma (GHOSH et al., 2017). Análises de expressão gênica em larga escala da planta sob estresse, seja abiótico e/ou biótico, permitem inferir através de quais vias a planta se contrapõe nestas complexas

interações por mecanismos endógenos, e assim identificar genes candidatos específicos responsivos a tais condições que podem ser úteis no melhoramento (SANGHA et al., 2013; BUCHANAN; GRUISSEM; JONES, 2015; KUMAR et al., 2016).

O avanço rápido no sequenciamento de genomas, através da Genômica, por meio de tecnologias de alto desempenho, revelou a necessidade do preenchimento de lacunas em relação ao entendimento da função dos genes e sua regulação nos mais diversos contextos. Isto impulsionou o desenvolvimento de ferramentas para análise da expressão gênica em larga escala, que tem sido realizada principalmente por meio da Transcriptômica (análise de transcritos) e Proteômica (análise de (LIEBLER, 2002; VOGEL; MARCOTTE, 2012)

Em função da sua posição no fluxo da informação genética, a análise proteômica é bastante relevante entre as análises de expressão gênica, por ser capaz de identificar os produtos gênicos mais próximos de um fenótipo, que muitas vezes representam os produtos finais de processos regulatórios, fornecendo uma riqueza de informações adicionais da planta (FEUSSNER; POLLE, 2015). Além disso, nem sempre a análise quantitativa de transcritos reflete a abundância de proteínas, devido às modificações pós-transcricionais e pós-traducionais, e consequentemente pode não ser tão conclusiva sobre o papel dos produtos destes transcritos nas condições avaliadas, embora possa complementar à abordagem (ANDERSON; SEILHAMER, 1997; GYGI et al., 1999; GREENBAUM et al., 2003; VOGEL; MARCOTTE, 2012; AEBERSOLD; MANN, 2016; HUA et al., 2016).

O objeto de estudo da proteômica, o proteoma, é definido como o complemento proteico do genoma ou conjunto de todas as proteínas acumuladas a partir da expressão pelo genoma de um organismo, analisado por meio de suas células, tecidos ou organelas sob determinadas condições (WASINGER et al., 1995; WILKINS; GOOLEY, 1997; JUNGBLUT et al., 2008).

As análises de proteínas em proteômica podem ser realizadas por duas metodologias: *bottom-up*, onde as proteínas são fragmentadas por proteólise, e a partir da análise e identificação dos seus fragmentos peptídicos, obtém-se a identificação da proteína inteira, e *top-down*, que caracteriza proteínas intactas. Ambas as metodologias demandam a extração de proteínas por protocolos específicos, e sua posterior separação para então seguir à identificação. A *bottom-up*, é a mais utilizada devido à facilidade para fracionamento, ionização e fragmentação

de peptídeos que de proteínas intactas. Nesta metodologia a separação de proteínas ou peptídeos tem sido realizada de maneira geral por dois mecanismos, que podem ter variações: eletroforese em gel bidimensional (2-DE, *Bidimensional Electrophoresis*) e, mais recentemente, cromatografia líquida (também chamada de *gel-free* ou proteômica *shotgun*, que parte de uma mistura complexa de proteínas (JORRIN-NOVO et al., 2019).

Na separação por 2-DE, após coloração e quantificação relativa, segue-se para a digestão e espectrometria de massas (MS). Já na cromatografia líquida, onde o equipamento é diretamente acoplado à MS, realiza-se a digestão da mistura complexa previamente à separação, seguindo à identificação de espectros de massa e por fim a obtenção dos espectros (LIEBLER, 2002).

O desenvolvimento da espectrometria de massas permitindo a análise de biomoléculas de proteínas, acelerou exponencialmente o campo da proteômica em variadas espécies (HURKMAN; TANAKA, 2007). Associado a isso, o crescimento dos bancos de dados de genomas e a evolução de *softwares* e tecnologias de Bioinformática tanto para quantificação relativa como para a identificação de proteínas, foram cruciais para mais rápida ampliação do seu alcance (MOCHIDA; SHINOZAKI, 2011).

A técnica 2-DE tem sido a mais acessível em muitos laboratórios, em comparação com a técnica de proteômica *shotgun*, principalmente devido aos custos dos equipamentos, sendo por isto ainda a mais utilizada em proteômica de plantas (JORRIN-NOVO et al., 2019).

2.4.1 Eletroforese em Gel Bidimensional

A evolução de técnicas de separação simultânea de proteínas por eletroforese foi o que impulsionou a abordagem de estudo de proteínas em larga escala de forma conjunta, como complexos que interagem entre si, e não apenas estudadas isoladamente, como feito anteriormente na chamada “química de proteínas” (BRADSHAW, 2008).

A Eletroforese em Gel Bidimensional (*Two-dimensional Gel Electrophoresis*, 2DE ou 2D-PAGE), considerada uma técnica clássica em proteômica, permite a separação das proteínas por ponto isoelétrico (pI) através de focalização isoelétrica

(IEF) acoplada à separação por massa molecular através de eletroforese em gel de poliacrilamida-dodecilsulfato de sódio (SDS-PAGE) (RIGHETTI et al., 2008).

A IEF é realizada a partir de gel imobilizado em forma de fita sobre suporte plástico, contendo anfólitos que geram um gradiente de pH, permanecendo imobilizados sob campo elétrico, e que age como tampão. Os valores da faixa de pH de 1 a 13 podem ser sintetizados de forma linear ou não-linear, variando em sua amplitude e no comprimento da fita, afetando a resolução final. Este gel é hidratado com a solução de proteínas por tempo adequado, e submetido a campo elétrico, de modo que as proteínas absorvidas migram até a região do gradiente onde sua carga líquida é zero (PERGANDE; COLOGNA, 2017).

A etapa de IEF reduz a complexidade de misturas complexas de amostras ao fracioná-las pelos valores de pI (PERGANDE; COLOGNA, 2017). No entanto, é bastante sensível a substâncias que podem estar presentes na amostra, como altos níveis de sal, lipídios, polissacarídeos, mucinas, polifenóis e ácidos nucleicos, os quais se não forem removidos, resultam em perda de *spots* proteicos, estrias horizontais e verticais e pobre focalização. Assim, especialmente em plantas, que possuem maior variedade e quantidade de interferentes, é necessária sua eliminação, ao mesmo tempo em que se preserva a integridade das proteínas durante a extração. Após a focalização da amostra, é necessário realizar a redução e alquilação de grupos –SH, evitando a formação de oligômeros e mantendo a molécula distendida (RIGHETTI et al., 2008).

A segunda dimensão é feita através de aparato vertical com lâminas (ou pratos) de vidro montados para preparar um gel de até 20 x 20 cm para separação por tamanho (massa molecular), resultando em uma distribuição de componentes proteicos (*spots*, geralmente em forma circular, oval ou elíptica), como um mapa, separando até 10.000 proteínas em um único gel. As duas dimensões da separação por 2DE fornecem um repertório das principais proteínas presentes no sistema em análise (tecido, célula, fluido) e permite assim correlacioná-las a valores de massa e ponto isoelétrico (RIGHETTI et al., 2008).

Após a separação em gel, os métodos de coloração para a visualização das proteínas utilizam tradicionalmente prata ou Coomassie para que se prossigam às análises posteriores de quantificação e identificação dos *spots* (LIEBLER, 2002).

Diante da complexidade do proteoma, e com objetivo de aumentar a velocidade e a reprodutibilidade das análise, foram desenvolvidas variantes da técnica da técnica 2D-PAGE, destacando-se dentre estas a Eletroforese em Gel Bidimensional Diferencial (*Two-dimensional Differential in-Gel Electrophoresis*, 2D-DIGE), que engloba o uso de diferentes corantes fluorescentes nas diferentes amostras sob análise. Ao corar cada amostra e um *pool* formado pela mistura das amostras diferentemente, esta técnica permite misturá-las e realizar uma única corrida eletroforética para analisá-las, permitindo que todas as identificações e quantificações sejam feitas sob as mesmas condições experimentais. Apesar de aumentar a reprodutibilidade da análise, este método nem sempre está disponível devido aos custos envolvidos nos equipamentos de identificação dos corantes e mesmo dos próprios corantes (ISSAQ; VEENSTRA, 2013).

Softwares específicos para análise de imagens dos géis bidimensionais, devidamente escaneados por densitometria, permitem a identificação de proteínas diferencialmente acumuladas (DAPs, *differential accumulated proteins*) nos géis, tornando possível realizar sua quantificação relativa. De forma simplificada, o programa realiza a sobreposição das imagens dos géis de diferentes tratamentos, detectando *spots* exclusivos (presentes em apenas um tratamento) e *spots* comuns (apresentam diferença no acúmulo entre os tratamentos comparados). A determinação das DAPs é feita considerando no mínimo três géis (réplicas técnicas) de cada tratamento, pela análise estatística do volume normalizado dos *spots*, que determina aqueles com diferença significativa entre tratamentos. Geralmente, entre as proteínas comuns, são consideradas diferenciais as com acúmulo igual ou superior a 50% em relação ao tratamento em comparação (*ratio* ≥1,5) (SARASWATHY; RAMALINGAM, 2011).

Uma vez detectadas as DAPs, procede-se à excisão individual de *spots* (que em geral corresponde a um polipetídeo). Seguindo a metodologia mais usada, *bottom-up*, os polipeptídeos excisados em gel serão submetidos à descoloração, purificação e digestão para fragmentação em peptídeos por meio de enzimas específicas (digestão enzimática), para que sejam então submetidos à identificação por espectrometria de massas. A técnica 2D-PAGE pode ter variações, como por exemplo, sendo combinada com a cromatografia líquida antes da espectrometria de

massas (BERGMÜLLER; BAGINSKY; GRUISSEM, 2008; JORRIN-NOVO et al., 2019)

2.4.2 Espectrometria de Massas

A ferramenta corrente para identificação de proteínas após a separação tem sido a espectrometria de massas. Na abordagem *bottom-up*, esta é precedida pela digestão, e consiste na medida da razão massa-carga (*m/z*) de íons moleculares característicos de um dado composto, de acordo com um padrão de fragmentação. Tendo se desenvolvido e estabelecido como método para análise de biomoléculas diversas no início do século XX, aperfeiçoaram-se técnicas para identificação de compostos grandes (proteínas e peptídeos), com a introdução dos métodos de ionização suave: ionização por dessorção à laser associada à matriz (MALDI- *matrix assisted laser desorption/ionization*) e ionização por *electrospray* (ESI- *electrospray ionization*) (BERGMÜLLER; BAGINSKY; GRUISSEM, 2008)**.**

Peptídeos de um *spot* digerido submetidos à MS geram espectros de massa (*m/z versus* intensidade do spot), que constituem a impressão digital de peptídeos (PMF, do inglês, *peptide mass fingerprint*) de uma proteína. A PMF de cada proteína é comparada com bancos de dados de proteínas da espécie ou de grupos taxonômicos próximos, cujas proteínas são submetidas à fragmentação teórica (*in silico*) (HENZEL; WATANABE; STULTS, 2003; BERGMÜLLER; BAGINSKY; GRUISSEM, 2008).

A espectrometria de massas tem permitido a identificação imparcial de proteínas em misturas altamente complexas, que verdadeiramente permitiu a expansão dessas determinações, além de capacitar a identificação de modificações pós-traducionais (uma lacuna da genômica e transcriptômica), com uma facilidade e escala que métodos anteriores não poderiam abordar. Atualmente esta técnica tem se firmado na identificação de biomoléculas devido à sua alta sensibilidade, acurácia e alta resolução de massa e análise rápida de amostras (BERGMÜLLER; BAGINSKY; GRUISSEM, 2008).

2.4.3 Aplicações em Estudos de Estresses Vegetais

A análise proteômica de plantas tem alguns desafios quando se compara à de outros organismos, sendo os principais a existência de genomas sequenciados de espécies não-modelo, para que através do proteoma predito se proceda à identificação mais precisa de proteínas. Além disso, há a necessidade de sistematização de protocolos de extração proteica, os quais podem exigir particularidades dependendo das caraterísticas da espécie, garantindo não interferência nas análises posteriores (WIJK, 2001; REINDERS et al., 2004; CHEN; HARMON, 2006; HEAZLEWOOD; MILLAR, 2006).

Apesar destas dificuldades, análises de proteômica de plantas tem mostrado resultados positivos em variadas espécies vegetais, indicando classes de proteínas mais envolvidas na avaliação de resposta a estresses bióticos e abióticos que podem funcionar como candidatos para o melhoramento, além de fornecer informações sobre como a planta lida com tais estresses em variadas vias metabólicas ao mesmo tempo (ARORA; SANDHU, 2017; BADOWIEC; WEIDNER, 2014; BELTRAN *et al.*, 2017; ČERNÝ *et al.*, 2016; ZADRAŽNIK *et al.*, 2013; SILVA et al., 2017)

Análise de proteínas em arroz sob infestação da cigarrinha marrom (*Nilaparvata lugens*), ao identificar proteínas em maior abundância na variedade resistente em comparação à variedades sensível e selvagem, permitiram conhecer proteínas e vias envolvidas na resistência da planta, como as relacionadas ao metabolismo, à resposta ao estresse e síntese de proteínas (SANGHA et al., 2013). Já em *Arabidopis thaliana* sob ataque de *Myzus persicae*, foram identificadas proteínas mais diferencialmente abundantes relacionadas ao metabolismo de aminoácidos, carboidratos, energia e fotossíntese (TRUONG et al., 2015). Trigo diploide exibindo resistência à afídio (*Sitobion avenae*) em campo foi submetido à analise proteômica, revelando-se como valioso recurso para uso no melhoramento, com a abundância de proteínas relacionadas à processos metabólicos, como fotossíntese e regulação transcricional, e apenas na variedade resistente, proteínas de resposta à estresse, como NBR-LRR-like, de resposta a estresse oxidativo e de síntese, reparo e replicação de DNA (GUAN et al., 2015).

Em relação ao déficit hídrico a proteômica também tem revelado padrões de expressão de proteínas e um panorama das vias metabólicas envolvidas, como por

exemplo com a identificação de proteínas relacionadas à fotossíntese mais diferencialmente acumuladas, alterando processos de regulação da Rubisco, transporte de elétrons e o ciclo de Calvin em soja (DAS et al., 2016); e em trigo, com proteínas relacionadas à defesa celular e detoxificação em raízes e relacionadas à fotossíntese em folhas (HAO et al., 2015).

Na família das cactáceas relata-se poucos estudos com análise proteômica, os quais é possível listar: relativos à análise diferencial de tecidos de calos de *Cereus peruvianus* crescidos em diferentes meios (MANGOLIN; OTTOBONI; MACHADO, 1999); análise da fração de N-glicanos a partir de gel de eletroforese de *Mamillaria gracilis* (BALEN et al., 2005); glicoproteoma de *M. gracilis* crescido *in vitro* (BALEN et al., 2006); avaliação de proteínas diferencialmente expressas em resposta a estresse salino e osmótico, demonstrando dificuldade de *M. gracilis* ativar processos protetivos sob as condições avaliadas (ROGIĆ et al., 2015); avaliação dos mecanismos moleculares de biossíntese de betalaína em frutos de *Hylocereus polyrhizus* ao longo de estágios de maturação (HUA et al., 2016); expressão diferencial de proteínas marcadoras da embriogênese somática em duas linhagens de calos de *Stephanocereus luetetzelburgii* (MARCHI, 2016); identificação de peptídeos ricos em cisteína em *Pereskia bleo* com importante papel na defesa em plantas (LOO et al., 2017). Já especificamente na subfamília *Opuntioideae*, os estudos utilizando análise proteômica são ainda mais limitados, citando-se por exemplo pesquisa identificando proteínas diferencialmente abundantes em *Nopalea cochenilifera* sob déficit hídrico (REIS, 2009); identificação de expressão diferencial de proteínas associadas a diferentes estágios de maturação do fruto (ROSAS-CÁRDENAS; PAREDES-LÓPEZ; CRUZ-HERNÁNDEZ, 2012) e a avaliação de 5 espécies do gênero para análise de mudanças metabólicas decorrentes da domesticação (PICHEREAUX et al., 2016). No entanto, poucos destes estudos se referem à resposta a estresses bióticos e abióticos com estas promissoras espécies.

Atribui-se os poucos estudos com cactáceas a nível molecular de forma geral, à dificuldade no estabelecimento de protocolos de análise e validação, devido à presença em maior quantidade de metabólitos secundários como mucopolissacarídeos, pigmentos, ceras e terpenóides nos seus tecidos que requerem maior especificidade para eliminação destes interferentes; e além disso, a importância econômica mais reduzida em relação à grandes culturas, contribuindo para manter as

pesquisas com tais espécies à margem. No entanto com a crescente expansão e importância da cultura nos últimos anos, faz-se necessário a aplicação de modernas técnicas de biotecnologia para melhorar seu cultivo, especialmente quanto à questões que afetam sua produção (VALDERRAMA-CHÁIREZ; CRUZ-HERNÁNDEZ; PAREDES-LÓPEZ, 2002; LLEDÍAS et al., 2017; MARTÍNEZ-GONZÁLEZ et al., 2017).

Artigo 1 - Proteome response in prickly pear (*Opuntia stricta*) interaction with cactus scale in different water conditions

Mara Danielle Silva do Carmo[1], Melquisedec de Sousa Oliveira[1], Elton Pedro Nunes Pena[1], Amaro de Castro Lira Neto[2], Djalma Cordeiro dos Santos[2], José de Paula Oliveira[2], Fabiana Aparecida Cavalcante Silva[1], Tercilio Calsa Jr.[1]*

[1]Laboratório de Genômica e Proteômica de Plantas, Departamento de Genética, Centro de Biociências, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego 1235, Cidade Universitária, Recife, Pernambuco, 50670-901.
[2]Instituto Agronômico de Pernambuco, Sede, Av. Gal. San Martin, 1371, San Martin, Recife, Pernambuco, 50761-000.

* Corresponding: tercilio@ufpe.br; Tel.: +55-081-2126-7829

Highlights:

- Prickly pear exhibits different pathways to circumvent cactus scale under different water regime, being more affected under drought condition
- In response to plague, proteins associated to jasmonic acid are more accumulated under irrigated condition
- Prickly pear, a CAM plant, suffers drought stress above 30 days without water

Abstract: Prickly pear is a relevant crop in arid and semi-arid regions with diverse uses to fruit and cladode, standing out in places where economy is based on cattle raising and uncertainty regarding the rains, that seriously affects the animal feed. Although it is a rustic crop, it has cactus scale as one of its main pests, with no records of resistant varieties. This work evaluated the differential expression of proteins in prickly pear (*Opuntia stricta* (Haw.) Haw. subsp. esparzae Scheinvar) under attack of cactus scale. Plants of 1-year-old IPA200016 variety were submitted to infestation and maintained under water deficit for 35 days, in four treatments [irrigated non-infested (HS), irrigated infested (HC), drought non-infested (SS), drought infested (SC)]. The differential proteome was examined using two-dimensional gel electrophoresis. Forty-four protein spots were found to be differentially regulated in irrigated condition and 34 in water deficit. Mass spectrometric analysis allowed the identification of 67 differentially expressed proteins, presumably involved in a variety of functions including transcription, light and transduction signaling, revealing molecular mechanisms involving both regulatory and effector proteins. This work, for the first time, provides insights into the interaction between the prickly pear and cactus scale at translational and post-translational levels.

Keywords: Cactaceae; *Diaspis echinocacti*; biotic stress; 2D-PAGE; defense response.

1. Introduction

Arid and semiarid regions are the most extensive on the planet, accounting for 5 billion hectares. One of the most relevant crops in these areas is prickly pear (*Opuntia* spp.), a Cactaceae species in expansion of their crops, adapted to attend such areas demand for food supply, specially helping economically low-income populations through animal feed [1–3]. Despite their tolerance to environmental adversities such as high temperatures and drought conferred by anatomical, physiological and morphological adaptations, prickly pear species suffer with diseases and pests. The cactus scale (*Diaspis echinocacti* Bouché, 1833) is a relevant pest to prickly pear [4–6]. They are sucking insects that feed on the sap, at the same time as can act as vectors of viruses and pathogenic microorganisms. Despite the small size, 1 to 3 mm, under favorable environmental conditions, they can spread rapidly, through sexual and asexual reproduction [5,7–9].

The interrelation between plants and insects is complex and intricate, with a dynamic communication. Insects have specialized to feed on particular species of plants, that, in turn, have developed a wide range of morphological and biochemical characteristics to protect from those herbivores [10]. The understanding of the host plant-aphid relationship has been advanced through technologies at molecular level, since is possible to measure stress-induced changes at transcript expression level (transcriptomics), protein complement level (proteomics) and the consequent effect on metabolism (metabolomics) [11]. Even during susceptibility, the plant defenses are activated, although not enough to completely protect from insect attack. This basal defense appears to be due to the incomplete attenuation of pest pattern triggered induced (PTI) mechanisms and may be due to inefficient pest-derived effector recognition, thus leading to a weak effector triggered immunity (ETI) [12]. Some works about response to insect-pest attack have revealed candidate molecules for resistance, through identification of genes and proteins responsive in these conditions, providing a better picture of what pathways are underlying the resistance [13–16], but species such as prickly pear have been little explored in such interaction. In this study, we used proteomic tools over prickly pear (*Opuntia stricta*) under infestation by cactus scale (*Diaspis echinocacti*) and water deficit for the identification of differential proteins, focusing on the selection of candidates as defense response markers.

2. Materials and Methods

2.1. Plant Cultivation and Treatment

The experiment was carried out in a greenhouse located at Agronomic Institute of Pernambuco (IPA), Recife, Brazil (-8.063129º, -34.926315º), from August to October 2016. Clones from *Opuntia stricta* (variety IPA 200016), 1 year old, with 3 cladodes, obtained from in vitro culture, were transplanted to pots with soil classified as sandy loam (coarse sand 55%, fine sand 33% and clay 11%) as substrate.

Plants were submitted to the following treatments: 1 - irrigated at 100% pot capacity and without infestation (HS); 2 - irrigated at 100% pot capacity and with infestation (HC); 3 - irrigation suppression and no infestation (SS) and 4 - suppression of irrigation and infestation (SC). The treatments were kept isolated by anti-aphidic grid. The infestation was carried out by

continuous contact during 30 days with infested prickly pear fragments collected from IPA Experimental fields (São Bento do Una, Arcoverde, Ibimirim and Caruaru municipalities, state of Pernambuco, Brazil). After establishment of infestation, irrigation was suppressed for 35 days in water deficit treatments, while it was kept in other treatments three times a week at 100% pot capacity. Six plants were used in each treatment (biological replicates). After the stress period, the insects were removed from the infested plants and the cladode samples were collected, immediately frozen in liquid nitrogen and stored at -80˚C for further analysis.

2.2. Relative water content, biochemistry, reactive oxygen species and enzymatic analysis

Relative water content (RWC) was determined following the Barrs & Weatherley (1962) method. In each replicate, three known samples (1 cm long and 1 cm wide) were taken from the central region of the cladode and their fresh mass (MF) was determined on an analytical balance. These samples were then kept in deionized water for 24 h to obtain the turgid mass (MT). After that, the samples were dried at 70 ° C for 48 h to determine the dry mass (MS) of the tissue. The RWC values were determined by the equation: (MF-MS) / (MT-MS) × 100.

Biochemical, enzymatic and reactive oxygen species (ROS) analyzes were performed according to the following established protocols: Carbohydrates: Dubois et al. [18]; Proteins: Bradford [19]; Total Free Amino Acids: Moore & Stein [20]; Proline: Bates [21]; Chlorophyll a, b, total, carotenoids: Lichtenthaler [21]; Hydrogen peroxide (H_2O_2): Alexieva et al.[21]; Lipid peroxidation (malondialdehyde-MDA content): Heath; Packer [21], with modifications; Catalase activity (CAT): Havir & McHale [24], with modifications according to Azevedo et al. [26]; Ascorbate peroxidase (APX): Nakano & Asada [27]; Superoxide dismutase activity (SOD): Giannopolotis & Ries [27].

Experimental design was factorial 2x2. Respecting prerequisites of normality and homogeneity (RWC, total soluble carbohydrates, free amino acids, proline, proteins and chlorophyll), data were analyzed using the software Statistica 8.0 (StatSoft. Inc., Tulsa, OK 74104, USA), through Anova factorial and test Newman-Keuls. For the SOD, CAT, APX, H_2O_2, MDA data, it was used generalized linear models (GLMs) with Gaussian error distribution were used to analyze the relationships between the biochemical variables and the infestation and irrigation factors using in R *software*. A separate model was used for each response variable with the explanatory variables presence/absence of cactus scale and presence/absence of irrigation. The interactions between the explanatory variables were also considered.

2.3. Protein Sample Preparation, 2DE, and Protein Abundance Analyzes

Proteins from secondary cladodes under different treatments were extracted according to Wu et al. [29]. To separation in first dimension, isoelectric focusing (IEF) was performed on the Multiphor II electrophoresis system platform (GE Healthcare Life Sciences) using IPG strips (13 cm, pH 3-10, GE Healthcare Life Sciences) rehydrated in IPG-box for 16 hours, with 400

μg of proteins homogenized with rehydration buffer (Phenylmethane sulfonyl fluoride - PMSF), dithiothreitol (DTT), IPG buffer pH 3-10). IEF follow three steps: 300 V - 100 V/h, 3500 V - 2900 V/h, 3500V - 27000 V/h. Throughout the focusing, they were maintained in direct current (2 mA), 5 W of power and temperature of 10°C. Then, strips were reduced and alkylated with equilibrium buffer (urea 6M, Tris-HCl buffer 75 mM pH 8.8, glycerol 30%, SDS 2%) containing DTT 1% and iodoacetamide 2,5% (IAA), respectively. The second dimension was performed utilizing SDS-PAGE gels 12,5% (20x20 cm) in vertical plates, at 15°C in two steps: i) 300 V gradient, 15 mA, 30 W for 20 minutes; ii) 300 V, 30 mA, 30 W for approximately 2 h and 30 min. Gels were stained with Coomassie Brilliant Blue G-250 solution containing 0.01 $g.L^{-1}$ according to Candiano et al. [30]. At least three replicates of independent gels were generated by treatment. The acquisition of gel images was performed in Image Scanner III (GE Healthcare Life Sciences) through the LabScan 6.0 program, according to the default parameters, and image analysis of gels was performed using the Image Master 2D Platinum v.7.05 program (GE Healthcare Life Sciences). For the quantitative analysis, only reproducibly detected spots were considered. The volume of each spot was normalized against the total volume of the valid spots. Significant spots were selected as differentially accumulated protein (DAP) according to analysis of variance ($p \leq 0.05$) and the volume percentage ratio between control and stressed treatments greater than or equal to 1.5. Irrigated treatments (HS, control and HC, stressed) were compared, as well as water deficit treatments (SS, control and SC, stressed).

2.4. Protein Identification by MALDI-ToF/ToF MS and Database Searching

DAP spots were excised from gels and digested with trypsin as described by Webster & Oxley (2005), then, the samples were concentrated in a vacuum rotaevaporator at 30°C for 30 min. MS and MS/MS spectra were obtained using an MALDI-ToF/ToF Autoflex III mass spectrometer (Bruker Daltonics Inc., Bremen, Germany) in the Analytical Center of the Center for Strategic Technologies of Northeast (CETENE, Recife, Brazil). The pellet was solubilized in 5 μl of 0.1% trifluoroacetic acid (TFA). For each reading cycle, 2 μl of the sample were mixed with 2 μl of α-cyano-4-hydroxycinnamic acid matrix in acetonitril (ACN) and 3% TFA. The parameters were set to the positive ion reflection mode with acceleration voltage of 20kV, assuming a firing rate of 100 Hz, mass range of 700 to 5,000 Da, laser intensity ranged from 25 to 50% and 2,100 to 4,000 shots by spectrum. The equipment was calibrated using a peptide mixture $[M + H]^+$ ions for standard calibration. Peaklists files were generated using the FlexAnalysis 3.4 software (Bruker Daltonics). The identification of the spectra was performed through the MASCOT online software, initially by the public access version, based in peptide mass fingerprinting method (PMF) for MS spectra and MS/MS ion search method for MS/MS spectra, against the sub-banks Viridiplantae, *Arabidopsis thaliana*, *Oryza sativa* and other green plants from NCBIprotein and SwissProt database. Subsequently, additional identification was conducted through a private version of the MASCOT program, kindly made available for access in collaboration with the Center for Advanced Proteomics of the University of Washington (Seattle, Washington, USA (http://proteomicsresource.washington.edu/), against the sub-database Cactaceae.

The experimental error used for mass values was as follows: i) to MS spectra analysis: up to 1.2 Da of tolerance; ii) to MS/MS spectra: 100 to 200 ppm and 0.2 to 1.2 Da (for parental ion), and 100 to 200 ppm and 0.2 to 0.6 Da (for ion fragment). Another search parameters were set in both methods as following: carbamidomethylation of cysteines as fixed modification and methionine oxidation as variable modifications, allowing up to one missed cleavage. Protein identification was performed using the Mascot search probability-based molecular weight search (MOWSE) score. Only the identifications with calculated probability score equal or greater than to the cut-off value were considered significant. The score equals -10.log (P), where P is the probability that the similarity found is random. Peptides with a Mascot Score exceeding the threshold value corresponding to <5% false positive rate, calculated by the Mascot procedure.

Additional searches were performed with SearchGUI v.3.2.20 software using xml files obtained after analysis via MALDI-ToF/ToF that were individually converted to mzXML format through the FlexAnalysis software tool (Bruker Daltonics), and submitted to the presumptive identification [32], whose results were visualized using the program PeptideShaker v.1.16.15 [33]. The following parameters were used to search the SearchGUI: tolerance m/z of the parental ion: 0.25 Da; ion fragments m/z tolerance: 0.25 Da; charge of the parental ion: 1-1; isotope: 0-1; maximum missed cleavages: 1; fixed modifications: carbamidomethylation (C); variable modifications: oxidation (M). The other parameters remained in the default software mode. Reference proteomes available in UniProt were used as a database, initially from the Cactaceae family, and then if there was no identification, subsequent searches were carried out against *Spinacia oleracea* species (species with proteome available and taxonomically close to the genus *Opuntia*) and A. thaliana (model specie dicots). To correct identification MSQuant parameter was considered.

2.5. Protein Classification

For gene ontology (GO) analysis, the amino acid sequences of differentially accumulated proteins putatively identified in silico were recovered as multifasta format from Uniprot database. Fasta sequence from NCBI single accesses was retrieved from the nr (non-redundant) database and entered manually into multifasta file. Then, the multifasta file was used to map GO terms for biological processes in Mercator [34], which already uses the BLAST tool, to search the orthologous accesses correspondence in Arabidopsis thaliana; standard parameters, including the InterPro option (IPR), were used.

3. Results and Discussion

3.1. Biochemistry analysis

Plant-herbivore interactions are influenced by plant biochemistry and physiological performance, which may vary depending on the biotic and abiotic environment of the plants [35]. After 35 days under water deficit, the relative water content (RWC) reached a minimum of 48.34% in the treatment of drought and without infestation (SS), and with a slight increase to 59.26% in treatment under drought and infestation (SC) (Figure 1). The stress period for which

prickly pear was subjected may have led to the beginning of the desiccation, considering a visible reduction in the thickness of the cladodes (data not shown), which reflects reduction of the central aquifer parenchyma [36].

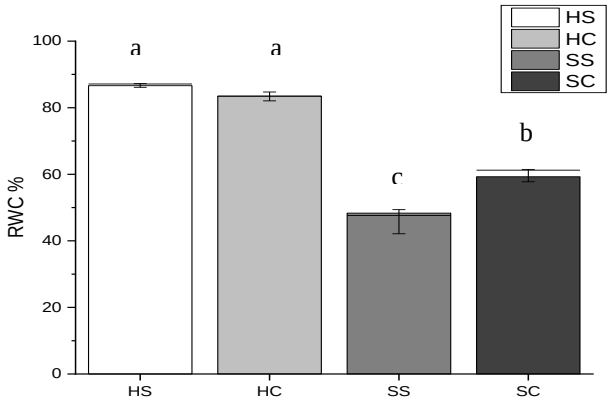

Figure 1. Mean values and standard error (vertical bars) of the RWC between treatments: HS; HC; SS; SC. Means followed by the same letter indicate that there is no significant difference between treatments (Test Newman-Keuls, $p < 0.05$).

A severe water stress is considered when RWC-value are 20 percentage points lower than well-irrigated plants, leading to desiccation when more than half of the water tissue is lost [37]. Although cactus scale infestation have had little effect on the RWC of prickly pear, the water deficit condition along the growth of insect colonies may have contributed to limit responses of metabolic pathways in pest control, affecting the primary metabolism, as reported below on biochemical and proteomic data. Plants under water deficit allocate resources to roots biomass to increase water uptake possibly at the expense of construction of photosynthetic tissue [38], which can also be reflected in the defense against herbivorous insects [39]. Depending on the intensity of the water stress, there may be positive, negative or neutral results on insect performance, which also depends on their eating habits and needs [40,41].

The decrease in RWC in *Opuntia* spp. and other succulent species has been observed under varied water deficit conditions, as by Habibi; Hajiboland [42], with 60% content after 20 days of drought, Pimienta-Barrios et al. [43] with 58% after 45 days of water deficit and by Scalisi et al. [44], reaching up to 44% in non-irrigated mother cladodes for 4 months. The RWC index is influenced by the number of daughter cladodes in relation to the mother cladode, indicating a flow of water to younger tissues that are more photosynthetically active; besides, it also depends on soil water potential, soil water content, atmospheric evaporative demand and plant species.

Although the cacti and succulents are able to withstand less water availability, as observed in long periods of dry season in the field, different varieties of *Opuntia* with different levels of domestication may present distinct tolerance to biotic stresses, such as water and salinity, and as the plant genotype [45]. Probably, *Opuntia stricta* supports less these conditions, which leads to the necessity of comparative evaluation of this aspect between different varieties of the genus *Opuntia*. Reports of Habibi and Hajiboland [46] show two species of succulent Crassulaceae of the same genus, with difference in RWC after water deficit. The variation in water adjustment

among a variety of species more adapted to the water deficit has also been observed when evaluating the water use efficiency through the accumulation of biomass or dry mass [46].

Aloe vera, with CAM metabolism, presented lower accumulation of biomass and gel production, both under higher and lower irrigation levels, in comparison to intermediate levels, which presented higher yield, evidencing more the water saving promoted by this metabolism [47]. In the case of CAM plants, adapted to the lower availability of water, the condition of excess water, besides not contributing to increase the biomass, can also cause oxidative and physiological stress [48].

As well as RWC, for soluble carbohydrates, free amino acids and protein content, treatments presented the same behavior, being increased in the irrigated condition, with higher levels in the irrigated treatment without infestation (HS), decreasing in the irrigated and infested treatment (HC) and sequentially in treatment with non-infested water deficit (SS) and with a slight increase in SC treatment. Irrigation was the only factor that promoted significant differences among treatments for all these biochemical parameters. (Figures 2a, 2b, 2c).

The significant reduction of soluble carbohydrates (Figure 2a), the main source of carbon skeletons during water deficit in CAM plants [49], in treatments under drought in relation to irrigated ones, reinforce the severity of water stress.

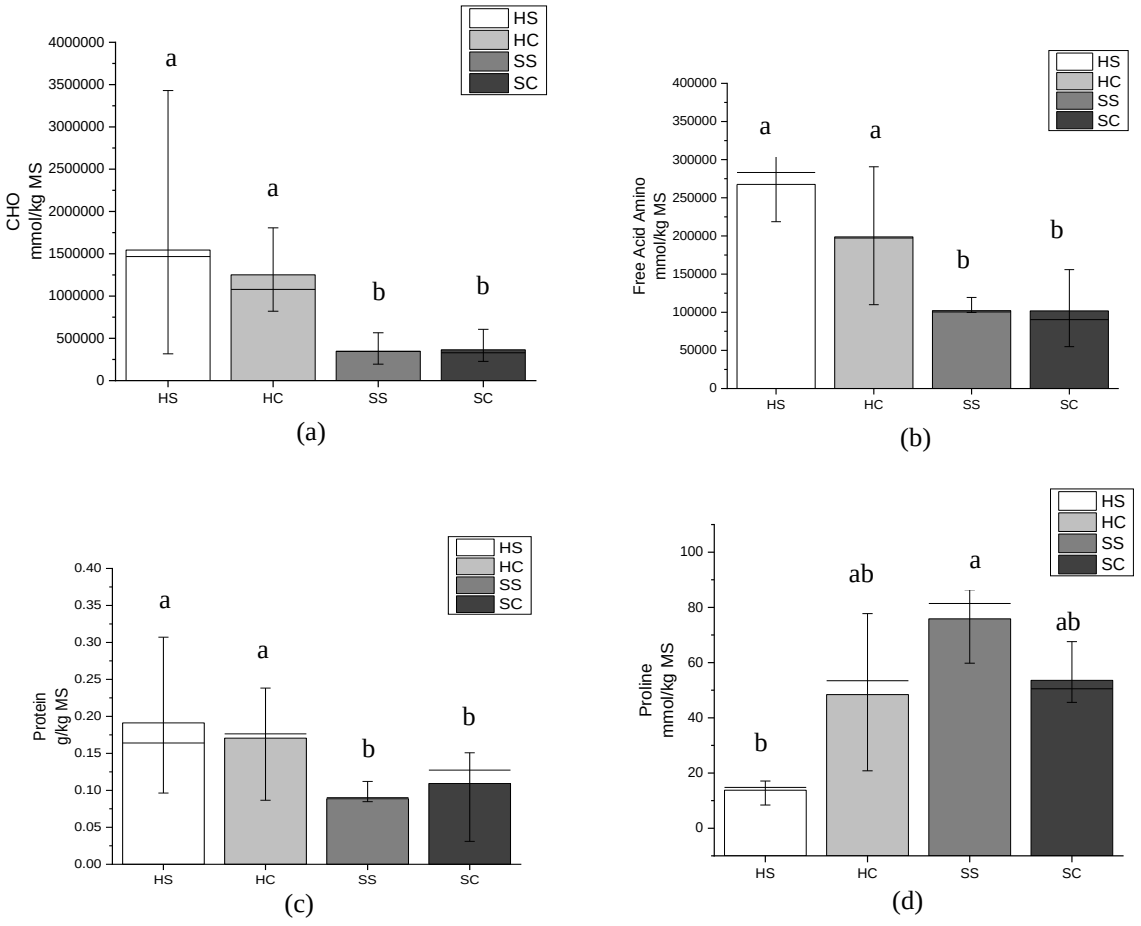

Figure 2. Mean values and standard error (vertical bars) of the soluble carbohydrates (a), free amino acids (b), protein content (c) and proline (d) between treatments: HS; HC; SS; SC. Means followed by the same letter indicate that there is no significant difference between treatments (Test Newman-Keuls, $p < 0.05$).

Among water deficit and herbivory, water stress generally is more harmful to the amino acid content, reducing it, and the presence of insects at low intensities is generally asymptomatic externally in the plant, varying according to the time of exposure to the infestation; even under intense infestations there is the hypothesis of the manipulation of essential amino acids by the insect, increasing its content to improve its nutrition [50]. What seems to happen between prickly pear and cactus scale is that on irrigated and infested condition there is a decreasing in total amino acid levels that would benefit the insect's diet (Figure 2b). Amino acids in addition to having various roles, regulate ion transport, modulate stomatal opening, participate in the detoxification of heavy metals, contribute to redox homeostasis, influence gene expression and affect the synthesis and activity of some enzymes [51].

In relation to the amino acid proline (Figure 2d), both irrigation and the interaction of irrigation and infestation factors promoted a difference between treatments. Under dry conditions and without infestation (SS), the proline content was the highest, as expected, reflecting its role in osmoregulation [51]. The irrigated and infested treatment (HC) had a significant increase of this amino acid in relation to the non-infested one (HS). For the treatment under dry and infested (SC), it did not increase, but was similar to HS, probably double stress incapacitated the plant to increase the levels of this amino acid. Proline exhibits high cross-tolerance to a wide range of different stresses [52], and may be increased under herbivory to constitute defense proteins [53,54]

The chlorophyll content is one of the most important factors in the determination of the photosynthesis rate and a useful indicator of both the productivity of the photosynthetic potential and the general vigor of the plant [55]. The impact of applied stresses on prickly pear relative to photosynthesis was observed by the significant decrease in levels of chlorophyll a, b, total and carotenoid pigments between treatments irrigated and under water deficit (Figures 3a, 3b, 3c, 3d). Among irrigated treatments, there was a slight increase in pigment content in infested treatment (HC), although not significant. Some plant-herbivore interactions reveal that resistance or tolerance requires the maintenance or increase of levels of chromophores under infestation [56]. The coverage of part of the leaf area by insect colonies with their waxy coating may have led to changes in the absorption of radiant light by the cladode and alteration in the photosynthetic activity of the plant, with lower absorption of photosynthetically active photon flux, leading to stress and damage to photosystem II [57]. The increase in HC treatment may result from an attempt by the plant to overcome oxidative damage resulting from infestation through antioxidant pigments, as well as by the greater induction of processes associated with photosynthesis, in order to increase the carbon input and may be part of the response to the pest, or insect manipulation to obtain more resources [58].

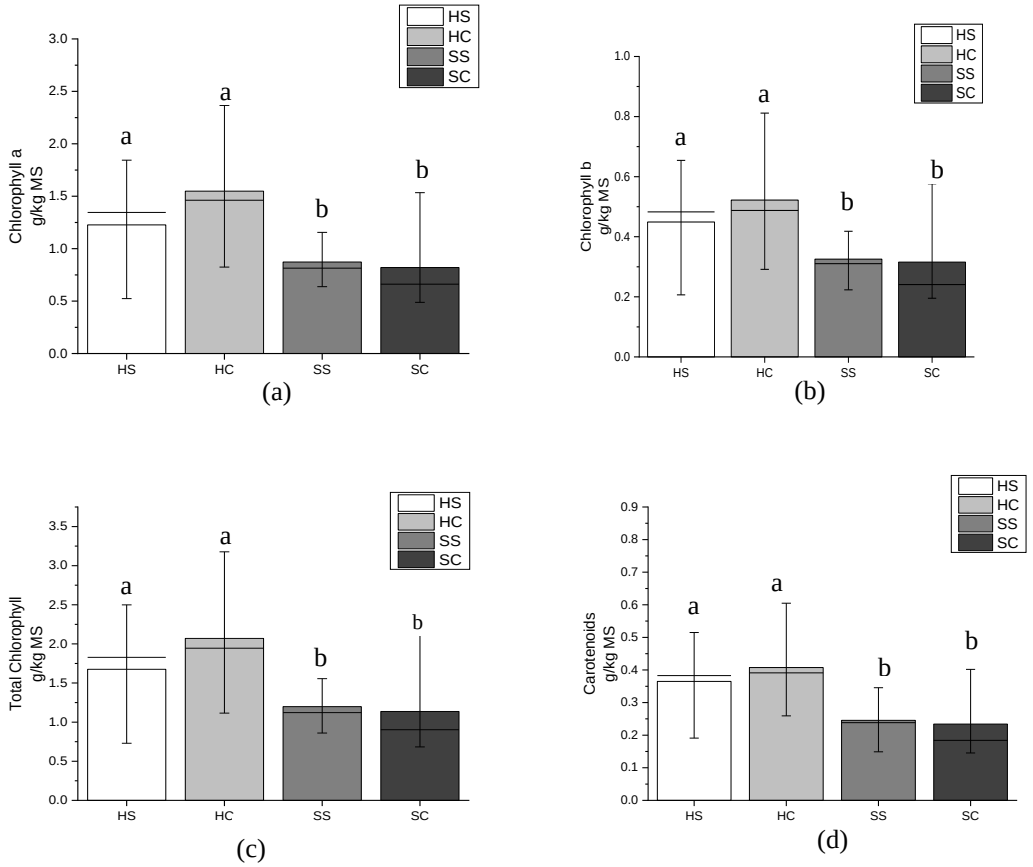

Figure 3. Mean values and standard error (vertical bars) of the chlorophyll a (a), chlorophyll b (b), chlorophyll total (c) and carotenoid pigments (d) between treatments: HS; HC; SS; SC. Means followed by the same letter indicate that there is no significant difference between treatments (Test Newman-Keuls, $p < 0.05$).

On the other hand, when the treatments under drought were compared to each other, a slight reduction of the pigments of the infested treatment under dry (SC) in relation to the non-infested under dry (SS) treatment was observed, revealing a reduced capacity of the plant to respond to the infestation when it is already affected by drought or even, changing its focus from synthesis of pigments for the synthesis of compounds of the secondary metabolism, thus a lower synthesis of chlorophylls, may be part of the adaptive response [58–60]. Several studies have reported a decrease in pigment levels in susceptible plants infested with scales and aphids insects, as well as under water deficit conditions. According to Ni et al. [61], chlorophyll degradation can frequently accompanies insect feeding damage in plants. Golan et al. [62]observed changes in pigment content in citrus and fern after six months of insect infestation by *Coccus hesperidum* scales, with increase in lower levels of infestation as an attempt to adapt and decrease with increased infestation.

The responses of the antioxidant activity to CAT and APX (Figure 4a, 4b) in the treatments under water deficit conditions in relation to the irrigated treatments were low and could reflect a severe stress condition, corroborated by the low RWC in only 35 days of water suppression, lower than the observed in larger periods for other species of the same genus under other conditions. The SOD enzyme (Figure 4c), however, increased its activity under the water

deficit conditions (SS and SC), being higher in treatment under drought and not infested (SS), but quite affected by the infestation, both in SC, as in HC.

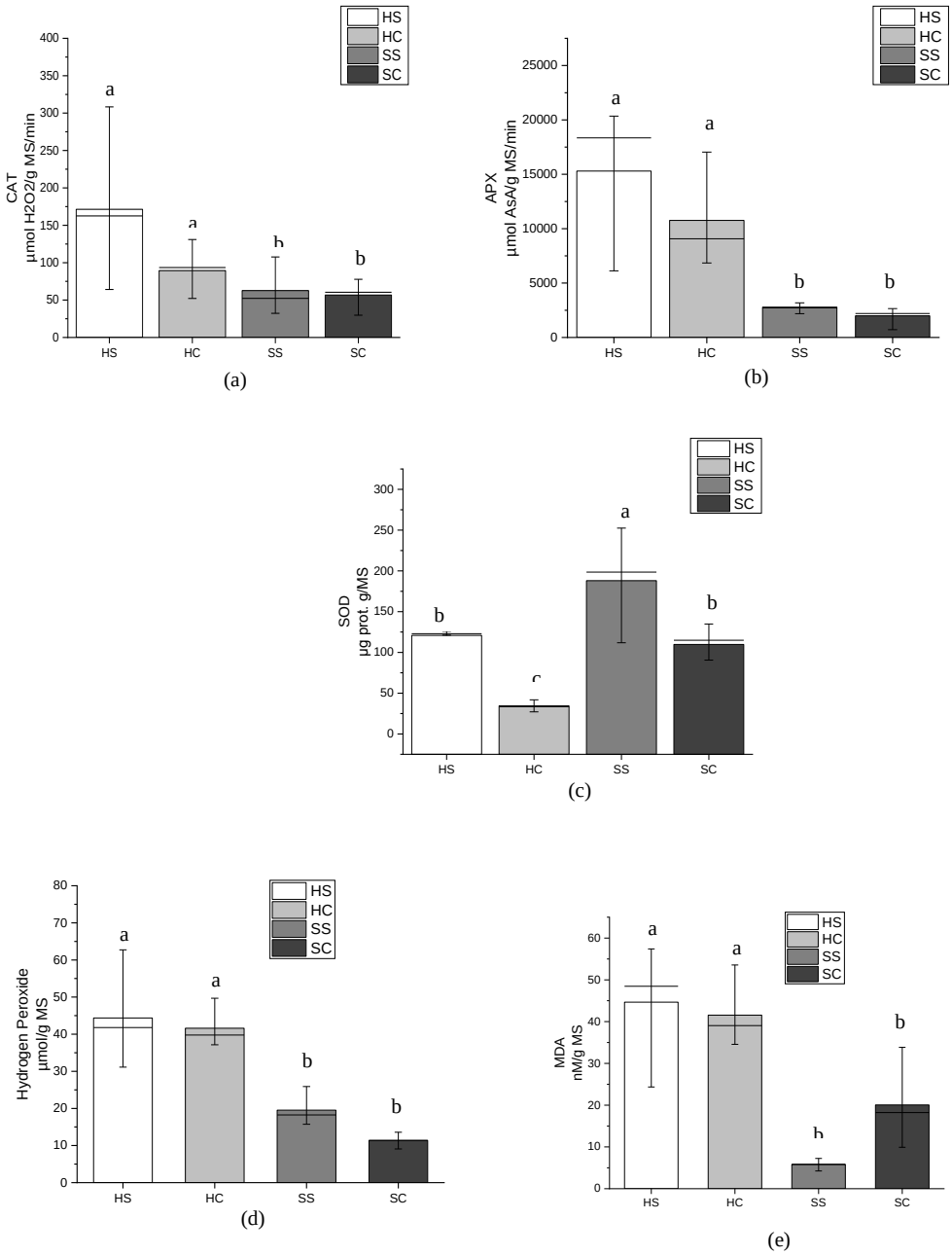

Figure 4. Mean values and standard error (vertical bars) of the CAT (a), APX (b), SOD (c), peroxide (d) and MDA (e) between treatments: HS; HC; SS; SC. Means followed by the same letter indicate that there is no significant difference between treatments (Test GLM, $p < 0.05$).

On the other hand, hydrogen peroxide and MDA levels (Figure 4d, 4e) in treatments under water deficit were significantly lower than in irrigated treatments. Both for antioxidant enzyme activity and for ROS, only the irrigation factor contributed to significantly differentiate the treatments. The lower accumulation of peroxide at that period may indicate alternation of the period of greatest need for signaling by the molecule. As well as the lower accumulation of MDA under dry conditions, it may reflect effective SOD activity to prevent H_2O_2 ROS-derived

from causing greater damage to the membrane, although the other enzymes (CAT and APX) did not follow the same pattern with accumulation at this time. Studies with water deficit in Crassulaceae show that these antioxidant enzymes appear to be more active at specific moments after stress, with more effective CAT activity in the first days, and more effective SOD and APX activities at the end of the period [42,46], demonstrating that this response varies with the intensity of the stress imposed [63].

Regarding the infestation, considering that the evaluation is a response after more than 35 days of infestation, it is noticed that the lower accumulation of peroxide in the infested treatments may reflect an attempt of its depletion by the antioxidant system, but less efficient, or even affected by the presence of the insect. Under conditions of biotic stress, depending on the perception of pathogens or herbivores elicitors by the plant, in general in the early stages there is an oxidative explosion, as well as a few hours later, with subsequent stabilization by the action of antioxidant enzymes [64].

In addition, herbivores can manipulate the resistance response by negatively regulating the accumulation of ROS, whose signaling pathway is interwoven with that of hormones responsive to biotic stress, jasmonic acid and salicylic acid [64,65]. In general, according to biochemical enzymatic data, prickly pear under water deficit has a reduced capacity to deal with biotic stress. Sequential evaluations and insect performance in the plant would aid in a better compression of this interaction affected by the environment.

3.2. Differentially abundant proteins in prickly pear infested with cactus scale irrigated and under water deficit

Two-dimensional gel electrophoresis-based (2D-PAGE) proteome analysis of irrigated *Opuntia stricta* infested or non-infested (here termed, Comparison 1) revealed a total of 855 protein spots, with an average of 142 spots per gel. Out from this total, 44 spots were differentially accumulated in response to pest attack (Figure 5), with 18 common to both conditions: but 6 more abundant in infested treatment and 12 more abundant in control condition. Were detected 24 exclusive spots in the infested condition and 2 exclusives in the control. Forty spots could be putatively identified: 13 in the control treatment (with 1 spot corresponding to two proteins) and 27 in the stressed one (with 2 spots corresponding to two proteins) (Table 1).

While under water deficit, infested or non-infested (here termed, Comparison 2), 2D-PAGE proteome analysis of *O. stricta* revealed a total of 1,207 protein spots, with an average of 201 spots per gel. Out from this total, 34 spots were differentially accumulated in response to the biotic stress factor (Figure 6), with 24 common to both conditions, but 6 more abundant in infested treatment and 18 more abundant in control condition; and yet 10 exclusives in infested condition. Twenty-one spots were putatively identified, 13 in control treatment (with 1 spot corresponding to two proteins) and 10 in stressed treatment (with 1 spot corresponding to two proteins) (Table 2). Proteins identified were grouped by gene ontology according to categories for biological process (Figures 7 and 8).

Most of proteins accumulated in both comparisons were related to RNA. In common between those treatments were 2 proteins (GATA-19 transcription factor, spot 81, in SC;

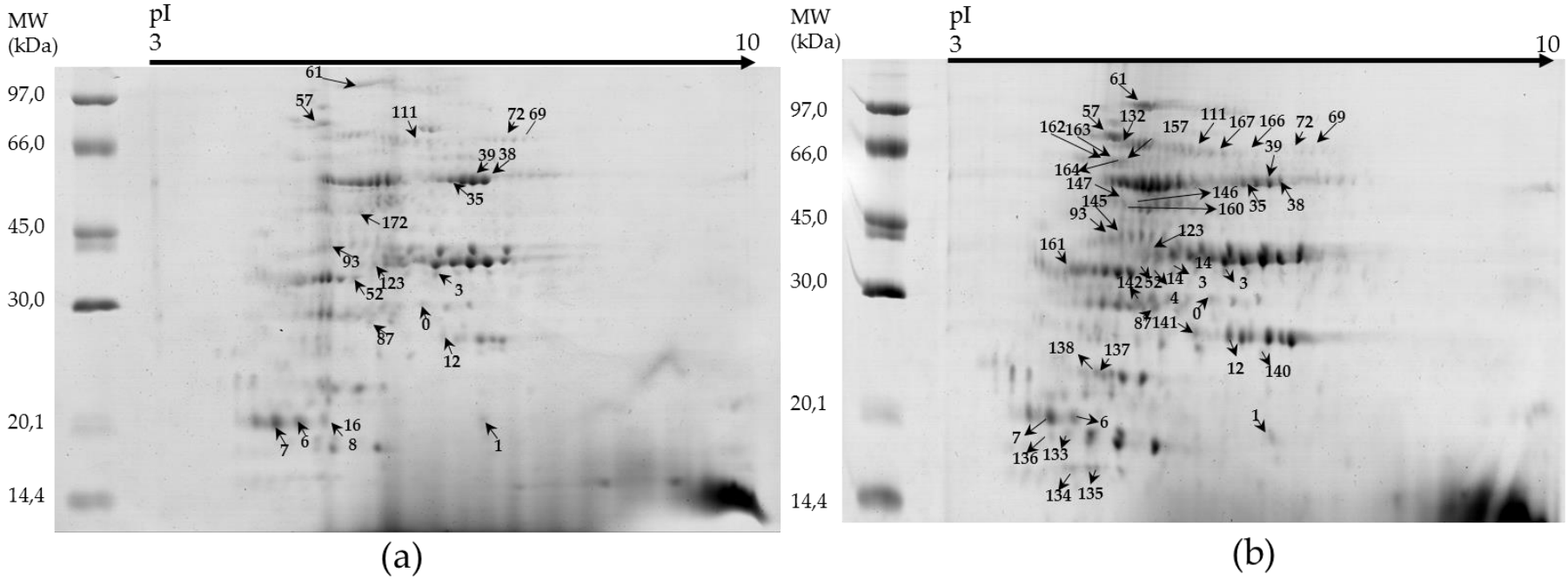

Figure 5. 2D-PAGE from prickly pear *Opuntia stricta* proteins (secondary cladodes) for irrigated control treatment: HS (a) and irrigated infested treatment: HC (b).

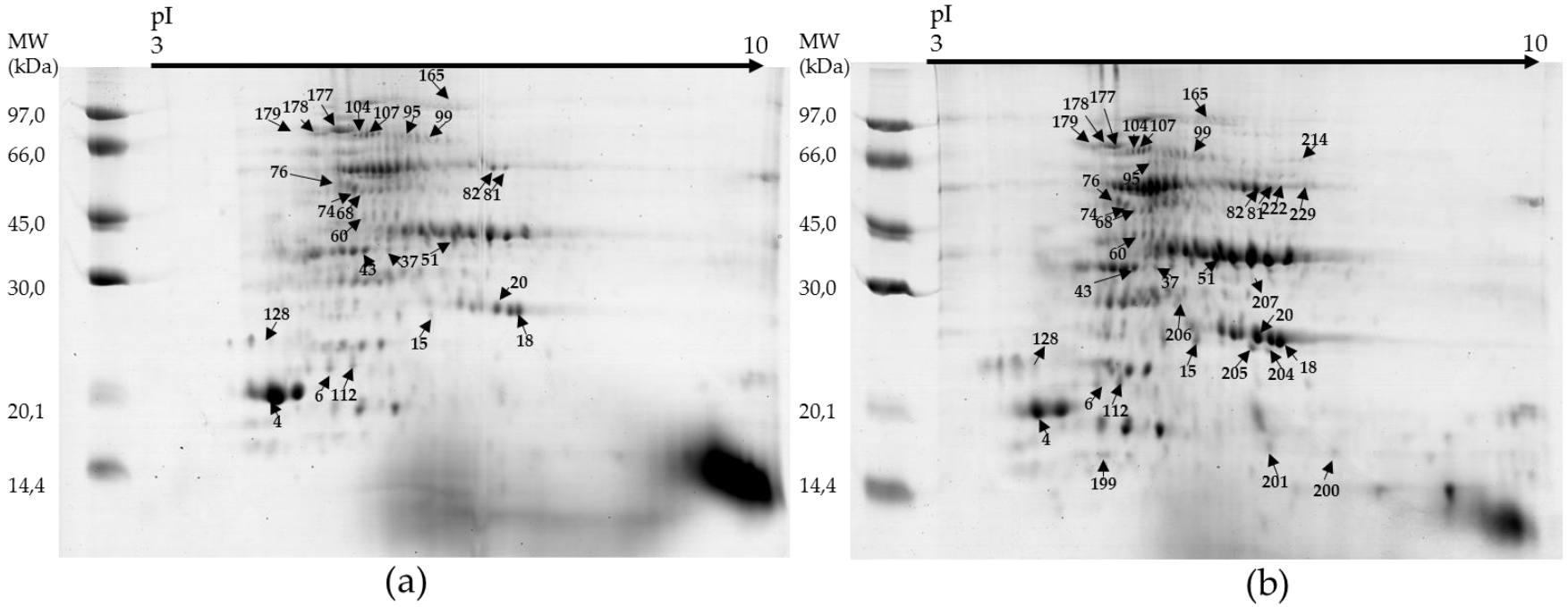

Figure 6. 2D-PAGE from prickly pear *Opuntia stricta* proteins (secondary cladodes) for water deficit control treatment: SS (a) and water deficit and infested treatment: SC (b).

Table 1. Proteins putatively identified from prickly pear *Opuntia stricta* (secondary cladodes) that significantly changed in abundance under conditions irrigated control treatment (HS) and irrigated infested treatment (HC), with designation of category of biological process according to Lohse et al. 2014.

Spot No.[1]	Assignment	Uniprot Access	Identification mode[2]	Experimental Mr/pI	Theoretical Mr/pI[3]	Score (limit) or Confidence[4]	Ratio[5]
Treatment Irrigated and Non-infested (HS)							(C/S)
Photosynthesis							
6	Ribulose bisphosphate carboxylase large chain	Prot1	MSMS/ SearchGUI	20,067.5/4.64	20,974/-	100	4.6
38	Ribulose bisphosphate carboxylase large chain	Prot2	MS/ Mascot	57,405.6/6.88	3,7276/8.13	89 (80)	2.7
39	Ribulose bisphosphate carboxylase large chain	Prot3	MS/ Mascot	57,071.1/6.73	47,2970/6.08	90 (80)	2.3
35	Ribulose bisphosphate carboxylase large chain	Prot4	MS/ Mascot	57,598.0/6.46	34,755/7.83	82 (80)	1.6
Protein							
72	Ycf1	Prot5	MSMS/ SearchGUI	70,707.67/7.09	39,701/-	100	6.2
111	Yeast cadmium factor 1	Prot6	MSMS/ SearchGUI	68,953.83/6.01	36,947/-	100	1.9
52	Arginyl-tRNA-protein transferase	Prot7	MSMS/ SearchGUI	35599.00/5.34	71,149/-	100	1.9
Signaling							
168	Phytochrome C	Prot8	MSMS/ SearchGUI	20,176/4.99	39,410/-	100	exclusive
7	Phytochrome C	Prot9	MSMS/ SearchGUI	20,297.83/4.38	23,577/-	100	2.4
7	Phospho-enol-pyruvate carboxylase	Prot10	MSMS/ SearchGUI	20,297.83/4.38	23,577/-	100	2.4

Lipid Metabolism							
3	Probable fatty acyl-CoA reductase 4	Prot11	MS/ Mascot	36,031.50/6.26	56,467/9.07	55 (54)	1.8
Hormone Metabolism							
69	ACC oxidase (ACO)	Prot12	MSMS/ SearchGUI	69,179.08/7.36	31,723/-	100	2.9
Unclassified							
0	Protein C2-domain ABA-related 7-like isoform X2	Prot13	MS/ Mascot	30,300.17/6.11	15,970/4.72	91 (80)	3.5
1	Uncharacterized protein P0046H03.11	Prot14	MS/ Mascot	19,599.50/6.8	18,622/11.65	72 (65)	1.7
Treatment Irrigated and Infested (HC)							(S/C)
RNA							
145	DNA-directed RNA polymerase subunit α	Prot15	MSMS/ SearchGUI	44,982.67/5.05	37,990/-	100	exclusive
147	DNA-directed RNA polymerase subunit β	Prot16	MSMS/ SearchGUI	53,078.00/5.07	121,368/-	100	exclusive
135	GATA transcription factor 19	Prot17	MS/ Mascot	16,704.00/4.82	29,479/6.14	51 (49)	exclusive
167	DNA-directed RNA polymerase subunit β	Prot18	MS/ Mascot	69,539.33/6.16	12,1611/8.68	62 (54)	exclusive
157	SWI/SNF-regulator chromatin/actin protein	Prot19	MSMS/ SearchGUI	65,610.67/5.07	80,163/-	100	exclusive
136	Maturase K	Prot20	MSMS/ SearchGUI	18,922.33/4.36	42,087/-	100	exclusive
Signaling							
136	Phytochrome C	Prot21	MSMS/ SearchGUI	18,922.33/4.36	23,451/-	100	exclusive

135	Protein enhanced disease resistance 2-like isoform X1	Prot22	MSMS/ SearchGUI	16,704.00/4.82	83,783/-	70.5	exclusive
142	Wall-associated receptor kinase-like 4	Prot23	MS/ Mascot	30,728.67/5.23	86,269/5.66	55 (54)	exclusive
Cell							
146	Actin-3	Prot24	MS/ Mascot	51,670.00/5.26	41,810/5.23	62 (55)	exclusive
160	Villin-3	Prot25	MS/ Mascot	51,849.67/5.16	10,9452/7.51	52 (49)	exclusive
Protein							
93	Protein TIC 214	Prot26	MSMS/ SearchGUI	42413.83/5.07	226,086/-	100	4.3
132	Phosphatidylinositol N-acetylglucosaminyltransferase subunit A (PIG-A)	Prot27	MSMS/ SearchGUI	73,630.33/5.1	50,424/-	100	exclusive
Transport							
57	V-type proton ATPase catalytic subunit A	Prot28	MS/ Mascot	78,815.67/4.93	68,923/5.3	65 (58)	3.4
133	Uncharacterized protein	Prot29	MS/ Mascot	19,164/4.52	58,838/5.94	67 (65)	exclusive
Photosynthesis							
134	NADH dehydrogenase subunit I	Prot30	MSMS/ SearchGUI	16,700.33/4.59	19,577/-	100	exclusive
Lipid Metabolism							
87	Acetyl-CoA carboxylase β subunit	Prot31	MSMS/ SearchGUI	28,939.67/5.45	20,472/-	100	7.1
Amino Acid Metabolism							
123	Probable acylpyruvase FAHD1, mitochondrial	Prot32	MS/ Mascot	40,189.00/5.46	24,325/6.44	55 (54)	8.3
Nucleotide Metabolism							
162	Xanthine dehydrogenase	Prot33	MSMS/ SearchGUI	65,632.33/4.88	17,603/-	100	exclusive
Electron Transport							

144	ATP synthase subunit α, mitochondrial	Prot34	MS/ Mascot	36,076.33/5.42	55,595/6.51	59 (55)	exclusive
TCA ORG Transformation							
161	Malic enzyme	Prot35	MSMS/ SearchGUI	35,258.00/4.44	70,490/-	100	exclusive
Minor CHO Metabolism							
163	Inositol-pentakisphosphate 2-kinase	Prot36	MSMS/ SearchGUI	65,217.33/4.95	53,239/-	100	exclusive
Unclassified							
140	U-box domain-containing protein 73	Prot37	MS/ Mascot	25,162.33/6.63	64,754/5.81	62 (49)	exclusive
143	Uncharacterized protein	Prot38	MS/ Mascot	35922.33/5.61	9,138/4.59	65 (62)	exclusive
12	Neurochondrin X1	Prot39	MSMS/ SearchGUI	26,647.17/6.33	69,787/-	99	1.8
164	Protein TIFY 9	Prot40	MSMS/ SearchGUI	65,216.00/5.01	21,813/-	100	exclusive
137	Omega-amidase, chloroplastic	Prot41	MSMS/ SearchGUI	23389.67/4.9	40,330/-	85	exclusive
141	Uncharacterized protein OSJNBa0084D17.16	Prot42	MS/ Mascot	27000/5.86	39,057/11.05	73 (62)	exclusive

411 [1]The spot number is the index in the reference gel; [2]Mode of spectra obtention and software used; [3]Proteins identified by MSMS/SearchGUI was not provided the pI; [4]Proteins
412 identified by MS/Mascot was provided the score and between parentheses the cut-off from databases search; [5]C/S: Accumulation ratio between non-infested treatment (HS) and
413 infested treatment (HC), both irrigated. S/C: Accumulation ratio between infested treatment (HC) and non-infested treatment (HS), both irrigated.
414

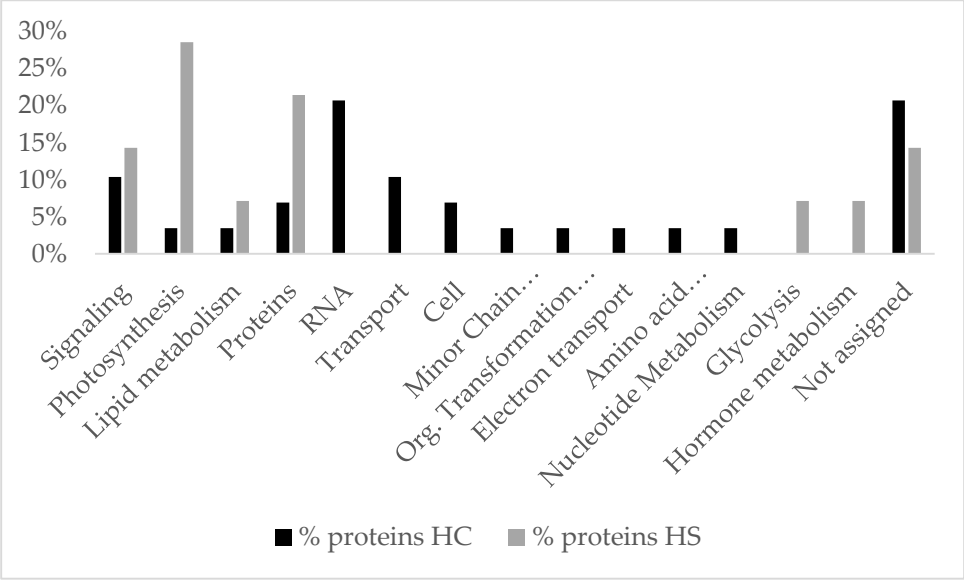

Figure 7. Gene ontology analysis of DAPs for the biological process of from prickly pear *Opuntia stricta* (secondary cladodes) for irrigated (HS) and irrigated and infested treatment (HC), with designation of category of biological process according to Lohse et al.[34].

Table 2. Proteins putatively identified from prickly pear *Opuntia stricta* (secondary cladodes) that significantly changed in abundance under conditions water deficit control treatment (SS) and water deficit and infested treatment (SC), with designation of category of biological process according to Lohse et al. 2014.

Spot No.[1]	Assignment	Uniprot Access	Identification mode[2]	Experimental Mr/pI	Theoretical Mr/pI[3]	Score (limit) or Confidence[4]	Ratio[5]
Treatment Water Deficit and Non-infested (SS)							**C/S**
Glycolysis							
74	Phosphoenolpyruvate carboxylase	Prot43	MSMS/ SearchGUI	53,976/5.11	28,294/-	100	1.5
60	Phosphoenolpyruvate carboxylase	Prot44	MSMS/ SearchGUI	45,824/5.2	23,577/-	100	1.5
104	Phosphoenolpyruvate carboxylase	Prot45	MSMS/ SearchGUI	76,528.67/5.19	45,887/-	100	2.0
RNA							
112	Maturase K	Prot46	MSMS/ SearchGUI	22,378.67/5.04	42,398/-	100	1.7
165	GATA transcription factor 19	Prot47	MS/ Mascot	102,303.33/6.13	29,487/6.14	53 (49)	3.1
Amino Acid Metabolism							
4	Delta 1-pyrroline-5-carboxylate synthetase	Prot48	MSMS/ SearchGUI	20,274.33/4.22	50,289/-	100	3.7
Signaling							
37	Phytochrome C	Prot49	MSMS/ SearchGUI	37,048/5.46	23,451/-	100	1.6
99	Phytochrome C	Prot50	MSMS/ SearchGUI	74,146.33/5.93	23,451/-	100	3.0
112	Phytochrome C	Prot51	MSMS/ SearchGUI	22,378.67/112	35,038/-	100	1.7
Hormone Metabolism							
6	Ethylene-overproduction protein 1 (ETO1)	Prot52	MSMS/ SearchGUI	22,265.67/4.79	112,942/-	99	1.8
Protein							
95	Calmodulin-binding receptor-like	Prot53	MS/ Mascot	68,251/5.38	46,378/9.26	58 (54)	1.7

	cytoplasmic kinase 2						
Cell Wall							
107	Pollen-specific leucine-rich repeat extensin-like protein 1	Prot54	MSMS/ SearchGUI	76,904/5.25	102,823/-	56	1.7
Lipid Metabolism							
51	Sterol 3-beta-glucosyltransferase UGT80A2-like	Prot55	MSMS/ SearchGUI	40,103/6.22	68,310/-	78	1.5
Not assigned							
43	TITAN-like protein	Prot56	MSMS/ SearchGUI	36,780/5.20	48,170/-	89	1.7
Treatment Water Deficit and Infested (SC)							**S/C**
Protein							
82	Ribosomal protein S4	Prot57	MSMS/ SearchGUI	55,610.67/6.65	21,709/-	100	1.6
15	Arginyl-tRNA-protein transferase	Prot58	MSMS/ SearchGUI	25,660.67/5.91	71,149/-	100	2.5
199	F-box protein At1g78100	Prot59	MSMS/ SearchGUI	16,992.67/4.83	36,553/-	100	exclusive
222	Putative U-box domain-containing protein 58	Prot60	MSMS/ SearchGUI	56,291.33/6.93	49,531/-	100	exclusive
200	Sec14p-like phosphatidylinositol transfer family protein	Prot61	MSMS/ SearchGUI	17,266/7.54	28,994/-	100	exclusive
RNA							
81	GATA transcription factor 19	Prot62	MS/ Mascot	55,729.33/6.8	29,487/6.14	58 (49)	1.5
128	Maturase K	Prot63	MSMS/ SearchGUI	23,584/4.13	54,107/-	100	6.0
REDOX							
205	Glutaredoxin-C13	Prot64	MS/ Mascot	24,811.67/6.6	11,583/8.57	54 (49)	exclusive
Phosphate Transport							
15	Uncharacterized protein	Prot65	MSMS/ SearchGUI	25,660.67/5.92	58,382/-	80	2.5

C1 Metabolism							
18	Protein arginine N-methyltransferase 1.5	Prot66	MSMS/ SearchGUI	25,431/6.93	71,870/-	79	1.7
Cell Division							
20	Cyclin-dependent kinase B1-1	Prot67	MS/ Mascot	25,756/6.66	34,754/8.26	58 (49)	1.5

[1]The spot number is the index in the reference gel; [2]Mode of spectra obtention and software used; [3]Proteins identified by MSMS/SearchGUI was not provided the pI; [4]Proteins identified by MS/Mascot was provided the score and between parentheses the cut-off from databases search; [5]C/S: Accumulation ratio between non-infested treatment (SS) and infested treatment (SC), both under water deficit. S/C: Accumulation ratio between infested treatment (SC) and non-infested treatment (SS), both under water deficit.

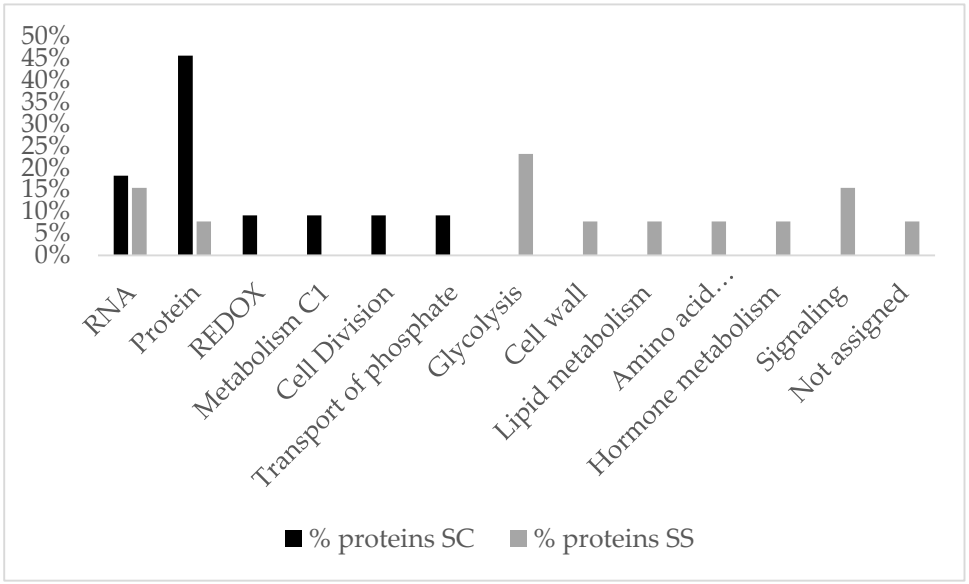

Figure 8. Gene ontology analysis of DAPs for the biological process of from prickly pear *Opuntia stricta* (secondary cladodes) for water deficit treatment (SS) and water deficit and infested treatment (SC), with designation of category of biological process according to Lohse et al. (2014).

spot 135, in HC; spot 165, in SS; and Maturase K, spot 128 in SC, spot 112 in SS and spot 136 in HC). They probably represent isoforms and indicate high positive regulation of specific genes responding to each stress condition.

GATA19 transcription factor contain TIFY and CCT domains and characteristic zinc finger region. TIFY domain belongs to TIFY family transcriptions factors plant-specific [66], which have been found in the JASMONATE ZIM-domain (JAZ) protein family, involved in jasmonic acid driven processes [66–69]. Protein GATA19 in prickly pear infested may be specifically associated to the induction of genes of biotic response in prickly pear, as seen in *Nicotiana tabacum* activating wound-responsive genes [70] and also in chickpea [71].

The SWI/SNF-regulator chromatin/actin protein (spot 157) was only detected in infested irrigated plants (HC), also may be involved in a host-mediated gene regulation towards pest insect control. It is involved in transcription regulation, altering interactions between histone octamers and DNA using energy derived from hydrolysis of ATP. Subunits of this complex has been found with significant role over biotic stress response genes, associated to JA- and SA-dependent [72,73].

Among proteins differentially accumulated with similar role in both comparisons under the two water conditions evaluated (irrigated: HS *versus* HC; water deficit: SS *versus* SC), there were those related to oxidative stress, phosphate regulation and ubiquitination.

To deal with the ROS accumulation, infested irrigated plants (HC) showed abundance of xanthine dehydrogenase, XDH (spot 162), from category of nucleotide metabolism. XDH catalyzes oxidation of hypoxanthine and xanthine to uric acid. Uric acid eliminating H_2O_2 was already seen contributing to the acclimation of *A. thaliana* to drought [74] and, an

inhibition of xanthine oxidase, an interconvertible form of XDH, also associated with ROS accumulation under aphids infestation in wheat, and when it is inhibited abolishes downstream defense responses, peroxidase and chitinase activities in resistant wheat strains [75]. The accumulation of XDH under cactus scale pest attack in prickly pear may be associated to the role of ROS scavenger, through its metabolite uric acid, which yet may be a nitrogen source to biosynthesis of defensive compounds.

In relation to treatments under water deficit, the infested treatment (SC), presented glutarredoxin (GRX) C13 (spot 205) exclusively in this treatment, classified in the biological process of redox metabolism. GRX can regulate the activity of proteins by reversible glutathionation or reduced disulfide bonds in dehydroascorbate, peroxiredoxin and methionine sulfoxide reductase, contributing to the removal of ROS and the repair of oxidative damage in macromolecules [76,77]. The active site type-CC present in protein GRX identified, has been associated with the reduction of sensitivity to the stress-related hormones and auxin, and increased tolerance to various abiotic stresses, such as oxidative, osmotic and saline [78]. This protein may be responding to the two stress factors, since both can lead to the accumulation of ROS by decreased water availability as well as a consequence of pest attack.

In the two comparisons, the hormone metabolism category presented proteins related to ethylene hormone, with accumulation of ethylene-overproduction protein 1 (ETO1) (spot 6), on drought non infested treatment (SS), that act on stability regulation of enzymes from ethylene biosynthesis [79,80]. While in treatment irrigated non infested (HS), there was accumulation of ACC oxidase (ACO) (spot 69), which catalyzes one of the final step on ethylene biosynthesis [81]. These results reveal the relevance of ethylene as a hub in any water conditions of prickly pear responding to cactus scale, as also seen in maize resistance to aphid [82]. More researches are necessary to understand if in drought condition (SS), ETO1 is an allele that promotes less or more ethylene accumulation. Less ethylene accumulation on SS treatment, if ETO1 was acting in degradation of enzyme involved in ethylene biosynthesis, may be justified by the fact that amino acid methionine is a precursor to ethylene was reduced, once protein levels were more reduced in SS treatment (Figure 2c), but lightly increased at SC treatment. While in irrigated infested treatment (HC), less ethylene may be important in avoidance of cell wall softening, to prevent access of the insect.

Cactus scale infestation seems affect the presence of phosphoenolpyruvate carboxylase (PEPC) (glycolysis category), a primary catalyst for atmospheric CO_2 fixation in CAM and C4 plants, that was more accumulated in controls treatments irrigated and non-irrigated (spot 7, HS, comparison 1 and spots 60, 74 e 104, SS, comparison 2), or yet as a strategy to diversion energy from growth metabolism to defense. Falcão et al. (2013) also observed a prickly pear variety with PEPC activity compromised for 12 months of field evaluation under carmine cochineal infestation, nevertheless presenting greater accumulation of soluble organic acids, carbohydrates and sugar, although with lower dry biomass, suggesting plant ability to bypass less PEPC accumulation or activity, and yet directing its products to the production of secondary metabolites deterrent for the insect.

Interestingly, at the same time, it was observed that the plant may maintain its performance, at least in the irrigated infested treatment (HC), with the accumulation of malic

enzyme (spot 161) (TCA category). This occurrence is probably by decarboxylating of accumulated malate and stored in vacuoles by PEPC during plant life prior to infestation or from biosynthesis by other sources. Malate decarboxylation by malic enzyme yields pyruvate, CO_2 and NADPH, products that may be involved in plant protection by different ways: to uptake of reducing power, by NADPH, to maintain processes with energy demand increased under stress; flavonoid and lignin biosynthesis or providing pyruvate to mitochondrial respiration to obtain ATP; or yet ROS synthesis to kill or damage the pest [84,85]. Also, non-photosynthetic isoforms from malic enzyme seems to function in plant defense responses, being accumulated under various stressing factors like salinity, wounding, radiation, fungal and nematode elicitors [84–87]. According to Buchanan et al. (2015), malate is the more effective substrate to fatty acid biosynthesis for membrane repair, through supplying of pyruvate that is decarboxylated into acetyl CoA, the substrate for that process. So, other hypothesis may be the accumulation of malic enzyme to supply membrane repair and other defense–related mechanisms to strike back cactus scale pest. It would be necessary a future study to know which isoform refers the one accumulated in this condition.

The infested and irrigated treatment (HC) has less carbon assimilation due to the lower accumulation of ribulose-1,5-bisphosphate carboxylase large chain (spots 6, 35, 38 and 39, in HS control irrigated treatment), component of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), probably indicating the use of nitrogen reserves from Rubisco, with longer turnover time [88], to defensive compounds. Despite this, infested and irrigated plants (HC) showed greater abundance of proteins related to energy intake, not present in the infested under dry condition (SC), in categories photosynthesis (with NADH dehydrogenase subunit I, spot 134), electron transport (mitochondrial ATP synthase α subunit, spot 144) and TCA (malic enzyme, spot 161). This seemed to give greater support to plants infested under irrigation to express proteins associated with the most effective biological processes against the cactus scale, which were not present in plants under water deficit, such as signaling, cell organization, lower chain carbohydrate metabolism and lipid metabolism, shown below.

From three proteins related to signaling (phytochrome C, enhanced disease resistance isoform X1 like-2 protein, wall-associated receptor kinase-like 4), the last two stand out. The enhanced disease resistance (EDR) isoform X1 like-2 protein (spot 135), induced exclusively in irrigated and infested treatment (HC), presents PH (putative pleckstrin homology) and START (steroidogenic acute regulatory protein-related lipid-transfer) domains, whose functions allows predicting that this protein may be connecting lipid signaling, mitochondria activity and the activation of programmed cell death processes as a hypersensitive reaction [89]. In plants, EDR has been reported accumulated in cotton under the sucking-pest whitefly [90] and conferring resistance to pathogenic fungi, in some cases in a response depending on SA, and promoting increased expression of defense genes (like PR-1) faster than in wild species [91,92]. Although some examples concern to pathogens, the gene induction pattern by sucking insects can be compared to that, because of their similar mode of action regarding toxins inoculation [93–95].

The wall-associated receptor kinase-like 4 protein, WAKL4 (spot 142), exclusively identified in HC treatment, belongs to RKL family (receptor kinase-like proteins), that

participates in several signaling processes in plants among which response to phytohormones, development regulation and biotic stressing factors, as mechanical injury. As a transmembrane protein, with an amino-terminal extracellular ligand-binding region, may contain various motifs to recognize variations in extracellular region [96,97]. This feature made WAKL4 a good candidate to cell wall receptor to link cytoplasm to diverse array of ligands, as already observed for mineral nutrients [98] or pathogens-derived elicitors, co-expressed with well characterized pathogen defense related genes [99].

Two proteins related to category *cell*, cytoskeleton organization were accumulated only on HC treatment. Villin 3 (spot 160), based on phylogenetic analyzes and conservation of Ca^{2+} binding sites, it is capable of breaking and aggregating actin filaments leading to the beam rotation [100]; and actin 3 (spot 14), higher-order cytoskeletal structures crucial for the maintenance of cell architecture and expansion, groups in filament bundles generated from individual actin filaments by the actions of aggregating proteins, such as villins [100]. The role of cytoskeleton actin in plant innate response against bacteria and fungi is associated with changes in cytoskeletal architecture during immune response, being considered a new conserved component of pattern-triggered immunity (PTI), through the polymerization that is required to increase actin density as a component for tolerance to pathogenic bacteria [101,102]. Studies has demonstrated that enzymes like actin depolymerizing factor (ADF) in both plants and insects are involved in actin polymerization, in some cases in plants increasing resistance to fungus through JA and SA signaling pathways [103]. Also, it was found that actin-binding and depolymerizing enzymes in aphid saliva may preventing phloem obstructions [104]. The higher abundance of villin 3 and actin 3 on infested prickly pear may be associated with the insect feeding apparatus insertion into the plant, promoting alteration of cytoskeleton by a specific herbivory-associated molecular pattern (HAMP), possibly through the involvement of ADF-like proteins which are known to act negatively over sap-sucking pests feeding efficiency [105], or yet by action of enzymes present in insect saliva over plant defense responses requiring actin polymerization.

Regarding to *lipid metabolism*, in control treatment HS, a putative fatty acyl-CoA reductase 4 (FAR4, spot 3) was almost twice more accumulated compared to infested treatment HC. This protein is involved in wax biosynthesis and can also be associated to suberin, a protective hydrophobic barrier [106]. During fungal infection, it has been observed that cuticle may reduce plant-pathogen recognition, and cuticular defects can enhance resistance to fungi [107]. The same might be happening with prickly pear under cactus scale attack, where cuticle thickness or structural dynamics may have a negative direct effect over the insect, by releasing cuticle-derived fragments that act as signaling molecules, recognized by the pest but also by the plant itself, who starts directing regulation from cuticle synthesis to defense compounds production. In counterpoint, also in this category, acetyl-CoA carboxylase (ACCase) β subunit (spot 87) was almost twice more accumulated in infested irrigated plants (HC). ACCases are present in plastids and in cytosol with a regulatory function: they catalyze the carboxylation of acetyl-CoA to malonyl-CoA, as the main substrate for the acetate-malonate pathway that leads to secondary metabolites and fatty acids biosynthesis and participate in shikimic acid, pentose and acetate-mevalonate pathways, for the synthesis of steroid, phenol, glycoside and [108–110]. Induction of these compounds has

been found during aphid feeding [111], then prickly pear may be synthesizing ACCase to defense modulation, generating compounds associated to membrane composition and defensive compounds.

The category of *minor carbohydrate metabolism* was represented by inositol-pentakisphosphate 2-kinase (IPK2) (spot 163), accumulated only in the infested irrigated treatment (HC). This protein does transference ATP-depending of phosphate groups in synthesis of phytic acid (IP6) [112], which here may be acting as a deterrent compound to cactus scale. In addition, it has roles associated with inorganic phosphate homeostasis [113] and may have a direct role in prickly pear infested as one of the mediators of JA-signaling, being a co-factor in a complex formed by coronatine insensitive 1 (COI1-JAZ) to induce degradation of transcriptional repressor JAZ proteins, seen in another species, through inositol polyphosphates $InsP_5$ and $InsP_8$, once JAZ act under defense genes JA-responsive [114–116].

Present as response to cactus scale in both comparisons, it was the *protein* category as one of the most represented class, especially in infested water deficit treatment (SC). In irrigated infested treatment TIC 214 (spot 93) and phosphatidylinositol N-acetylglucosaminyltransferase subunit A, PIG-A (spot 132) were more abundant. TIC proteins, translocons of the inner envelope of chloroplasts, are part of the chloroplast import complex, belong to the translocase family. Besides being a selective barrier for transport of many metabolites, controlling protein homeostasis, they can also support certain metabolic processes, as tocopherol, chlorophyll, carotenoid or lipid biosynthesis [117]. Thus, TIC 214 can be more directly associated with response mechanisms against cactus scale through the biosynthesis of these compounds, mostly involved in ROS scavenging and maybe some photosystem or membrane recompositing.

Phosphatidylinositol N-acetylglucosaminyltransferase subunit A (PIG-A) (spot 132) is designated into protein posttranslational modification subcategory. It is part of an enzyme that transfers N-acetylglucosamine to phosphatidylinositol in the first step of the glycosylphosphatidylinositol (GPI)-anchor synthesis. The subunit of this enzyme may be contributing in a wide way to defense, in which glycolipids or proteins that are anchored to the GPI are involved, such as maintaining the cuticular wax, in order to make the structure of its cuticular layer less diffuse [118]; or attached to the exterior surface of the plasma membrane participating into cell-to-cell interaction [119], in cell wall in secretory pathways or altering its composition increasing pectin levels [120,121] or even long-distance interactions by cleavage or releasing from plasma [119,122].

In the water deficit infested treatment (SC), among the accumulated proteins, four were highlighted. The sec14p-like phosphatidylinositol transfer family protein (spot 200), classified in a subdivision of targeting of secretory pathways, regulating signaling interfaces between lipid metabolism and membrane trafficking. It has a CRAL-TRIO domain that can be involved in lipid transport (to one domain) or more complex functions in signal transduction, transport and organelle biology, integrating lipid metabolism with other biochemical processes (to multidomains) [123]. Its silencing is associated to reduction in JA level, JA-dependent defense against pathogen and phospholipid turnover [124]. Probably, this protein was involved in trafficking of phosphoinositide for specific signaling purposes,

according signals from JA. Proteins related to phosphoinositide, for both infested treatments irrigated as or dry, such as PIG-A, IPK2, sec14p-like phosphatidylinositol, were accumulated, indicating that phospholipid-based signaling cascades are well conserved in prickly pear immune response to combat cactus scale insect.

Still in SC treatment, there were three proteins specifically targeted for degradation. F-box (spot 199) and U-box domain-containing protein 58 (spot 222) are components of one of the major E3 ubiquitin ligase, the SCF complex. This complex mediates ubiquitination and subsequent proteasomal degradation of target proteins, then it is involved in molecular networks to post-translational regulation, including response to biotic and abiotic stresses [125]. According to the carboxyl-terminus of F-box proteins, different substrates are recognized, and yet these motifs can mediate some protein-protein interactions, besides they have been associated with plant hormone responses, like JA and SA [126,127]. Under cactus scale infestation, these proteins may be directly related to a response associated to the recognizing of the insect, as seen in other plants, when a F-box protein is required for many JA-dependent responses, as insect attack and wounding, through COI1 complex [114,115,128]. Other studies in order to know its targets in this interaction plant-insect will be relevant.

Other protein that can be related to protein degradation is arginyl-tRNA-protein transferase (spot 15), that was accumulated almost twice in water deficit and infested treatment (SC). It is involved in the post-translational conjugation of arginine to the N-terminal aspartate or glutamate of a given protein. This arginylation is required for degradation via the ubiquitin pathway, through the identity of the amino-terminal residue of specific protein substrates [129,130]. This protein was also accumulated in irrigated control treatment (spot 52), and may be an isoform from alternative splicing, that results in differential expression, activity, and localization (Kwon et al. 1999). Then these isoforms, accumulated in such different conditions, reflect targeting to degradation of different proteins.

Similar to that observed in SC, in HC treatment there was accumulation of protein associated with degradation, protein 73 with U-box domain (spot140), but it was classified by GO as *not assigned*. However according domains and motifs and other studies it is possible infer its role. It has ubiquitin E3 protein ligase activity in vitro [131]. Depending on the gene to which it is directed, this protein can act as positive or negative regulator [67,128,132].

Even in different water regimes, proteins that interact in response to cactus scale infestation, may be explaining successful infestation. Treatment under water deficit infested (SC) presented protein associated with cell division, the cyclin-dependent kinase B1-1 (CBK1;1) (spot 20), 50% more accumulated. Usually, this protein is found less accumulated in stress conditions when plants tend to drive their metabolism from growth and development to defense [133,134].

On the other hand, in irrigated infested treatment (HC), it was found the TIFY9 or JAZ10 (spot 164), that contains a ZIM-jasmonate (JAZ) domain. This domain is part of the TIFY family, sharing a conserved TIFY3G sequence within of ZIM motif and harboring proteins JAZ [66]. JAZ proteins are transcription repressors of JA-responsive genes involved in

several biological processes, being reported as highly expressed in response to wounding and herbivory, and also interacting with genes involved with ABA and GA [67,68,128,132,135] [90]. Not surprisingly their degradation is crucial to activating genes defense [128], although its absence prevents the activation of these genes [67]. Thus, its presence may be a consequence of slow turnover of its complex of degradation, COI1 complex, which depends on specific inositol polyphosphates (InsP) as cofactors [114,136]. In some stress responses, not all InsP are suitable as cofactor to COI [115]. Here IPK2 that catalyzes $InsP_6$ was also found accumulated in prickly pear infested treatments, if it is involved with COI, it may have had a lower efficiency. In addition, other studies identified JAZ-isoforms with lack of Jas domain highly resistant to JA-induced degradation [69].

Under infested conditions, the accumulated presence of JAZ proteins, as well as proteins related to favoring cell division, indicate possible interaction between JAZ and intermediaries that induce plant expansion, such as DELLA proteins. In addition, JAZ also suppress ethylene-related transcription factors, a hormone whose pathway also indicated inhibition in irrigated infested conditions [137]. It is noticed that the metabolic pattern of this variety of prickly pear, through such JAZ proteins, may be favoring the proliferation of the insect, by improving its nutrition by favoring cell division.

Differences in reorganization of functional categories of proteins accumulated under infestation between irrigated (13 categories) and dry (7 categories) treatments revealed that plant uses different mechanisms to circumvent the cactus scale attack when under combined stresses (biotic and abiotic). Although prickly pear is intrinsically tolerant to water deficit, under exposition to the experimental condition the plant showed a visible weakening, for example in relation to the cladodes thickness (not shown data) and the RWC.

When under pest attack and water deficit treatment, the plant presented proteins related to redox and cell division, categories that were not present in irrigated and infested treatment, revealing a more intensive action to combat oxidative stress, but also a possible attempt of manipulate the plant metabolism by the insect, in order to increase its supply feeding. As a common response for both situations, water deficit and irrigated infested, however in different categories, prickly pear invested in accumulation of protein involved in ROS scavenging, protein ubiquitination and post translational modifications. In irrigated treatment, plant seems to have more conditions to combat cactus scale, through specific proteins linked to lipid, minor carbohydrate and nucleotide metabolisms, cell organization, organic acid transformation, signaling and photosynthesis. In this condition prickly pear placing as secondary some processes like wax and ethylene biosynthesis, directing its metabolism and physiology towards regulation controlled by specific transcription factors, probably more associated with genes of defense; energy production and the biosynthesis of fatty acids, phytates and secondary metabolites.

Noteworthy, the cultivar used in this study is not considered resistant against cactus scale (according literature, none commercially available cultivar in Brazil and world has this feature), so the general proteome response observed here reflects possible plant molecular and physiological attempts to tolerate the pest action when well-hydrated. From the molecular mechanisms seen here, as well as their key components, it’s possible conclude that water deficit seems to favor the infestation, but it is possible comprise a set of potential

candidates for regulatory or effector agents towards the resistance against this pest, specially that observed in irrigated condition, when the plant were more responsive to one stress.

The proteins herein identified in response do cactus scale open ways to deep investigations to better understanding to this interaction, as focusing in how they can be influenced by phytohormones biotic-responsive, revealing crosstalk in signaling pathways, through other molecular techniques, e.g. microarray; if the protein are more or less accumulated as a consequence of infestation, or as a direct plant response, through transcriptomic, for example; visualize the performance of insect under both conditions over time; detect which isoform is present. Yet, one of contributions through the biomarkers candidates observed here, it will be possible screening other prickly pear varieties that present these proteins early under cactus scale infestation.

Author Contributions: T.C.J. and M.D.S.C. conceived and designed the experiments. M.D.S.C., E.P.N.P., J.P.O. and D.C.S. performed the experiments in greenhouse. M.D.S.C., E.P.N.P., M.S.O., F.A.C.S and T.C.J. performed the experiments on proteomics, data analysis and prepared the figures and tables. A.C.L.N., J.P.O., D.C.S., and T.C.J. provided plant and insect material. M.D.S.C., E.P.N.P., M.S.O., F.A.C.S. and T.C.J. helped on the analysis and interpretation of protein data. M.D.S.C. and T.C.J. wrote the original draft. M.D.S.C., E.P.N.P., M.S.O., F.A.C.S, A.C.L.N., J.P.O., D.C.S. and T.C.J. reviewed and edited the paper. All authors read and approved the final manuscript.

Conflicts of interest: The authors declare no conflicts of interest.

Acknowledgements: Authors are grateful to Agronomical Institute of Pernambuco (IPA) for providing the experimental station and all the support to install the experiment. We particularly thank to Dr. Rodrigo Leandro Braga de Castro Coitinho and Dr. Antônio Felix da Costa for providing cactus scale-infested samples.

References

[1] G. Onaga, K. Wydra, Advances in Plant Tolerance to Biotic Stresses, in: Plant Genomics, 2017: pp. 229–272. https://doi.org/dx.doi.org/10.5772/64351.

[2] C. Sáenz, Opuntias as a natural resource, in: C. Saenz, H. EBerger, A. Rodríguez-Félix, L. Galetti, E. Sepúlveda, M.T. Varnero, V. García de Cortázar, R. Cuevas García, E. Arias, C. Mondragón, I. Higuera, C. Rosell (Eds.), Agro-Industrial Util. Cactus Pear, FAO, Rome, 2013: p. 168. www.fao.org/publications.

[3] P. Inglese, Cactus pear Opuntia ficus-indica L. (Mill.) for fruit production: an overview, Cactus Net Newsletter, Mekelle (Ethiopia), 2010.

[4] E.B. Lopes, Palma forrageira: cultivo, uso atual e perspectivas de utilização no semiárido nordestino, EMEPA/FAEPA, João Pessoa, 2007.

[5] J.F. Warumby, G.P. Arruda Filho, V.A.L.B. Cavalcanti, Pragas da palma, in: R.S.C.

762 Menezes, D.A. Simões, E.V.S.B. Sampaio (Eds.), A Palma No Nord. Do Bras., 2005:
763 pp. 65–80.

764 [6] L.E. Claps, M.E. de Haro, Coccoidea (Insecta: Hemiptera) associated with Cactaceae
765 in Argentina, J. Prof. Assoc. Cactus Dev. 4 (2001) 77–83.

766 [7] G.P. Arruda, Aspectos etológicos da cochonilha da palma forrageira Diaspis
767 echinocacti (Bouche, 1833) (Homoptera, Diaspididae), Universidade Federal Rural de
768 Pernambuco, 1983.

769 [8] S. Longo, C. Rapisarda, Pragas da Palma Forrageira, in: G. BARBERA, P. INGLESE,
770 E.P. BARRIOS (Eds.), Agroecol. Cultiv. e Usos Da Palma Forrageira,
771 SEBRAE-PB/FAO, João Pessoa, 2001: p. 241.

772 [9] G.P. Arruda Filho, G.P. Arruda, Manejo integrado da cochonilha Diaspis echinocacti
773 praga da palma forrageira em Brasil, Manejo Integr. Plagas y Agroecol. (2002) i–iv.

774 [10] E. Stahl, O. Hilfiker, P. Reymond, Plant–arthropod interactions: who is the winner?,
775 Plant J. 93 (2018) 703–728. https://doi.org/10.1111/tpj.13773.

776 [11] J.S. Bale, K.L. Ponder, J. Pritchard, Coping with stress, CABI Publishing, 2007.
777 https://doi.org/10.1079/9780851998190.0001.

778 [12] B.B. Buchanan, W. Gruissem, R.L. Jones, Biochemistry & molecular biology of
779 plants, 2nd ed., American Society of Plant Physiologists, Willey Blackwell, Rockville,
780 Md., 2015.

781 [13] A. Barnes, J. Bale, C. Constantinidou, P. Ashton, A. Jones, J. Pritchard, Determining
782 protein identity from sieve element sap in Ricinus communis L. by quadrupole time of
783 flight (Q-TOF) mass spectrometry, J. Exp. Bot. 55 (2004) 1473–1481.
784 https://doi.org/10.1093/jxb/erh161.

785 [14] S. Kumar, M. Kanakachari, D. Gurusamy, K. Kumar, P. Narayanasamy, P. Kethireddy
786 Venkata, A. Solanke, S. Gamanagatti, V. Hiremath, I.S. Katageri, S. Leelavathi, P.A.
787 Kumar, V.S. Reddy, Genome-wide transcriptomic and proteomic analyses of
788 bollworm-infested developing cotton bolls revealed the genes and pathways involved
789 in the insect pest defence mechanism, Plant Biotechnol. J. 14 (2016) 1438–1455.
790 https://doi.org/10.1111/pbi.12508.

791 [15] J.S. Sangha, H.C. Yolanda, J. Kaur, W. Khan, Z. Abduljaleel, M.S. Alanazi, A. Mills,
792 C.B. Adalla, J. Bennett, B. Prithiviraj, G.C. Jahn, H. Leung, Proteome analysis of rice
793 (Oryza sativa L.) mutants reveals differentially induced proteins during brown
794 planthopper (Nilaparvata lugens) infestation, Int. J. Mol. Sci. 14 (2013) 3921–3945.
795 https://doi.org/10.3390/ijms14023921.

796 [16] S. Hoffmann-Benning, D.A. Gage, L. McIntosh, H. Kende, J.A.D. Zeevaart,
797 Comparison of peptides in the phloem sap of flowering and non-flowering Perilla and
798 lupine plants using microbore HPLC followed by matrix-assisted laser
799 desorption/ionization time-of-flight mass spectrometry, Planta. 216 (2002) 140–147.
800 https://doi.org/10.1007/s00425-002-0916-0.

[17] H.D. Barrs, P.E. Weatherley, A re-examination of the relative turgidity technique for estimating water deficits in leaves, Aust. J. Bbiological Sci. 15 (1962) 413–428. https://doi.org/10.1071/bi9620413.

[18] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric Method for Determination of Sugars and Related Substances, Anal. Chem. 28 (1956) 350–356. https://doi.org/10.1021/ac60111a017.

[19] M.M. Bradford, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, Anal. Biochem. 72 (1976) 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

[20] B.Y.S. Moore, H. Stein, Photometric ninhydrin method for use in the chromatography of amino acids, (1948).

[21] L.S. Bates, Rapid determination of free proline for water - stress studies, Plant Soil. 39 (1973) 205–207.

[22] H.K. Lichtenthaler, Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes, Methods Enzymol. 148 (1987) 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

[23] V. Alexieva, I. Sergiev, S. Mapelli, E. Karanov, The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat, Plant, Cell Environ. 24 (2001) 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

[24] R. Heath, L. Packer, Photoperoxidation in isolated Chloroplasts of Fatty Acid Peroxidation chlorophyll, Arch. Biochem. Biophisics. 126 (1968) 189–198.

[25] E.A. Havir, N.A. McHale, Biochemical and Developmental Characterization of Multiple Forms of Catalase in Tobacco Leaves, Plant Physiol. 84 (1987) 450–455. https://doi.org/10.1104/pp.84.2.450.

[26] R.A. Azevedo, R.M. Alas, R.J. Smith, P.J. Lea, Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and root of wild-type and a catalase-deficient mutant of barley, Physiol. Plant. (1998) 280–292.

[27] Y. Nakano, K. Asada, Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts, Plant Cell Physiol. 22 (1981) 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

[28] C.N. Giannopolotis, S.K. Ries, Superoxide Dismutases, Plant Physiol. (1977) 309–314. https://doi.org/dx.doi.org/10.1104/pp.59.2.309.

[29] X. Wu, E. Xiong, W. Wang, M. Scali, M. Cresti, Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis, Nat. Protoc. 9 (2014) 362–374. https://doi.org/10.1038/nprot.2014.022.

[30] G. Candiano, M. Bruschi, L. Musante, L. Santucci, G.M. Ghiggeri, B. Carnemolla, P.

839 Orecchia, L. Zardi, P.G. Righetti, Blue silver: A very sensitive colloidal Coomassie
840 G-250 staining for proteome analysis, Electrophoresis. 25 (2004) 1327–1333.
841 https://doi.org/10.1002/elps.200305844.

842 [31] J. Webster, D. Oxley, Peptide mass fingerprinting: protein identification using
843 MALDI-TOF mass spectrometry, Methods Mol Biol. 310 (2005) 227–240.

844 [32] M. Vaudel, H. Barsnes, F.S. Berven, A. Sickmann, L. Martens, SearchGUI: An
845 open-source graphical user interface for simultaneous OMSSA and X!Tandem
846 searches, Proteomics. 11 (2011) 996–999. https://doi.org/10.1002/pmic.201000595.

847 [33] M. Vaudel, J.M. Burkhart, R.P. Zahedi, E. Oveland, F.S. Berven, A. Sickmann, L.
848 Martens, H. Barsnes, PeptideShaker enables reanalysis of MS-derived proteomics
849 data sets: To the editor, Nat. Biotechnol. 33 (2015) 22–24.
850 https://doi.org/10.1038/nbt.3109.

851 [34] M. Lohse, A. Nagel, T. Herter, P. May, M. Schroda, R. Zrenner, T. Tohge, A.R.
852 Fernie, M. Stitt, B. Usadel, Mercator: A fast and simple web server for genome scale
853 functional annotation of plant sequence data, Plant, Cell Environ. 37 (2014) 1250–
854 1258. https://doi.org/10.1111/pce.12231.

855 [35] B. Gutbrodt, S. Dorn, K. Mody, Drought stress affects constitutive but not induced
856 herbivore resistance in apple plants, Arthropod. Plant. Interact. 6 (2012) 171–179.
857 https://doi.org/10.1007/s11829-011-9173-0.

858 [36] R.I. Ventura-Aguilar, E. Bosquez-Molina, S. Bautista-Baños, F. Rivera-Cabrera,
859 Cactus stem (Opuntia ficus-indica Mill): anatomy, physiology and chemical
860 composition with emphasis on its biofunctional properties, J. Sci. Food Agric. 97
861 (2017) 5065–5073. https://doi.org/10.1002/jsfa.8493.

862 [37] T.C. Hsiao, Plant Responses To, Ann Rev. Plant Pysiol. 24 (1973) 519–570.
863 https://doi.org/10.1016/j.bmcl.2011.09.127.

864 [38] Z. Zlatev, F. Lidon, Effects of water deficit on plant growth, water relations and
865 photosynthesis, Biol. Veg. e Agro-Industrial. 31 (2005) 235–252.

866 [39] S.Y.S. Lisar, R. Motafakkerazad, M.M. Hossain, I.M.M. Rahman, Water Stress in
867 Plants: Causes, Effects and Responses, in: I.M.M. Rahman, H. Hasegawa (Eds.),
868 Water Stress, IntechOpen, Rijeka, Croatia, 2012: p. 310.
869 https://doi.org/10.5772/39363.

870 [40] A.F. Huberty, R.F. Denno, Plant water stress and its consequences for herbivorous
871 insects: A new synthesis, Ecology. 85 (2004) 1383–1398.
872 https://doi.org/10.1890/03-0352.

873 [41] W.B. Sconiers, M.D. Eubanks, Not all droughts are created equal? The effects of
874 stress severity on insect herbivore abundance, Arthropod. Plant. Interact. 11 (2017)
875 45–60. https://doi.org/10.1007/s11829-016-9464-6.

876 [42] G. Habibi, R. Hajiboland, Comparison of water stress and UV radiation effects on
877 induction of CAM and antioxidative defense in the succulent rosularia elymaitica

878 (Crassulaceae), Acta Biol. Cracoviensia Ser. Bot. 53 (2011) 15–24.
879 https://doi.org/10.2478/v10182-011-0020-5.

880 [43] E. Pimienta-Barrios, J. Zañudo-Hernandez, V.C. Rosas-Espinoza, A.
881 Valenzuela-Tapia, P.S. Nobel, Young daughter cladodes affect CO2 uptake by mother
882 cladodes of Opuntia ficus-indica, Ann. Bot. 95 (2005) 363–369.
883 https://doi.org/10.1093/aob/mci034.

884 [44] A. Scalisi, B. Morandi, P. Inglese, R. Lo Bianco, Cladode growth dynamics in Opuntia
885 ficus-indica under drought, Environ. Exp. Bot. 122 (2016) 158–167.
886 https://doi.org/10.1016/j.envexpbot.2015.10.003.

887 [45] B. Mengesha, F. Mekbib, E. Abraha, In Vitro Screening of Cactus Opuntia
888 ficus-indica Mill. Genotypes for Drought Tolerance, Am. J. Plant Sci. 07 (2016)
889 1741–1758. https://doi.org/10.4236/ajps.2016.713163.

890 [46] G. Habibi, R. Hajiboland, Comparison of photosynthesis and antioxidative protection
891 in Sedum album and Sedum stoloniferum (Crassulaceae) under water stress,
892 Photosynthetica. 50 (2012) 508–518. https://doi.org/10.1007/s11099-012-0066-y.

893 [47] H. Silva, S. Sagardia, O. Seguel, C. Torres, C. Tapia, N. Franck, L. Cardemil, Effect of
894 water availability on growth and water use efficiency for biomass and gel production
895 in Aloe Vera (Aloe barbadensis M.), Ind. Crops Prod. 31 (2010) 20–27.
896 https://doi.org/10.1016/j.indcrop.2009.08.001.

897 [48] D. Rhodes, A. Nadolska-Orczyk, Plant Stress Physiology, Encycl. Life Sci. (2002) 1–
898 7. https://doi.org/10.1038/npg.els.0001297.

899 [49] J. Ceusters, A.M. Borland, M.P. De Proft, Drought adaptation in plants with
900 crassulacean acid metabolism involves the flexible use of different storage
901 carbohydrate pools, Plant Signal. Behav. 4 (2009) 212–214.

902 [50] V. Florencio-Ortiz, S. Sellés-Marchart, J. Zubcoff-Vallejo, G. Jander, J.L. Casas,
903 Changes in the free amino acid composition of Capsicum annuum (pepper) leaves in
904 response to Myzus persicae (green peach aphid) infestation. A comparison with water
905 stress, PLoS One. 13 (2018) 1–19. https://doi.org/10.1371/journal.pone.0198093.

906 [51] V.K. Rai, Role of amino acids in plant responses to stresses, Biol. Plant. 45 (2002)
907 481–487. https://doi.org/10.1023/A:1022308229759.

908 [52] G. Kaur, B. Asthir, Proline: a key player in plant abiotic stress tolerance, Biol. Plant.
909 59 (2015) 609–619. https://doi.org/10.1007/s10535-015-0549-3.

910 [53] F. Ren, Y.T. Lu, Overexpression of tobacco hydroxyproline-rich glycopeptide
911 systemin precursor A gene in transgenic tobacco enhances resistance against
912 Helicoverpa armigera larvae, Plant Sci. 171 (2006) 286–292.
913 https://doi.org/10.1016/j.plantsci.2006.04.001.

914 [54] F. Dardeau, M. Body, A. Berthier, F. Miard, J.P. Christidès, M. Feinard-Duranceau, F.
915 Brignolas, D. Giron, F. Lieutier, A. Sallé, Effects of fertilisation on amino acid
916 mobilisation by a plant-manipulating insect, Ecol. Entomol. 40 (2015) 814–822.

https://doi.org/10.1111/een.12274.

[55] P.J. Zarco-Tejada, J.R. Miller, G.H. Mohammed, T.L. Noland, P.H. Sampson, Vegetation stress detection through chlorophyll a + b estimation and fluorescence effects on hyperspectral imagery, J. Environ. Qual. (2002) 1–2.

[56] T.M. Heng-Moss, X. Ni, T. Macedo, J.P. Markwell, F.P. Baxendale, S.S. Quisenberry, V. Tolmay, Comparison of Chlorophyll and Carotenoid Concentrations among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines, J. Econ. Entomol. 96 (2003) 475–481. https://doi.org/10.1093/jee/96.2.475.

[57] A.D. Neves, R.F. Oliveira, J.R.P. Parra, A new concept for insect damage evaluation based on plant physiological variables, An. Acad. Bras. Cienc. 78 (2006) 821–835. https://doi.org/10.1590/S0001-37652006000400015.

[58] S. Zhou, Y.-R. Lou, V. Tzin, G. Jander, Alteration of plant primary metabolism in response to insect herbivory, Plant Physiol. 169 (2015) 1488–1498. https://doi.org/10.1104/pp.15.01405.

[59] G.D. Buntin, S.K. Braman, D.A. Gilbertz, D. V. Phillips, Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury, J. Econ. Entomol. 89 (1996) 990–995. https://doi.org/10.1093/jee/89.4.990.

[60] S. Goławska, R. Krzyzanowski, I. łukasik, Relationship between aphid infestation and chlorophyll content in fabaceae species, Acta Biol. Cracoviensia Ser. Bot. 52 (2010) 76–80. https://doi.org/10.2478/v10182-010-0026-4.

[61] X. Ni, S.S. Quisenberry, J. Markwell, T. Heng-Moss, L. Higley, F. Baxendale, G. Sarath, R. Klucas, In vitro enzymatic chlorophyll catabolism in wheat elicited by cereal aphid feeding, Entomol. Exp. Appl. 101 (2001) 159–166. https://doi.org/10.1023/A:1019273626873.

[62] K. Golan, K. Rubinowska, K. Kmieć, I. Kot, E. Górska-Drabik, B. Łagowska, W. Michałek, Impact of scale insect infestation on the content of photosynthetic pigments and chlorophyll fluorescence in two host plant species, Arthropod. Plant. Interact. 9 (2014) 55–65. https://doi.org/10.1007/s11829-014-9339-7.

[63] K. Apel, H. Hirt, Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction, Annu. Rev. Plant Biol. 55 (2004) 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701.

[64] A. Block, S.A. Christensen, C.T. Hunter, H.T. Alborn, Herbivore-derived fatty-acid amides elicit reactive oxygen species burst in plants, J. Exp. Bot. 69 (2017) 1235–1245. https://doi.org/10.1093/jxb/erx449.

[65] P.I. Kerchev, B. Fenton, C.H. Foyer, R.D. Hancock, Plant responses to insect herbivory: Interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways, Plant, Cell Environ. 35 (2012) 441–453. https://doi.org/10.1111/j.1365-3040.2011.02399.x.

[66] B. Vanholme, W. Grunewald, A. Bateman, T. Kohchi, G. Gheysen, The tify family previously known as ZIM, Trends Plant Sci. 12 (2007) 239–244. https://doi.org/10.1016/j.tplants.2007.04.004.

[67] A. Chini, S. Fonseca, G. Fernández, B. Adie, J.M. Chico, O. Lorenzo, G. García-Casado, I. López-Vidriero, F.M. Lozano, M.R. Ponce, J.L. Micol, R. Solano, The JAZ family of repressors is the missing link in jasmonate signalling, Nature. 448 (2007) 666–673. https://doi.org/10.1038/nature06006.

[68] H.S. Chung, A.J.K. Koo, X. Gao, S. Jayanty, B. Thines, A.D. Jones, G.A. Howe, Regulation and Function of Arabidopsis JASMONATE ZIM-Domain Genes in Response to Wounding and Herbivory, Plant Physiol. 146 (2008) 952–964. https://doi.org/10.1104/pp.107.115691.

[69] H.S. Chung, G.A. Howe, A Critical Role for the TIFY Motif in Repression of Jasmonate Signaling by a Stabilized Splice Variant of the JASMONATE ZIM-Domain Protein JAZ10 in Arabidopsis, Plant Cell Online. 21 (2009) 131–145. https://doi.org/10.1105/tpc.108.064097.

[70] K. Sugimoto, S. Takeda, H. Hirochika, Transcriptional activation mediated by binding of a plant GATA-type zinc finger protein AGP1 to the AG-motif (AGATCCAA) of the wound-inducible Myb gene NtMyb2, Plant J. 36 (2003) 550–564. https://doi.org/10.1046/j.1365-313X.2003.01899.x.

[71] A. Pandey, S. Chakraborty, A. Datta, N. Chakraborty, Proteomics Approach to Identify Dehydration Responsive Nuclear Proteins from Chickpea (*Cicer arietinum* L.), Mol. Cell. Proteomics. 7 (2008) 88–107. https://doi.org/10.1074/mcp.M700314-MCP200.

[72] R. Archacki, R. Yatusevich, D. Buszewicz, K. Krzyczmonik, J. Patryn, R. Iwanicka-Nowicka, P. Biecek, B. Wilczynski, M. Koblowska, A. Jerzmanowski, S. Swiezewski, Arabidopsis SWI/SNF chromatin remodeling complex binds both promoters and terminators to regulate gene expression, Nucleic Acids Res. 45 (2016) 3116–3129. https://doi.org/10.1093/nar/gkw1273.

[73] J.W. Walley, H.C. Rowe, Y. Xiao, E.W. Chehab, D.J. Kliebenstein, D. Wagner, K. Dehesh, The chromatin remodeler SPLAYED regulates specific stress signaling pathways, PLoS Pathog. 4 (2008) 1–9. https://doi.org/10.1371/journal.ppat.1000237.

[74] S. Watanabe, A. Nakagawa, S. Izumi, H. Shimada, A. Sakamoto, RNA interference-mediated suppression of xanthine dehydrogenase reveals the role of purine metabolism in drought tolerance in Arabidopsis, FEBS Lett. 584 (2010) 1181–1186. https://doi.org/10.1016/j.febslet.2010.02.023.

[75] J.M. Berner, A.J. Van der Westhuizen, Inhibition of Xanthine Oxidase Activity Results in the Inhibition of Russian Wheat Aphid-Induced Defense Enzymes, J. Chem. Ecol. 36 (2010) 1375–1380. https://doi.org/10.1007/s10886-010-9879-y.

[76] S. Morita, Y. Yamashita, M. Fujiki, R. Todaka, Y. Nishikawa, A. Hosoki, C. Yabe, J. Nakamura, K. Kawamura, I.N. Suwastika, M.H. Sato, T. Masumura, Y. Ogihara, K.

Tanaka, S. Satoh, Expression of a rice glutaredoxin in aleurone layers of developing and mature seeds: subcellular localization and possible functions in antioxidant defense, Planta. 242 (2015) 1195–1206. https://doi.org/10.1007/s00425-015-2354-9.

[77] S. Sha, K. Minakuchi, N. Higaki, Purification and characterization of glutaredoxin (thioltransferase) from rice (Oryza sativa L.), J. Biochem. 848 (1997) 842–848. http://jb.oxfordjournals.org/content/121/5/842.short.

[78] R. Sharma, P. Priya, M. Jain, Modified expression of an auxin-responsive rice CC-type glutaredoxin gene affects multiple abiotic stress responses, Planta. 238 (2013) 871–884. https://doi.org/10.1007/s00425-013-1940-y.

[79] H. Yoshida, M. Nagata, K. Saito, K.L.C. Wang, J.R. Ecker, Arabidopsis ETO1 specifically interacts with and negatively regulates type 2 1-aminocyclopropane-1-carboxylate synthases, BMC Plant Biol. 13 (2005) 1–13. https://doi.org/10.1186/1471-2229-5-14.

[80] L.K.C. Wang, H. Yoshida2, C. Lurin, J.R. Ecker, Regulation of ethylene gas biosynthesis by the Arabidopsis ETO1 protein, Nature. 428 (2004) 945–950. https://doi.org/10.1038/nature02458.1.

[81] K.L. Wang, H. Li, J.R. Ecker, Ethylene Biosynthesis and Signaling Networks, Plant Cell. 14 (2002) 131–152. https://doi.org/10.1105/tpc.001768.S-.

[82] J. Louis, S. Basu, S. Varsani, L. Castano-Duque, V. Jiang, W.P. Williams, G.W. Felton, D.S. Luthe, Ethylene Contributes to *maize insect resistance1* -Mediated Maize Defense against the Phloem Sap-Sucking Corn Leaf Aphid, Plant Physiol. 169 (2015) 313–324. https://doi.org/10.1104/pp.15.00958.

[83] H.M. Falcão, M.T. Oliveira, A.C. Mergulhão, M. V. Silva, M.G. Santos, Ecophysiological performance of three Opuntia ficus-indica cultivars exposed to carmine cochineal under field conditions, Sci. Hortic. (Amsterdam). 150 (2013) 419–424. https://doi.org/10.1016/j.scienta.2012.11.021.

[84] P. Casati, M.F. Drincovich, G.E. Edwards, C.S. Andreo, Malate metabolism by NADP-malic enzyme in plant defense, Photosynth. Res. 61 (1999) 99–105. https://doi.org/10.1023/A:1006209003096.

[85] M.F. Drincovich, P. Casati, C.S. Andreo, NADP-malic enzyme from plants: A ubiquitous enzyme involved in different metabolic pathways, FEBS Lett. 490 (2001) 1–6. https://doi.org/10.1016/S0014-5793(00)02331-0.

[86] N.K. Gautam, S.S. Marla, N. Mirza, Z. Khan, B. Singh, D.P. Wankhede, B.H. Gawade, Evaluation of field pea accessions for root-knot nematode resistance and possible role of NADP dependent malic enzyme gene in host resistance, Indian J. Genet. Plant Breed. 77 (2017) 556–563. https://doi.org/10.5958/0975-6906.2017.00073.6.

[87] V.G. Maurino, M. Saigo, C.S. Andreo, M.F. Drincovich, Non-photosynthetic “malic enzyme” from maize: A constitutively expressed enzyme that responds to plant defence

1035 inducers, Plant Mol. Biol. 45 (2001) 409–420.
1036 https://doi.org/10.1023/A:1010665910095.

1037 [88] D.D. Bilgin, J.A. Zavala, J. Zhu, S.J. Clough, D.R. Ort, E.H. Delucia, Biotic stress
1038 globally downregulates photosynthesis genes, Plant, Cell Environ. 33 (2010) 1597–
1039 1613. https://doi.org/10.1111/j.1365-3040.2010.02167.x.

1040 [89] D. Tang, J. Ade, C.A. Frye, R.W. Innes, Regulation of plant defense responses in
1041 Arabidopsis by EDR2, a PH and START domain-containing protein, Plant J. 44
1042 (2005) 245–257. https://doi.org/10.1111/j.1365-313X.2005.02523.x.

1043 [90] M. Ibrahim, S. Yasmeen, G. Zaman, L. Bin, M. Ibrahim, Protein profiling analysis of
1044 Gossypium hirsutum (Malvales : Malvaceae) leaves infested by cotton whitefly
1045 Bemisia tabaci (Homoptera: Aleyrodidae), Appl. Entomol. Zool. 51 (2016) 599–607.
1046 https://doi.org/10.1007/s13355-016-0436-1.

1047 [91] C.A. Frye, R.W. Innes, An Arabidopsis Mutant with Enhanced Resistance to Powdery
1048 Mildew, Plant Cell Online. 10 (1998) 947–956. https://doi.org/10.1105/tpc.10.6.947.

1049 [92] K. Hiruma, T. Nishiuchi, T. Kato, P. Bednarek, T. Okuno, P. Schulze-Lefert, Y.
1050 Takano, Arabidopsis ENHANCED DISEASE RESISTANCE 1 is required for
1051 pathogen-induced expression of plant defensins in nonhost resistance, and acts
1052 through interference of MYC2-mediated repressor function, Plant J. 67 (2011) 980–
1053 992. https://doi.org/10.1111/j.1365-313X.2011.04651.x.

1054 [93] P.J. Moran, Y. Cheng, J.L. Cassell, G.A. Thompson, Gene expression profiling of
1055 Arabidopsis thaliana in compatible plant-aphid interactions, Arch. Insect Biochem.
1056 Physiol. 51 (2002) 182–203. https://doi.org/10.1002/arch.10064.

1057 [94] L. Taiz, E. Zeiger, I.M. Moller, A. Murphy, Fisiologia e Desenvolvimento Vegetal,
1058 6th ed., Artmed, Porto Alegre, 2017.

1059 [95] L.L. Walling, The myriad plant responses to herbivores, J. Plant Growth Regul. 19
1060 (2000) 195–216. https://doi.org/10.1007/s003440000026.

1061 [96] J. Verica, Z. He, The Cell Wall-Associated Kinase (WAK) andWAK-Like Kinase
1062 Gene Family, Plant Physiol. 129 (2002) 455–459.
1063 https://doi.org/10.1104/pp.011028.1.

1064 [97] J.A. Verica, L. Chae, H. Tong, P. Ingmire, Z.-H. He, Tissue-specific and
1065 developmentally regulated expression of a cluster of tandemly arrayed cell
1066 wall-associated kinase-like kinase genes in Arabidopsis., Plant Physiol. 133 (2003)
1067 1732–46. https://doi.org/10.1104/pp.103.028530.

1068 [98] X. Hou, H. Tong, J. Selby, J. Dewitt, X. Peng, Z.H. He, Involvement of a cell
1069 wall-associated kinase, WAKL4, in Arabidopsis mineral responses, Plant Physiol. 139
1070 (2005) 1704–1716. https://doi.org/10.1104/pp.105.066910.

1071 [99] S. Meier, O. Ruzvidzo, M. Morse, L. Donaldson, L. Kwezi, C. Gehring, The
1072 arabidopsis wall associated kinase-like 10 gene encodes a functional guanylyl cyclase
1073 and is co-expressed with pathogen defense related genes, PLoS One. 5 (2010).

https://doi.org/10.1371/journal.pone.0008904.

[100] P. Khurana, J.L. Henty, S. Huang, A.M. Staiger, L. Blanchoin, C.J. Staiger, Arabidopsis VILLIN1 and VILLIN3 Have Overlapping and Distinct Activities in Actin Bundle Formation and Turnover, Plant Cell. 22 (2010) 2727–2748. https://doi.org/10.1105/tpc.110.076240.

[101] B. Day, J.L. Henty, K.J. Porter, C.J. Staiger, The Pathogen-Actin Connection: A Platform for Defense Signaling in Plants, Annu. Rev. Phytopathol. 49 (2011) 483–506. https://doi.org/10.1146/annurev-phyto-072910-095426.

[102] S.M. Schmidt, R. Panstruga, Cytoskeleton functions in plant-microbe interactions, Physiol. Mol. Plant Pathol. 71 (2007) 135–148. https://doi.org/10.1016/j.pmpp.2008.01.001.

[103] N. Inada, T. Higaki, S. Hasezawa, Nuclear Function of Subclass I Actin Depolymerizing Factor Contributes to Susceptibility in Arabidopsis to an Adapted Powdery Mildew Fungus, Plant Physiol. (2016) pp.01265.2015. https://doi.org/10.1104/pp.15.01265.

[104] S.J. Nicholson, S.D. Hartson, G.J. Puterka, Proteomic analysis of secreted saliva from Russian Wheat Aphid (Diuraphis noxia Kurd.) biotypes that differ in virulence to wheat, J. Proteomics. 75 (2012) 2252–2268. https://doi.org/10.1016/j.jprot.2012.01.031.

[105] H.A. Mondal, J. Louis, L. Archer, M. Patel, V.J. Nalam, S. Sarowar, V. Sivapalan, D.D. Root, J. Shah, Arabidopsis ACTIN-DEPOLYMERIZING FACTOR3 is required for controlling aphid feeding from the phloem, Plant Physiol. 176 (2018) 879–890. https://doi.org/10.1104/pp.17.01438.

[106] F. Domergue, S.J. Vishwanath, J. Joubes, J. Ono, J.A. Lee, M. Bourdon, R. Alhattab, C. Lowe, S. Pascal, R. Lessire, O. Rowland, Three Arabidopsis Fatty Acyl-Coenzyme A Reductases, FAR1, FAR4, and FAR5, Generate Primary Fatty Alcohols Associated with Suberin Deposition, Plant Physiol. 153 (2010) 1539–1554. https://doi.org/10.1104/pp.110.158238.

[107] S. Raffaele, A. Leger, D. Roby, Very long chain fatty acid and lipid signaling in the response of plants to pathogens, Plant Signal. Behav. 4 (2009) 94–99. https://doi.org/10.4161/psb.4.2.7580.

[108] Y. Sasaki, Y. Nagano, Plant Acetyl-CoA Carboxylase: Structure, Biosynthesis, Regulation, and Gene Manipulation for Plant Breeding, Biosci. Biotechnol. Biochem. 68 (2004) 1175–1184. https://doi.org/10.1271/bbb.68.1175.

[109] P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach, 2009. https://doi.org/10.1016/j.jep.2009.05.024.

[110] A.A. Giweli, A.M. Dzamic, M. Sokovic, M.S. Ristic, P. Janackovic, P.D. Marin, The chemical composition, antimicrobial and antioxidant activities of the essential oil of Salvia fruticosa growing wild in Libya, Arch. Biol. Sci. 65 (2013) 321–329.

https://doi.org/10.2298/ABS1301321G.

[111] C.M. Smith, X. Liu, L.J. Wang, X. Liu, M.S. Chen, S. Starkey, J. Bai, Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory, J. Chem. Ecol. 36 (2010) 260–276. https://doi.org/10.1007/s10886-010-9756-8.

[112] J.I. Baños-Sanz, J. Sanz-Aparicio, C.A. Brearley, B. González, Expression, purification, crystallization and preliminary X-ray diffraction analysis of the apo form of InsP5 2-K from Arabidopsis thaliana, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 68 (2012) 701–704. https://doi.org/10.1107/S1744309112017307.

[113] H.F. Kuo, T.Y. Chang, S.F. Chiang, W. Di Wang, Y.Y. Charng, T.J. Chiou, Arabidopsis inositol pentakisphosphate 2-kinase, AtIPK1, is required for growth and modulates phosphate homeostasis at the transcriptional level, Plant J. 80 (2014) 503–515. https://doi.org/10.1111/tpj.12650.

[114] L.B. Sheard, X. Tan, H. Mao, J. Withers, G. Ben-Nissan, T.R. Hinds, Y. Kobayashi, F.-F. Hsu, M. Sharon, J. Browse, S.Y. He, J. Rizo, G.A. Howe, N. Zheng, Jasmonate perception by inositol phosphate-potentiated COI1- JAZ co-receptor, Nature. 468 (2010) 400–405. https://doi.org/10.1038/nature09430.Jasmonate.

[115] A. Mosblech, C. Thurow, C. Gatz, I. Feussner, I. Heilmann, Jasmonic acid perception by COI1 involves inositol polyphosphates in Arabidopsis thaliana, Plant J. 65 (2011) 949–957. https://doi.org/10.1111/j.1365-313X.2011.04480.x.

[116] D. Laha, P. Johnen, C. Azevedo, M. Dynowski, M. Weiß, S. Capolicchio, H. Mao, T. Iven, M. Steenbergen, M. Freyer, P. Gaugler, M.K.F. de Campos, N. Zheng, I. Feussner, H.J. Jessen, S.C.M. Van Wees, A. Saiardi, G. Schaaf, VIH2 Regulates the Synthesis of Inositol Pyrophosphate InsP 8 and Jasmonate-Dependent Defenses in Arabidopsis, Plant Cell. 27 (2015) 1082–1097. https://doi.org/10.1105/tpc.114.135160.

[117] E. Gutierrez-Carbonell, D. Takahashi, G. Lattanzio, J. Rodríguez-Celma, J. Kehr, J. Soll, K. Philippar, M. Uemura, J. Abadía, A.F. López-Millán, The distinct functional roles of the inner and outer chloroplast envelope of pea (Pisum sativum) as revealed by proteomic approaches, J. Proteome Res. 13 (2014) 2941–2953. https://doi.org/10.1021/pr500106s.

[118] S.B. Lee, Y.S. Go, H.-J. Bae, J.H. Park, S.H. Sho, H.J. Cho, D.S. Lee, O.K. Park, I. Hwang, M.C. Suh, Disruption of Glycosylphosphatidylinositol-Anchored Lipid Transfer Protein Gene Altered Cuticular Lipid Composition, Increased Plastoglobules, and Enhanced Susceptibility to Infection by the Fngal Pathogen Alternaria brassicicola, Plant Physiol. 150 (2009) 42–54. https://doi.org/10.1104/pp.109.137745.

[119] U.F. Benning, B. Tamot, B.S. Guelette, S. Hoffmann-Benning, New Aspects of Phloem-Mediated Long-Distance Lipid Signaling in Plants, Front. Plant Sci. 3 (2012) 1–10. https://doi.org/10.3389/fpls.2012.00053.

[120] E. Ruiz-May, S.-J. Kim, F. Brandizzi, J.K.C. Rose, The Secreted Plant N-Glycoproteome and Associated Secretory Pathways, Front. Plant Sci. 3 (2012) 1–15. https://doi.org/10.3389/fpls.2012.00117.

[121] J.P. Vogel, T.K. Raab, C. Schiff, S.C. Somerville, PMR6, a Pectate Lyase – Like Gene Required for Powdery Mildew Susceptibility in Arabidopsis, Plant Cell. 14 (2002) 2095–2106. https://doi.org/10.1105/tpc.003509.sis.

[122] C. Schultz, P. Gilson, D. Oxley, J. Youl, A. Bacic, GPI-anchors on arabinogalactan-proteins: implications for signalling in plants, Trends Plant Sci. 3 (1998) 426–431.

[123] K. Saito, L. Tautz, T. Mustelin, The lipid-binding SEC14 domain, Biochim. Biophys. Acta - Mol. Cell Biol. Lipids. 1771 (2007) 719–726. https://doi.org/10.1016/j.bbalip.2007.02.010.

[124] A. Kiba, Y. Imanaka, M. Nakano, I. Galis, Y. Hojo, T. Shinya, K. Ohnishi, Y. Hikichi, Silencing of Nicotiana benthamiana SEC14 phospholipid transfer protein reduced jasmonic acid dependent defense against Pseudomonas syringae, Plant Biotechnol. 33 (2016) 111–115. https://doi.org/10.5511/plantbiotechnology.16.0503a.

[125] T. Cardozo, M. Pagano, The SCF ubiquitin ligase: Insights into a molecular machine, Nat. Rev. Mol. Cell Biol. 5 (2004) 739–751. https://doi.org/10.1038/nrm1471.

[126] H.S. Kim, T.P. Delaney, Arabidopsis SON1 Is an F-Box Protein That Regulates a Novel Induced Defense Response Independent of Both Salicylic Acid and Systemic Acquired Resistance, Plant Cell. 14 (2002) 1469–1482. https://doi.org/10.1105/tpc.001867.

[127] H. Yu, J. Wu, N. Xu, M. Peng, Roles of F-box proteins in plant hormone responses, Acta Biochim. Biophys. Sin. (Shanghai). 39 (2007) 915–922. https://doi.org/10.1111/j.1745-7270.2007.00358.x.

[128] B. Thines, L. Katsir, M. Melotto, Y. Niu, A. Mandaokar, G. Liu, K. Nomura, S.Y. He, G.A. Howe, J. Browse, JAZ repressor proteins are targets of the SCFCOI1complex during jasmonate signalling, Nature. 448 (2007) 661–665. https://doi.org/10.1038/nature05960.

[129] Y.T. Kwon, a S. Kashina, A. Varshavsky, Alternative splicing results in differential expression, activity, and localization of the two forms of arginyl-tRNA-protein transferase, a component of the N-end rule pathway., Mol. Cell. Biol. 19 (1999) 182–193. https://doi.org/10.1128/MCB.19.1.182.

[130] E. Graciet, F. Walter, D.O. Maoileidigh, S. Pollmann, E.M. Meyerowitz, A. Varshavsky, F. Wellmer, The N-end rule pathway controls multiple functions during Arabidopsis shoot and leaf development, Proc. Natl. Acad. Sci. 106 (2009) 13618–13623. https://doi.org/10.1073/pnas.0906404106.

[131] L.R. Zeng, C.H. Park, R.C. Venu, J. Gough, G.L. Wang, Classification, expression pattern, and E3 Ligase Activity Assay of Rice U-box-containing proteins, Mol. Plant.

1192 1 (2008) 800–815. https://doi.org/10.1093/mp/ssn044.

1193 [132] X. Zhou, S. Yan, C. Sun, S. Li, J. Li, M. Xu, X. Liu, S. Zhang, Q. Zhao, Y. Li, Y. Fan,
1194 R. Chen, L. Wang, A maize jasmonate Zim-domain protein, ZmJAZ14, associates
1195 with the JA, ABA, and GA signaling pathways in transgenic Arabidopsis, PLoS One.
1196 10 (2015) 1–21. https://doi.org/10.1371/journal.pone.0121824.

1197 [133] G. Kitsios, J.H. Doonan, Cyclin dependent protein kinases and stress responses in
1198 plants, Plant Signal. Behav. 6 (2011) 204–209. https://doi.org/10.4161/psb.6.2.14385.

1199 [134] G. West, D. Inze, D. Inzé, G. Beemster, D. Inze, Cell cycle modulation in the response
1200 of the primary root of Arabidopsis to salt stress, Plant Physiol. 135 (2004) 1050–1058.
1201 https://doi.org/10.1104/pp.104.040022.termining.

1202 [135] Y. Yan, S. Stolz, A. Chetelat, P. Reymond, M. Pagni, L. Dubugnon, E.E. Farmer, A
1203 Downstream Mediator in the Growth Repression Limb of the Jasmonate Pathway,
1204 Plant Cell Online. 19 (2007) 2470–2483. https://doi.org/10.1105/tpc.107.050708.

1205 [136] D. Laha, N. Parvin, M. Dynowski, P. Johnen, H. Mao, S.T. Bitters, N. Zheng, G.
1206 Schaaf, Inositol Polyphosphate Binding Specificity of the Jasmonate Receptor
1207 Complex, Plant Physiol. 171 (2016) 2364–2370. https://doi.org/10.1104/pp.16.00694.

1208 [137] A. Wager, J. Browse, Social network: JAZ protein interactions expand our knowledge
1209 of jasmonate signaling, Front. Plant Sci. 3 (2012) 1–11.
1210 https://doi.org/10.3389/fpls.2012.00041.

Artigo 2 - Effect of cactus scale infestation in prickly pear *Opuntia stricta* under water deficit

Mara Danielle Silva do Carmo[1], Melquisedec de Sousa Oliveira[1], Elton Pedro Nunes Pena[1], Amaro de Castro Lira Neto[2], Djalma Cordeiro dos Santos[2], José de Paula Oliveira[2], Sheyla Carla Barbosa da Silva Lima[1], Fabiana Aparecida Cavalcante Silva[1], Tercilio Calsa Jr.[1]*

[1]Laboratório de Genômica e Proteômica de Plantas, Departamento de Genética, Centro de Biociências, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego 1235, Cidade Universitária, Recife, Pernambuco, 50670-901.
[2]Instituto Agronômico de Pernambuco, Sede, Av. Gal. San Martin, 1371, San Martin, Recife, Pernambuco, 50761-000.

* Corresponding: tercilio@ufpe.br; Tel.: +55-081-2126-7829

Abstract: Cactus scale has affected prickly pear crops, reducing its productivity, in an aggravated way under drought. For a better understanding of interaction between cactus and prickly pear, this work evaluated the differential expression of proteins in prickly pear (*Opuntia stricta* (Haw.) Haw. subsp. esparzae Scheinvar) under attack of cactus scale in water deficit conditions. Plants of 1-year-old IPA200016 variety were submitted to infestation and maintained under water deficit for 35 days, in 2 treatments: irrigated non-infested (HS) and drought infested (SC). The differential proteome was examined using two-dimensional gel electrophoresis. Seventy-seven protein spots were found differentially regulated. Mass spectrometric analysis allowed the identification of 34 differentially abundant proteins (DAPs). Over-represented GO terms for the protein abundance included RNA, protein metabolism, secondary metabolism, redox and signaling, revealing molecular mechanisms involving in effectors proteins. This work provides insights into the interaction between the prickly pear and cactus scale at translational levels and also targets for further dissection about this interaction.

Keywords: *Diaspis echinocacti*; *Opuntia*; drought stress; biotic stress; 2D-PAGE; proteome response.

1. Introduction

Sucking insects promote one of the most harmful biotic stress responsible by reducing crop yield. Cactus scale are insect species very adapted to feed in Cactaceae. As phloem sucking, they reduce physiological plant performance at the same time as can act as vectors of viruses and pathogenic microorganisms. Despite of their small size, under favorable environmental conditions, they can spread rapidly through sexual and asexual reproduction (Arruda, 1983; Longo and Rapisarda, 2001; Arruda Filho and Arruda, 2002; Warumby, Arruda Filho and Cavalcanti, 2005).

Prickly pear (*Opuntia* spp.), a Cactaceae specie whose crops have expanded to attend demand for food and feed supply, especially in arid and semiarid areas (Inglese, 2010; Sáenz *et al.*, 2013; Arba *et al.*, 2017) has suffered with cactus scale *Diaspis echinocacti* Bouché, 1833 (Claps and Haro, 2001; Warumby, Arruda Filho and Cavalcanti, 2005; Lopes, 2007). There are no resistant varieties of the genus, and the current control search primarily rely on phenotypic evaluation of breeding materials, rather than selection based on targeted genotype (Vasconcelos *et al.*, 2009; Lopes *et al.*, 2010; Borges *et al.*, 2013).

Proteomic tools provide information on how plant responds to different conditions through proteins, important for elucidating the mechanisms for resistance and stress tolerance.

Even during susceptibility, plant defenses are activated, although not enough to completely protect from insect attack. This basal defense appears to be due to the incomplete attenuation of pest pattern triggered induced (PTI) mechanisms and may be due to inefficient pest-derived effector recognition, thus leading to a weak effector triggered immunity (ETI) (Buchanan, Gruissem and Jones, 2015)

Thus, the present study aimed use proteomic approach through bidimensional electrophoresis over prickly pear (*Opuntia stricta*) under simultaneous infestation by cactus scale (*Diaspis echinocacti*) and water deficit, for the identification of differential proteins, focusing on the selection of candidates as defense response markers.

2. Materials and Methods

2.1. Plant Cultivation and Treatment

The experiment was carried out in a greenhouse located at Agronomic Institute of Pernambuco (IPA), Recife, Brazil (-8.063129º, -34.926315º), from August to October 2016. Clones from *Opuntia stricta* (variety IPA 200016), 1 year old, with 3 cladodes, obtained from *in vitro* culture, were transplanted to pots with soil classified as sandy loam (coarse sand 55%, fine sand 33% and clay 11%) as substrate.

Plants were submitted to the following treatments: 1 - irrigated at 100% pot capacity (three times a week) and without infestation (HS); 2 - suppression of irrigation and infestation (SC). Treatments were kept isolated by anti-aphidic grid. The infestation was carried out by continuous contact during 55 days with infested prickly pear fragments collected from IPA Experimental fields (São Bento do Una, Arcoverde, Ibimirim and Caruaru municipalities, state of Pernambuco, Brazil). After establishment of infestation, irrigation was suppressed for 35 days in water deficit treatment, while it was kept in irrigated treatment three times a week at 100% pot capacity. Six plants were used in each treatment (biological replicates). After the stress period, the insects were removed from the infested plants and the cladode samples were collected, immediately frozen in liquid nitrogen and stored at -80˚C for further analysis.

2.2. Protein Sample Preparation, 2DE, and Protein Abundance Analyzes

Proteins from secondary cladodes under different treatments were extracted according to Wu et al. (2014). To separation in first dimension, isoelectric focusing (IEF) was performed on Multiphor II electrophoresis system platform (GE Healthcare Life Sciences) using IPG strips (13 cm, pH 3-10, GE Healthcare Life Sciences) rehydrated in IPG-box for 16 hours, with 400 μg of proteins homogenized with rehydration buffer (Phenylmethane sulfonyl fluoride (PMSF), dithiothreitol (DTT), IPG buffer pH 3-10). IEF follow three steps: 300 V - 100 V/h, 3500 V - 2900 V/h, 3500V - 27000 V/h. Throughout the focusing, they were maintained in direct current (2 mA), 5 W of power and temperature of 10°C. Then, strips were reduced and alkylated with equilibrium buffer (urea 6M, Tris-HCl buffer 75 mM pH 8.8, glycerol 30%, SDS 2%) containing DTT 1% and iodoacetamide 2,5% (IAA), respectively. The second dimension was performed utilizing SDS-PAGE gels 12,5% (20x20 cm) in vertical plates, at 15°C in two steps: i) 300 V gradient, 15 mA, 30 W for 20 minutes; ii) 300 V, 30 mA, 30 W for approximately 2 h and 30 min. Gels were stained with Coomassie Brilliant Blue G-250 solution containing 0.01 $g.L^{-1}$ according to Candiano et al. (2004). At least three replicates of independent gels were generated by treatment. The acquisition of gel images was performed in Image Scanner III (GE Healthcare Life Sciences) through the LabScan 6.0 program, according to the default parameters, and image analysis of gels was performed using the Image Master 2D Platinum v.7.05 program (GE Healthcare Life Sciences). For the quantitative analysis, only reproducibly detected spots were considered. The volume of each spot was normalized against the total volume of the valid spots. Significant spots were selected as differentially accumulated protein (DAP) according to analysis of variance ($p \leq 0.05$) and the volume percentage ratio between control and stressed treatments greater than

or equal to 1.5. Irrigated non-infested treatment (HS, control) were compared with water deficit infested treatment (SC, stressed).

2.3. Protein Identification by MALDI-ToF/ToF MS and Database Searching

DAPs spots were excised from gels and digested with trypsin as described by Webster & Oxley (2005), then, the samples were concentrated in a vacuum rotaevaporator at 30°C for 30 min. MS and MS/MS spectra were obtained using an MALDI-ToF/ToF Autoflex III mass spectrometer (Bruker Daltonics Inc., Bremen, Germany) in the Analytical Center of the Center for Strategic Technologies of Northeast (CETENE, Recife, Brazil). The pellet was solubilized in 5 μl of 0.1% trifluoroacetic acid (TFA). For each reading cycle, 2 μl of the sample were mixed with 2 μl of α-cyano-4-hydroxycinnamic acid matrix in acetonitril (ACN) and 3% TFA. The parameters were set to the positive ion reflection mode with acceleration voltage of 20kV, assuming a firing rate of 100 Hz, mass range of 700 to 5,000 Da, laser intensity ranged from 25 to 50% and 2,100 to 4,000 shots by spectrum. The equipment was calibrated using a peptide mixture $[M + H]^+$ ions for standard calibration. Peaklists files were generated using the FlexAnalysis 3.4 software (Bruker Daltonics).

The identification of the spectra was performed through the MASCOT online software, initially by the public access version, based in peptide mass fingerprinting method (PMF) for MS spectra and MS/MS ion search method for MS/MS spectra, against the sub-banks Viridiplantae, *Arabidopsis thaliana*, *Oryza sativa* and other green plants from NCBIprotein and SwissProt database. Subsequently, additional identification was conducted through a private version of the MASCOT program, kindly made available for access in collaboration with the Center for Advanced Proteomics of the University of Washington (Seattle, Washington, USA (http://proteomicsresource.washington.edu/), against the sub-database Cactaceae (from Uniprot) and *Opuntia ficus indica* and *Opuntia cochenillifera*, from a kind collaboration with University of Nevada.

The experimental error used for mass values was as follows: i) to MS spectra analysis: up to 1.2 Da of tolerance; ii) to MS/MS spectra: 100 to 200 ppm and 0.2 to 1.2 Da (for parental ion), and 100 to 200 ppm and 0.2 to 0.6 Da (for ion fragment). Another search parameter were set in both methods as following: carbamidomethylation of cysteines as fixed modification and methionine oxidation as variable modifications, allowing up to one missed cleavage. Protein identification was performed using the Mascot search probability-based molecular weight search (MOWSE) score. Only the identifications with calculated probability score equal or greater than to the cut-off value were considered significant. The score equals -10.log (P), where P is the probability that the similarity found is random. Peptides with a Mascot Score exceeding the threshold value corresponding to <5% false positive rate, calculated by the Mascot procedure.

Additional searches were performed with SearchGUI v.3.2.20 software using xml files obtained after analysis via MALDI-ToF/ToF that were individually converted to mzXML format through the FlexAnalysis software tool (Bruker Daltonics), and submitted to the presumptive identification (Vaudel *et al.*, 2011), whose results were visualized using the program PeptideShaker v.1.16.15 (Vaudel *et al.*, 2015). The following parameters were used to search the SearchGUI: tolerance *m/z* of the parental ion: 0.25 Da; ion fragments m/z tolerance: 0.25 Da; charge of the parental ion: 1-1; isotope: 0-1; maximum missed cleavages: 1; fixed modifications: carbamidomethylation (C); variable modifications: oxidation (M). The other parameters remained in the default software mode. Reference proteomes available in UniProt were used as a database, initially from the Cactaceae family, and then if there was no identification, subsequent searches were carried out against *Spinacia oleracea* species (species with proteome available and taxonomically close to the genus *Opuntia*) and *A. thaliana* (model specie dicots). To correct identification MSQuant parameter was considered.

2.4. Protein Classification

For gene ontology (GO) analysis, the amino acid sequences of differentially accumulated proteins putatively identified in silico were recovered as multifasta format from Uniprot database. Fasta sequence from NCBI single accesses was retrieved from the nr (non-redundant) database and entered manually into multifasta file. Then, the multifasta file was used to map GO terms for biological processes in Mercator (Lohse *et al.*, 2014), which already uses BLAST tool, to search orthologous accesses correspondence in Arabidopsis thaliana; standard parameters, including InterPro option (IPR), were used.

3. Results and Discussion

2D electrophoresis allowed detection of 1,002 reproductible spots, with an average of 167 spots per gel of the HS treatment, and 217 spots per gel in the SC treatment, of which 77 were detected as DAPs, totaling 58 exclusive spots: 3 in HS treatment and 53 in SC. The remaining 19 spots were detected in both treatments (common spots), out of which 16 were more accumulated in the control condition and 3 showed greater accumulation in the samples from the stress, SC treatment (Figure 1).

The analysis of the 77 spots selected as DAPs allowed the identification of 34 spots through mass spectrometry. Out from the 34 identified spots, only two were matched to unknown proteins whose cellular roles are not clear, while 32 non-redundant proteins were putatively annotated, allowing to infer possible functional aspects of the differential proteome, as well as its association with physiological and metabolic processes. Some proteins with similar annotations were identified from different spots, and some of them presented changes in the values of molecular mass and isoelectric point when compared to the predicted values in databases, likely indicating post-translational modification events (Table 1).

Based on GO categories to biological process, distribution obtained from differentially abundant proteins analysis regarding biological processes, DAPs were distributed into functional groups whose frequencies are presented in Figure 2. In this section, the categorization of total proteins identified showed that the most expressive and highlighted functional groups during stress were: RNA metabolism (35%), protein metabolism (25%) and secondary metabolism (5%).

3.1. Proteins from HS treatment

Proteins identified in the control (HS) treatment were mainly distributed in classes of RNA, protein and photosynthesis and, to a lesser extent, with proteins related to transport and hormone metabolism, indicating the main mechanisms involved in the maintenance of the plant basal machinery.

Photosynthesis. Subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco (spots 43, 47 e 48), the main molecule of the photosynthetic process, were found to be more accumulated in the control (HS) treatment. There are several possible interpretations for this result. This may reflect degradation in treatment under stress, as a function of ROS accumulation, or yet by synthesis of proteases to its degradation to reallocating nitrogen Rubisco-derived to synthesis of amino acids with osmoregulatory capacity (seen by Aranjuelo et al. (2011) or to defense resources, are also suggested by Zhou et al. (2015). This may results in the decrease of carbon assimilates by the plant under stress at the same time that is a mechanism to hamper herbivore performance by reducing the nutritive value of plant tissue (Zhou *et al.*, 2015). Besides regulation as a direct consequence of double stresses, it may be occurring according to variation in activity of other enzymes related to the photosynthetic process, such as PEPC, also affected in these conditions (spot 149). According to Griffiths et

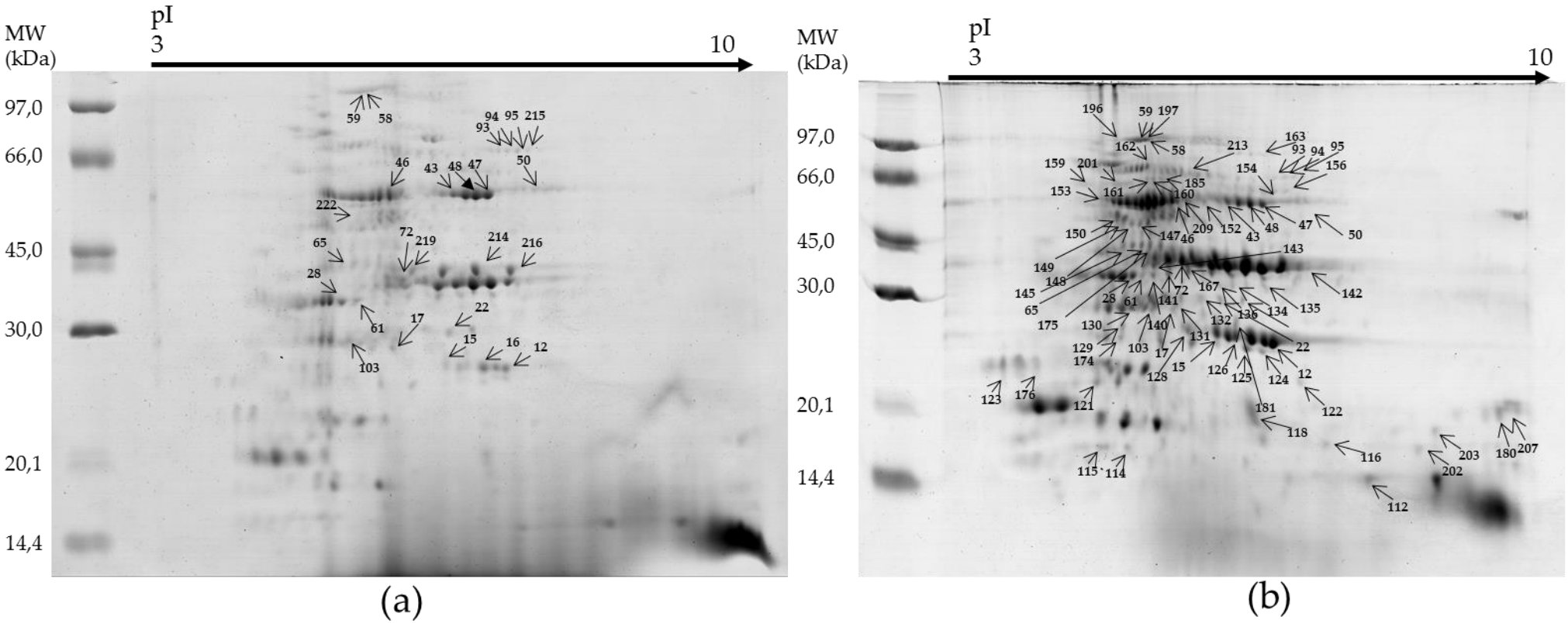

Figure 1. 2D-PAGE from prickly pear *Opuntia stricta* proteins (secondary cladodes) for water irrigated control treatment: HS (a) and water deficit and infested treatment: SC (b).

Table 1. Proteins putatively identified from prickly pear *Opuntia stricta* (secondary cladodes) that significantly changed in abundance under conditions irrigated control treatment (HS) and water deficit and infested treatment (SC), with designation of category of biological process according to Lohse et al. (2014).

N. Spot	Ratio	ID Uniprot	Assignment protein	pI experimental	MW experimental
CONTROL - HS	**C/S**				
Photosynthesis					
43	1.60183	Access1	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial	6.46	56,764.33
47	2.27929	Access2	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial	6.87	56,286.50
48	2.46014	Access3	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial	6.73	56,148.17
RNA					
17	1.73307	Access4	Remorin	5.77	28,275.40
65	1.53752	Access5	Remorin	5.32	43,322.00
216	exclusive	Access6	Remorin	7.19	40,944.67
50	1.58068	Access7	Remorin isoform X2	7.34	57,273.00
58	1.89752	Access8	GATA transcription factor 19	5.50	105,362.67
22	1.81276	Access9	Factor sigma	6.37	29,832.17
Protein					
61	3.42574	Access10	Arginyl-tRNA-protein transferase	5.36	34,993.00
94	1.57394	Access11	Ycf1	7.14	67,647.50
72	1.567	Access12	Translation initiation factor IF-1, chloroplastic	5.88	38,638.67
50	1.58068	Access13	Uncharacterized protein LOC4324954 isoform X1	7.34	57,273.00
Transport					
214	exclusive	Access14	Probable envelope ADP, ATP carrier protein, chloroplastic	6.78	40,929.00
Hormone Metabolism					
215	exclusive	Access15	ACC oxidase	7.47	70,004.67
Not assigned					
95	1.78879	Access16	Full insert sequence	7.25	67,572.50
STRESS - SC	**S/C**				
RNA					
112	exclusive	Access17	Remorin	8.07	14,916.67
114	exclusive	Access18	Remorin	5.11	16,984.33

122	exclusive	Access19	Remorin	7.23	22,104.67
141	exclusive	Access20	Helix-loop-helix DNA-binding superfamily protein, putative isoform 1	5.63	35,027.67
131	exclusive	Access21	GATA transcription factor 19	5.73	29,425.33
176	exclusive	Access22	Maturase K	4.13	23,584.00
12	1.70419	Access23	Helicase	7.03	25,862.50
Glycolysis					
149	exclusive	Access24	Phosphoenolpyruvate carboxylase	5.07	51,469.00
Secondary metabolism					
136	exclusive	Access25	Caffeoyl-CoA O-methyltransferase	6.27	32,293.00
Redox metabolism					
125	exclusive	Access26	Glutaredoxin-C13	6.60	24,811.67
C1 metabolism					
12	1.70419	Access27	Protein arginine N-methyltransferase 1.5	7.03	25,862.50
Protein					
115	exclusive	Access28	F-box protein At1g78100	4.83	16,992.67
116	exclusive	Access29	Sec14p-like phosphatidylinositol transfer family protein	7.54	17,266.00
132	exclusive	Access30	Poly [ADP-ribose] polymerase 2	6.04	30,292.67
138	exclusive	Access31	Non-specific, serine/threonine protein kinase	5.81	33,309.67
161	exclusive	Access32	Calmodulin-binding receptor-like cytoplasmic kinase	5.36	64,669.00
Signaling					
213	exclusive	Access33	Phytochrome C	5.88	69,041.33
140	exclusive	Access34	Phytochrome C	5.44	35,084.00
Not assigned					
135	exclusive	Access35	Uncharacterized protein	6.81	32,588.33
121	exclusive	Access36	Ethylene-overproduction protein 1	4.74	22,180.33

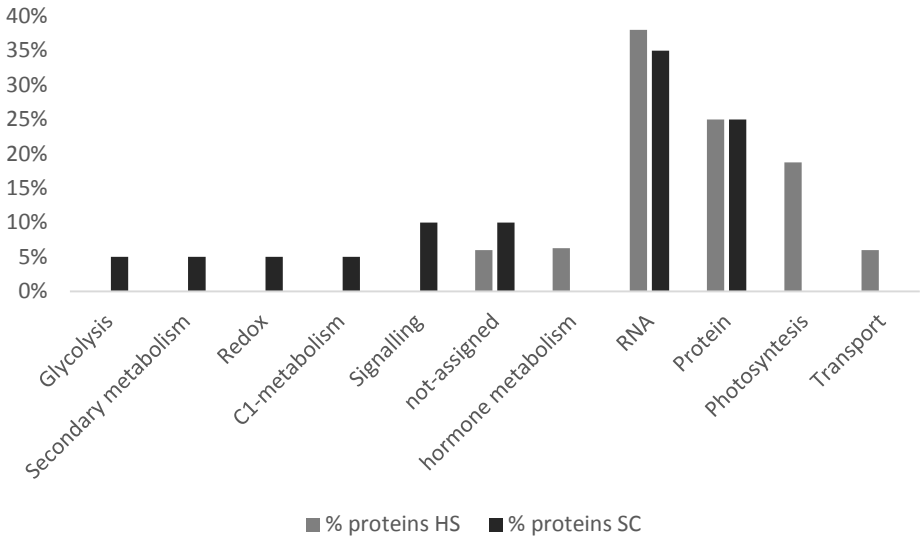

Figure 2. Gene ontology analysis of DAPs for the biological process of from prickly pear *Opuntia stricta* (secondary cladodes) for irrigated non-infested treatment (HS) and under water deficit and infested treatment (SC), with designation of category of biological process according to Lohse et al. (2014).

al. (2002) the carboxylase activity of Rubisco in CAM plants is higher when PEPC activity is lower due to conditions such as drought.Several proteomic studies and analysis of gene expression have observed inhibition of Rubisco accumulation and/or expression under conditions of water deficit (Yu et al., 2016, in soybean genotypes susceptible to drought; Parry et al., 2002; Aranjuelo et al., 2011) and sucking insect infestation (Nabity, Zavala and DeLucia, 2009).

RNA. Remorin proteins (spots 17, 65, 216) and remorin X2 isoform (spot 50) were accumulated in the control treatment, but also in the stress treatment (spots 112, 114, 122), indicating that different isoforms are regulating each condition in a specific way. It has a N-terminal region that is highly variable or absent or with a proline-rich N-terminal region (Marín and Ott, 2012). The coiled-coil domain provides indications about its function in protein–protein interaction and recognitions (Burkhard, Stetefeld and Strelkov, 2001). They were initially identified on the plasma membrane in the phosphorylated form (Farmer, Pearce and Ryan, 1989), and subsequent studies have suggested its association, specifically in plasma membrane fractions detergent resistant (Mongrand *et al.*, 2004; Laloi *et al.*, 2006; Lefebvre *et al.*, 2007). This association also suggests its role as a facilitator of cell signal transduction by direct interaction with signaling proteins such as receptor-like kinases (Marín and Ott, 2012).

It has been reported more than 100 members of the remorin protein family in poplar tissues (Song *et al.*, 2011). Li et al. (2013) in studies related to growth and development, have found remorin related to inhibition of xylem and phloem size and wall cell biosynthesis genes. They have also been observed in response to various biotic and abiotic stresses, such as in the plant-microbe interaction, and its role in the recognition of pathogen elicitors has been proposed (Liu *et al.*, 2009; Widjaja *et al.*, 2009; Marín and Ott, 2012) in interactions with nodular symbiotic bacteria (Lefebvre *et al.*, 2010). In response to herbivory by aphid and caterpillar separately it was identified being inhibited, but when the two stresses were considered simultaneously it was induced (Rodriguez-Saona *et al.*, 2010). In addition to interacting physically with viral protein in potato, leading to reduced movement of the virus, which associated with its location has suggested one of its functional roles (Raffaele *et al.*, 2009).

Regarding the abiotic stress, remorin was registered optimizing the growth of *Arabidopsis* under conditions of osmotic stress, resulting in tolerance to dehydration and salinity, probably by interacting with other signaling proteins (Checker and Khurana, 2013); was also differentially expressed during water deficit (Bray, 2002; Wang *et al.*, 2016).

The accumulated isoforms in the control condition in prickly pear are probably associated with maintenance of basal metabolism related to plant growth and development, which may have been inhibited during stress, with the accumulation of other isoforms, probably with different phosphorylation patterns, considering that stimulus-dependent phosphorylation has been shown for some remorins (Benschop *et al.*, 2007; Widjaja *et al.*, 2009; Marín and Ott, 2012). While in the stress condition isoforms accumulated may be associated with the identification of elicitors from cactus scale and, or the perception of osmotic stress coming from water deficit.

Sigma factor (spot 22) was almost twice as accumulated in the control treatment. This core-encoded protein is one of the subunits of plastid-encoded RNA polymerase (PEP). It regulates PEP through the donation of promoter recognition specificity and the ability to initiate transcription to the core complex. Then, sigma subunit is crucial for RNA polymerase function, and hence for the biogenesis and maintenance of chloroplasts, having its role controlled by the nucleus (Ishihama, 1988; Lonetto, Gribskov and Gross, 1992; Kanamaru and Tanaka, 2004; Chi *et al.*, 2015). Some isoforms of this proteins were already found associated to stress as well as their losses were associated with losses to plant, through harms in chloroplasts (Nagashima *et al.*, 2004; Lerbs-Mache, 2011; Sheibani-Tezerji *et al.*, 2015). In this case, the present isoform may be affected by both stresses, once it was less accumulated in stress treatment.

Transcription factor GATA19 (spot 58), accumulated in control treatment, it was also identified as accumulated exclusively in the stress treatment (spot 131), according the experimental MW and pI pattern, probably with a different isoform, thus controlling different aspects referent to the exposed conditions.

Protein metabolism. Prickly pear, under the stress conditions (SC), seems have inhibited some proteins, as seen with less accumulation of arginyl-tRNA-protein transferase (spot 61). It is involved in post-translational conjugation of arginine to the N-terminal aspartate or glutamate of a given protein. This arginylation is required for degradation via the ubiquitin pathway, through the identity of the amino-terminal residue of specific protein substrates (Kwon, Kashina and Varshavsky, 1999; Graciet *et al.*, 2009) and in this context reflect a regulation of target proteins specific.

3.2. Proteins from SC treatment

The stresses to which the plant was submitted triggered the accumulation of proteins involved in several classes, especially RNA and protein metabolism, but also proteins of secondary metabolism, redox, C1, glycolysis and signaling. The highlighted proteins are described below.

RNA. Higher protein accumulation related to RNA metabolism may be associated with higher intensity of transcriptional control, with proteins: remorin (spots 112, 114 e 122), maturase k (spot 176), helicase (spot 12), helix-loop-helix DNA-binding superfamily protein putative isoform 1 (spot 141) and GATA19 (spot 58).

Transcription factor helix-loop-helix DNA-binding superfamily protein, putative isoform 1 (HLH 1) as a transcriptional regulatory protein is a key player in a wide array of developmental processes (Massari and Murre, 2000). They may be associated with biotic and abiotic stresses. It was found accumulated under drought, heat and salt stress (Mao *et al.*, 2017; Wang *et al.*, 2018; Zhang *et al.*, 2018) and in involved in jasmonate, which signaling is also related to biotic and abiotic stress (Goossens, Mertens and Goossens, 2017).

Maturase K is encoded by a gene inside trnK intron of chloroplast genome, and it has been related to RNA splicing activity by the recognition of multiple regions and may represent a model for an early activity of trans-action nuclear splicing (Zoschke *et al.*, 2010). Some studies report accumulation of this protein under abiotic stresses, as in maize roots under salt stress in dephosphorylated form (Zörb, Schmitt and Mühling, 2010), in *Brassica napus* under phosphorus deficit stress (Yao *et al.*, 2011) and in chloroplasts suffering high reactive oxygen species pressure by dehydration (Pandey *et al.*, 2017). The presence of this proteins, related to plastids, reveals that chloroplast gene expression was not completely affected by the stresses, as a way to overcome the stresses alterations and maintain the plant metabolism.

Helicase (spot 12), according to data from the Uniprot database, presents the Q-motif, ATP-binding and C-terminal domains and belongs to the DEAD-box helicase family. Helicases are ubiquitous motor proteins that catalyze the unwinding of duplex DNA (DNA helicases) or rearrange secondary structures of duplex RNA (RNA helicases) in an ATP-dependent manner. Then, playing essential roles in basic cellular processes, such as DNA replication, repair, recombination, transcription, ribosome biogenesis, and translation initiation. Abiotic stresses affect the mechanism of cellular gene expression, then that is possible for molecules involved in nucleic acid metabolism, including helicases, to be affected. Thus, helicases may be regulating plant growth and development under stress conditions through regulating stress-induced pathways (Vashisht and Tuteja, 2006). A study by Sanan-Mishra et al. (2005) relates the construction of saline-tolerant tobacco plants with overexpression of a helicase gene, suggested as a new pathway for the engineering of plant stress tolerance. The exact mechanism of helicase-mediated stress tolerance is not currently understood. Owttrim (2006), associated RNA helicase to abiotic stresses in plants (cold, hormone stress). Li et al. (2008) demonstrated that DEAD-box helicase plays a role in modulating responses to pathogen defense and oxidative stress.

Secondary metabolism. Caffeoyl-CoA O-methyltransferase, CCoAOMT (spot 136), was observed exclusively under stress condition. This protein methylates caffeoyl-CoA to feruloyl-CoA, which participate in one stage of monolignol biosynthesis pathway for lignin biosynthesis in plants (Ghosh *et al.*, 2014). Not only, the accumulation of lignin is consistent with other findings of stress conditions (Ye *et al.*, 2001; Boerjan, Ralph and Baucher, 2003; Moura *et al.*, 2010; Miedes *et al.*, 2014; Castrillón-Arbeláez and Frier, 2016; Liu, Luo and Zheng, 2018), but also the accumulation of enzyme CCoAMT and genes associated with lignin deposition were found in response to salinity and water deficit (Moura *et al.*, 2010; Srivastava *et al.*, 2015) and biotic stresses (Chen *et al.*, 2000; Brahim *et al.*, 2010; Hamann, 2012; Santiago, Barros-Rios and Malvar, 2013; Miedes *et al.*, 2014; Zhao and Dixon, 2014). In addition, in rice, feruloyl-CoA, product of CCoAOMT enzyme, was most preferred in monolignol pathway as substrate by cinnamoyl-CoA reductase (CCR) than OsCCR20 and 21 isoforms, which are also responsive to biotic and abiotic stresses (Park *et al.*, 2017).

Lignin may be adding to prickly pear, physical and chemical rigidity, that provides reduction of transpiration and maintaining cell osmotic balance to minimize water losses (Liu, Luo and Zheng, 2018), increasing drought tolerance (Hu *et al.*, 2009), and yet reducing accessibility to cactus scale, already seen to another plant species to pests and diseases (Boerjan, Ralph and Baucher, 2003; Santiago, Barros-Rios and Malvar, 2013; Duan *et al.*, 2014; Jannoey *et al.*, 2015; Wang *et al.*, 2017).

Redox metabolism. In this category glutarredoxin protein (GRX) C13 (spot 125) was accumulated, exclusively in this treatment. GRX can regulate the activity of proteins by reversible glutathionation or reduced disulfide bonds in dehydroascorbate, peroxiredoxin and methionine sulfoxide reductase, contributing to the removal of ROS and the repair of oxidative stress (Sha, Minakuchi and Higaki, 1997; Morita *et al.*, 2015). The active site type-

CC present in protein GRX identified, has been associated with the reduction of sensitivity to the stress-related hormones and auxin, and increased tolerance to various abiotic stresses, such as oxidative, osmotic and saline (Sharma, Priya and Jain, 2013). This protein may be responding to the two stress factors, since both can lead to the accumulation of ROS. But in addition to removing ROS, GRX involvement in stress response may be associated with the regulation of gene expression by controlling the redox state of transcription factors or by other target proteins (Ndamukong *et al.*, 2007; Gutsche *et al.*, 2015).

C1 metabolism. Other protein that can be related to protein degradation is arginine N-methyltransferase (spot 12) that was almost twice accumulated in water deficit and infested treatment (SC). The conserved arginine methyltransferase family (PRMTs) may play several roles in transcriptional regulation and in other vital cellular processes by histone and non-histone protein methylation *in vivo*. They often target proteins bearing glycine-rich motifs and arginine, and act both by inducing and inhibiting genes related to biotic and abiotic stresses. They also regulate the alternative processing of stress-associated proteins by the methylation of spliceosomal components. Post-translational methylation of arginine residues profoundly affects the structure and function of the protein and thus implicates in a multitude of essential cellular processes that may be affected, such as signal transduction, mRNA splicing and transcriptional regulation (Ahmad, Dong and Cao, 2011; Hernando *et al.*, 2015). Further research is needed to indicate which genes may be regulated by this PRMT, but probably they are directly involved in the stress condition under which prickly pear was submitted.

Glycolysis. The presence of phosphoenolpyruvate carboxylase (PEPC, spot 149), a primary catalyst for atmospheric CO_2 fixation in CAM and C_4 plants, more accumulated in stressed treatment, shows that water deficit associated to infestation in prickly pear may have inducting photosynthesis process, revealing a great demand for carbon fixation products.

Protein metabolism. In order to respond to the water deficit and cactus scale, the prickly pear was showed to require signaling mediated by calmodulin-binding receptor-like cytoplasmic kinase 2, CRCK2 (spot 161). This protein belongs to the family of receptor like kinases (RLKs), specifically to receptor-like cytoplasmic kinases (RLCKs), which lack the transmembrane domain with an extracellular region ligand-binding. They have emerged as a major class of signaling proteins and are mainly implicated in pathways associated with the stress responses in plants and endogenous extracellular signaling molecules (Liang and Zhou, 2018).

Recent advances suggest a key role of RLCKs in concert with receptor kinase (RK) and receptor-like proteins (RLP) located to plasma membrane, mediating signaling as a way of regulate downstream of plant innate immunity, adaptation to abiotic stresses, hormone signaling, sexual reproduction, stomatal patterning, maintenance of shoot and root meristems, differentiation of vascular tissues, petal abscission and other developmental processes (Lin *et al.*, 2013).

Accumulation or induction of CRCK expression has been commonly observed in response to abiotic stresses such as water deficit, salinity, cold, and in some cases conferring resistance (Yang *et al.*, 2004; Vij *et al.*, 2008), and also it was observed in resistance gene to leaf spot diseases in cultivated peanut they were found CRCK2 up- and down-regulated (Dang *et al.*, 2018). As seen in *Arabidopsis*, with CRCK1 (Yang *et al.*, 2004), CRCK2 accumulated herein may be involved in perception of cytoplasmic calcium levels that promotes calmodulin binding, and then, enhances its kinase activity. This enables signal transduction in downstream signaling of genes defense-responsive, both for the abiotic and for the biotic stress, trough protein phosphorylation regulated by calcium/calmodulin.

Sec14p-like phosphatidylinositol transfer family protein (spot 116), classified in a subdivision of targeting of secretory pathways, regulating signaling interfaces between lipid metabolism and membrane trafficking, was accumulated in a way exclusive. It has a CRAL-

TRIO domain that can be involved in lipid transport (to one domain) or more complex functions in signal transduction, transport and organelle biology, integrating lipid metabolism with other biochemical processes (to multidomains) (Saito, Tautz and Mustelin, 2007). Its silencing is associated to reduction in JA level, JA-dependent defense against pathogen and phospholipid turnover (Kiba *et al.*, 2016). Probably, this protein was involved in trafficking of phosphoinositide for specific signaling purposes, according signals from JA responding to infestation.

Poly [ADP-ribose] polymerase 2 (spot 132), present exclusively in the treatment of stress, is part of the superfamily poly [ADP-ribose] polymerase (PARP), involved in the base excision repair pathway, by catalyzing the poly(ADP-ribosyl)ation of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism. Poly(ADP-ribosyl)ation is the covalent attachment of ADP-ribose subunits from NAD(+) to target proteins (Lamb, Citarelli and Teotia, 2012).

PARPs have important functions in many biological processes including DNA repair, epigenetic regulation and transcription. However, these roles are not always associated with enzymatic activity. In plants, members of the PARP family and/or poly(ADP-ribosyl)ation have been linked to DNA repair, mitosis, innate immunity and stress responses (Lamb, Citarelli and Teotia, 2012), as observed by Amor et al. (1998), which under medium oxidative stress in *Arabidopsis* it was DNA repair and PCD inhibition, and under severe oxidative stress it was overexpression of PARP, increase in breaks of DNA and cell death.

Other studies have observed that inhibition of PARP2 increased resistance to abiotic stresses, suggesting this response due to the maintenance of energy homeostasis due to the reduced consumption of NAD(+), later attributed to changes in abscisic acid levels, which facilitated the induction of a set of genes responsive to stress (Vanderauwera *et al.*, 2007). On the other hand, according to Briggs et al. (2017), the inhibition of PARPs disrupted a set of plant defenses against pathogens in *Arabidopsis* due to the lower recognition of microbe-associated molecular patterns (MAMPs). Song et al. (2015) also found the PARP2 isoform associated with organism viability in response to genotoxic stress and the restriction of pathogenic infection.

These results reveal that depending on the biotic interaction, this enzyme can act in different ways, according to Rissel et al. (2017), PARPs described so far for *A. thaliana* do not play a universal role in stress response, but proteins with PARP activity not yet described may be relevant. In prickly pear, the putative PARP2 appears to act as a defense response, and may be used in future investigations as to which genes its overexpression or knockout might affect. F-box protein At1g78100 (spot 115), is part of SCF complex (ASK-cullin-F-box) E3 ubiquitin ligase, that can mediate the ubiquitination and subsequent proteasomal degradation of target proteins selectively through the ubiquitin 26S proteasome system (Hua and Vierstra, 2011; Callis, 2014).

In this predominant plant complex, the F-box protein is the variable part and act as substrate adaptors, each interacting with and recruiting unique targets. Thus, F-box proteins have pivotal roles in diverse physiological processes, including hormone perception and signaling, development, reproduction, defense, light perception, and the circadian clock (Callis and Vierstra, 2000).

Gene expression studies reveal an important role of F-box proteins in plants in response to biotic and abiotic stresses and stress-related hormones such as JA, ethylene, ABA and SA, however, their roles and targets are mostly unknown (Kim and Delaney, 2002; Mazzucotelli *et al.*, 2006; Fang *et al.*, 2015; Chen *et al.*, 2017; Gonzalez *et al.*, 2017; Li *et al.*, 2018).

The plant genome is shown to be enriched with F-box proteins compared to non-plant organisms (Gagne *et al.*, 2002; Hua and Vierstra, 2011; Hua *et al.*, 2011; Jia *et al.*, 2013) in

Arabidopsis corresponding about 5% (Mazzucotelli *et al.*, 2006), which is suggested by Gonzaléz et al. (2017) constitute an extensive repertoire of plants as sessile organisms to deal with environmental challenges. This hypothesis is corroborated by what occurs in chickpea for example, with 83% of F-box genes differentially expressed in at least one stress condition (Gupta *et al.*, 2015).

Beside examples where the expression of F-box increases stress tolerance (Jiang *et al.*, 2014), there are also contrary situations, as observed by Yan et al. (2011), where the overexpression of F-box proteins reduced tolerance to abiotic stress. In this context, further investigation of associated genes, such as targets, or stress response genes directly affected by the expression of F-box, is necessary to verify whether this role would be positive or negative in the fight against cactus scale and water deficit in prickly pear.

Non-specific serine/threonine protein kinase (spot 138) is part of largest protein phosphatase family in various plant genomes and are known to play important roles in eukaryotic signal transduction pathways for stress responses and development (Cui et al., 2013). Serine/threonine protein kinase play a central role in signaling during pathogen recognition, in effector-triggered immunity (ETI) of plants, through phosphorylation of the hidroxyl groups of serine or threonine residues leading to a functional change of the target protein and activation of plant defense mechanisms and developmental control (Hardie, 1999; Romeis, 2001; Afzal, Wood and Lightfoot, 2008).

They are regarded as receptors that recognize elicitors of the oral secretion of insects entering the plant during feeding, having been found 53 serine/threonine protein kinase genes upregulated and downregulated in response to *Ectropis oblique* feeding (Wang *et al.*, 2016). They were also found in non-stressed but neighboring plants under the influence of plant hormonal peptide and with injuries or under attack of herbivores, as an indicator of plant preparation through plant-to-plant communication (Coppola *et al.*, 2017). In addition to being observed in the modulation of antifungal defense (Krishnan *et al.*, 2015); in response to salt stress as a positive regulator (Sun *et al.*, 2013) or negative (Cui *et al.*, 2013); water deficit (Luo *et al.*, 2018); against tomato pathogens, involved both in the recognition of elicitors and in phosphorylation (Bogdanove and Martin, 1999).

Induction of phosphorylation or dephosphorylation of proteins largely participates in the regulation of stress in plants. This modification results from an initial perception of a primary extracellular signal or a set of signals that alter the receptor interaction partners. After this multifactorial binding event, there is the transmission of secondary signals through the plasma membrane, with the accumulation of intracellular signaling molecules leading to the induction of specific cascades of phosphorylation and dephosphorylation. This leads to specific plant responses with activation of genes involved in particular defense responses or alterations to developmental processes (Gachomo, Shonukan and Kotchoni, 2003).

The presence of this accumulated protein under stress in the prickly pear reflects the phosphorylation of specific molecules for subsequent induction of genes. It is essential to identify your targets in subsequent studies.

In the treatment under stress, it is possible see how the proteins may be interacting in sequence, in an attempt to combat the water deficit and the cactus scale (Figure 3). The remorin protein in one of its phosphorylated isoforms seems to be one of those that participate in the perception of stress, because it is present attached to plasma membrane, this leads signal to the cytoplasm, which can be perceived by proteins as non-specific serine/threonine protein kinase and calmodulin-binding receptor-like cytoplasmic kinase. From this, signal transduction can occur, generating alterations in gene expression, with the accumulation of proteins that participate in the regulation of transcription, as transcription factors: GATA19, HLH1; DNA unwinding proteins for transcription, helicases, and which may act specifically for stress conditions, ubiquitination proteins, F-box. These once may contribute to the

accumulation through the gene expression of ROS eliminating proteins with GRX C13; proteins that strengthen transport tissues, such as CCoAOMT, which participates in lignin biosynthesis; or enzymes that perform post-translational modifications to target functions of target proteins, such as Arginine-N-methyltransferase. At the same time there is regulation of energy maintenance of the plant, through PEPC accumulation.

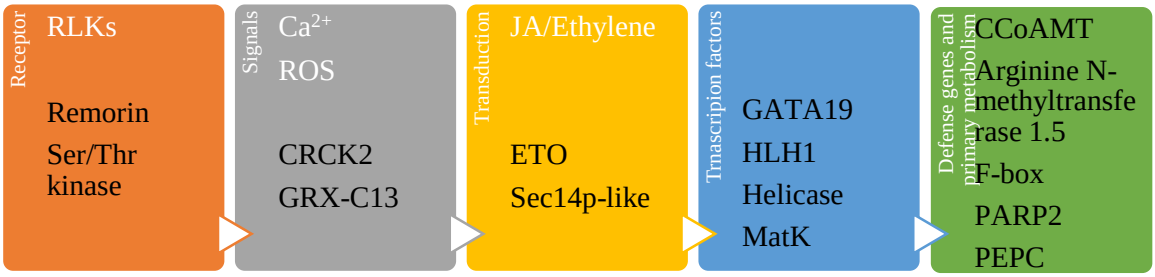

Figure 3. A schematic diagram illustrating the defense mechanisms induced by drought and *Diaspis echinocacti* in prickly pear.

4. Conclusion

Once that in agricultural ecosystems can occur variable stresses concomitantly and, in many intensities, to crops from semiarid it is important evaluation of how plant responds to combined stresses. According the relevance of the processes in which they participate to combat stresses, as well as reports from the literature of the involvement of these proteins in response to the insect, the proteins accumulated on stressed condition can be indicated as biomarkers to search in molecular level in other varieties.

The identified proteins, especially in the treatment under double stress, open a framework for further evaluation about this plant-insect interaction, to obtain more detailed later information, considering that many questions remaining, specially about how signaling between theses biotic and abiotic stresses cross-talk.

Author Contributions: T.C.J. and M.D.S.C. conceived and designed the experiments. M.D.S.C., E.P.N.P., J.P.O. and D.C.S. performed the experiments in greenhouse. M.D.S.C., E.P.N.P., M.S.O., F.A.C.S and T.C.J. performed the experiments on proteomics, data analysis and prepared the figures and tables. A.C.L.N., J.P.O., D.C.S., and T.C.J. provided plant and insect material. M.D.S.C., E.P.N.P., M.S.O., S.C.B.S.L., F.A.C.S. and T.C.J. helped on the analysis and interpretation of protein data. M.D.S.C. and T.C.J. wrote the original draft. M.D.S.C., E.P.N.P., M.S.O., F.A.C.S, S.C.B.S.L., A.C.L.N., J.P.O., D.C.S. and T.C.J. reviewed and edited the paper. All authors read and approved the final manuscript.

Conflicts of interest: The authors declare no conflicts of interest.

Acknowledgements: Authors are grateful to Agronomical Institute of Pernambuco (IPA) for providing the experimental station and all the support to install the experiment. We particularly thank to University of Nevada for providing database from Opuntia; Dr. Rodrigo Leandro Braga de Castro Coitinho and Dr. Antônio Felix da Costa for providing cactus scale-infested samples.

5. References

Afzal, A. J., Wood, A. J. and Lightfoot, D. A. (2008) 'Plant Receptor-Like Serine Threonine Kinases: Roles in Signaling and Plant Defense', *Molecular Plant-Microbe Interactions*, 21(5), pp. 507–517. doi: 10.1094/MPMI-21-5-0507.

Ahmad, A., Dong, Y. and Cao, X. (2011) 'Characterization of the PRMT gene family in rice

reveals conservation of arginine methylation', *PLoS ONE*, 6(8). doi: 10.1371/journal.pone.0022664.

Amor, Y. *et al.* (1998) 'The involvement of poly(ADP-ribose) polymerase in the oxidative stress responses in plants', *FEBS Letters*, 440(1–2), pp. 1–7. doi: 10.1016/S0014-5793(98)01408-2.

Aranjuelo, I. *et al.* (2011) 'Plant physiology and proteomics reveals the leaf response to drought in alfalfa (Medicago sativa L.)', *Journal of Experimental Botany*, 62(1), pp. 111–123. doi: 10.1093/jxb/erq249.

Arba, M. *et al.* (2017) 'Biology, flowering and fruiting of the cactus Opuntia spp.: A review and some observations on three varieties in Morocco', *Brazilian Archives of Biology and Technology*, 60, pp. 1–11. doi: 10.1590/1678-4324-2017160568.

Arruda Filho, G. P. and Arruda, G. P. (2002) 'Manejo integrado da cochonilha Diaspis echinocacti praga da palma forrageira em Brasil', *Manejo Integrado de Plagas y Agroecología*, pp. i–iv.

Arruda, G. P. (1983) *Aspectos etológicos da cochonilha da palma forrageira Diaspis echinocacti (Bouche, 1833) (Homoptera, Diaspididae)*. Universidade Federal Rural de Pernambuco.

Benschop, J. J. *et al.* (2007) 'Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis', *Molecular & Cellular Proteomics*, 6(7), pp. 1198–1214. doi: 10.1074/mcp.M600429-MCP200.

Boerjan, W., Ralph, J. and Baucher, M. (2003) 'Lignin biosynthesis', *Annual Review of Plant Biology*, 54(1), pp. 519–546. doi: 10.1146/annurev.arplant.54.031902.134938.

Bogdanove, A. J. and Martin, G. B. (1999) 'AvrPto-dependent Pto-interacting proteins and AvrPto-interacting proteins in tomato'.

Borges, L. R. *et al.* (2013) 'Selection of cactus pear clones regarding resistance to carmine cochineal Dactylopius opuntiae (Dactylopiidae)', *Acta Horticulturae*, 1, pp. 359–366. doi: 10.17660/ActaHortic.2013.995.47.

Brahim, S. *et al.* (2010) 'Leaf proteome responses of Arabidopsis thaliana exposed to mild cadmium stress', *Journal of Plant Physiology*, 167(4), pp. 247–254. doi: 10.1016/j.jplph.2009.09.015.

Bray, E. A. (2002) 'Abscisic acid regulation of gene expression during water- deficit stress in the era of the Arabidopsis genome', *Plant, Cell and Environment*, (25), pp. 153–161. Available at: http://www.thehindu.com/todays-paper/tp-national/tp-karnataka/gunashekar-castigates-bbmp-for-not-forming-tree-authority/article3495227.ece.

Briggs, A. G. *et al.* (2017) 'A transcriptomics approach uncovers novel roles for poly(ADP-ribosyl)ation in the basal defense response in Arabidopsis thaliana', *PLoS ONE*, 12(12), pp. 1–30. doi: 10.1371/journal.pone.0190268.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2015) *Biochemistry & molecular biology of plants*. 2nd edn. Edited by L. John Wiley & Sons. Rockville, Md.: American Society of Plant Physiologists, Willey Blackwell.

Burkhard, P., Stetefeld, J. and Strelkov, S. V (2001) 'Coiled coil: a highly versatile protein folding motif', *Trends in Cell Biology*, 11(2), pp. 82–88.

Callis, J. (2014) ‘The Ubiquitination Machinery of the Ubiquitin System’. doi: 10.1199/tab.0174.

Callis, J. and Vierstra, R. D. (2000) ‘Protein degradation is signaling’, *Cell signalling and gene regulation Figure*, (3), pp. 381–386. doi: 10.1007/s12190-012-0628-y.

Candiano, G. *et al.* (2004) ‘Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis’, *Electrophoresis*, 25(9), pp. 1327–1333. doi: 10.1002/elps.200305844.

Castrillón-Arbeláez, P. A. and Frier, J. P. D. (2016) ‘Secondary Metabolism in Amaranthus spp. — A Genomic Approach to Understand Its Diversity and Responsiveness to Stress in Marginally Studied Crops with High Agronomic Potential’, in Shanker, A. K. and Shanker, C. (eds) *Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives*. Rijeka, Croatia, pp. 183–228. doi: 10.5772/60477.

Checker, V. G. and Khurana, P. (2013) ‘Molecular and functional characterization of mulberry EST encoding remorin (MiREM) involved in abiotic stress’, *Plant Cell Reports*, 32(11), pp. 1729–1741. doi: 10.1007/s00299-013-1483-5.

Chen, C. *et al.* (2000) ‘Cell-Specific and Conditional Expression of Caffeoyl-Coenzyme A-3-O-Methyltransferase in Poplar’, *Plant Physiology*, 123(3), pp. 853–868. doi: 10.1104/pp.123.3.853.

Chen, L. H. *et al.* (2017) ‘A major facilitator superfamily transporter- mediated resistance to oxidative stress and fungicides requires Yap1, Skn7, and MAP kinases in the citrus fungal pathogen alternaria alternata’, *PLoS ONE*, 12(1), pp. 1–16. doi: 10.1371/journal.pone.0169103.

Chi, W. *et al.* (2015) ‘Plastid sigma factors: Their individual functions and regulation in transcription’, *Wood Science and Technology*. Elsevier B.V., pp. 770–778. doi: 10.1016/j.bbabio.2015.01.001.

Claps, L. E. and Haro, M. E. de (2001) ‘Coccoidea (Insecta: Hemiptera) associated with Cactaceae in Argentina’, *Journal of the Professional Association for Cactus Development*, 4, pp. 77–83.

Coppola, M. *et al.* (2017) ‘Plant-to-plant communication triggered by systemin primes anti-herbivore resistance in tomato’, *Scientific Reports*. Springer US, pp. 1–13. doi: 10.1038/s41598-017-15481-8.

Cui, M. H. *et al.* (2013) ‘An Arabidopsis R2R3-MYB transcription factor, AtMYB20, negatively regulates type 2C serine/threonine protein phosphatases to enhance salt tolerance’, *FEBS Letters*. Federation of European Biochemical Societies, 587(12), pp. 1773–1778. doi: 10.1016/j.febslet.2013.04.028.

Dang, P. M. *et al.* (2018) ‘Identification of expressed R -genes associated with leaf spot diseases in cultivated peanut’, *Molecular Biology Reports*. Springer Netherlands, 0(0), p. 0. doi: 10.1007/s11033-018-4464-5.

Dowd, P. F., Funnell-Harris, D. L. and Sattler, S. E. (2016) ‘Field damage of sorghum (Sorghum bicolor) with reduced lignin levels by naturally occurring insect pests and pathogens’, *Journal of Pest Science*. Springer Berlin Heidelberg, 89(4), pp. 885–895. doi: 10.1007/s10340-015-0728-1.

Duan, C. *et al.* (2014) ‘Induced defense responses in rice plants against small brown planthopper infestation’, *Crop Journal*. Crop Science Society of China and Institute of Crop

Sciences, CAAS, 2(1), pp. 55–62. doi: 10.1016/j.cj.2013.12.001.

Fang, X. *et al.* (2015) 'Proteomic dissection of plant responses to various pathogens', *Proteomics*, 15(9), pp. 1525–1543. doi: 10.1002/pmic.201400384.

Farmer, E. E., Pearce, G. and Ryan, C. A. (1989) 'In vitro phosphorylation of plant plasma membrane proteins in response to the proteinase inhibitor inducing factor', *Proceedings of the National Academy of Sciences of the United States of America*, 86(March), pp. 1539–1542. doi: 10.1073/pnas.86.5.1539.

Gachomo, E. W., Shonukan, O. O. and Kotchoni, S. O. (2003) 'The molecular initiation and subsequent acquisition of disease resistance in plants', *African Journal of Biotechnology*, 2(2), pp. 26–32.

Gagne, J. M. *et al.* (2002) 'Basic and Clinical Pharmacology 11th ed. - B. Katzung, et. al., (Lange, 2009) WW.pdf', 99(17). doi: 10.1073/pnas.162339999.

Ghosh, R. *et al.* (2014) 'Characterization of developmental- and stress-mediated expression of Cinnamoyl-CoA reductase in kenaf (Hibiscus cannabinus L.)', *The Scientific World Journal*. Hindawi Publishing Corporation. doi: 10.1155/2014/601845.

Gonzalez, L. E. *et al.* (2017) 'Transcriptome analysis uncovers Arabidopsis F-BOX STRESS INDUCED 1 as a regulator of jasmonic acid and abscisic acid stress gene expression', *BMC Genomics*. BMC Genomics, 18(1), pp. 1–15. doi: 10.1186/s12864-017-3864-6.

Goossens, J., Mertens, J. and Goossens, A. (2017) 'Role and functioning of bHLH transcription factors in jasmonate signalling', *Journal of Experimental Botany*, 68(6), pp. 1333–1347. doi: 10.1093/jxb/erw440.

Graciet, E. *et al.* (2009) 'The N-end rule pathway controls multiple functions during Arabidopsis shoot and leaf development', *Proceedings of the National Academy of Sciences*, 106(32), pp. 13618–13623. doi: 10.1073/pnas.0906404106.

Griffiths, H. *et al.* (2002) 'Regulation of Rubisco activity in crassulacean acid metabolism plants: better late than never', *Functional Plant Biology*, 29, pp. 689–696. doi: 10.1071/PP01212.

Gupta, S. *et al.* (2015) 'Genome-wide survey and expression analysis of F-box genes in chickpea', *BMC Genomics*, 16(1), pp. 1–15. doi: 10.1186/s12864-015-1293-y.

Gutsche, N. *et al.* (2015) 'Plant-specific CC-type glutaredoxins: Functions in developmental processes and stress responses', *Biological Chemistry*, 396(5), pp. 495–509. doi: 10.1515/hsz-2014-0300.

Hamann, T. (2012) 'Plant cell wall integrity maintenance as an essential component of biotic stress response mechanisms', *Frontiers in Plant Science*, 3(April), pp. 1–5. doi: 10.3389/fpls.2012.00077.

Hardie, D. G. (1999) 'Plant Protein Serine/Threonine Kinases: Classification and Functions', *Annual Review of Plant Physiology and Plant Molecular Biology*, 50(1), pp. 97–131. doi: 10.1146/annurev.arplant.50.1.97.

Hernando, C. E. *et al.* (2015) 'Genome wide comparative analysis of the effects of PRMT5 and PRMT4/CARM1 arginine methyltransferases on the Arabidopsis thaliana transcriptome', *BMC Genomics*, 16(1), pp. 1–15. doi: 10.1186/s12864-015-1399-2.

Hu, Y. *et al.* (2009) 'Differential expression of candidate genes for lignin biosynthesis under

drought stress in maize leaves', *Journal of Applied Genetics*, 50(3), pp. 213–223. doi: 10.1007/BF03195675.

Hua, Z. *et al.* (2011) 'Phylogenetic comparison of F-Box (FBX) gene superfamily within the plant kingdom reveals divergent evolutionary histories indicative of genomic drift', *PLoS ONE*, 6(1). doi: 10.1371/journal.pone.0016219.

Hua, Z. and Vierstra, R. D. (2011) 'The Cullin-RING Ubiquitin-Protein Ligases', *Annual Review of Plant Biology*, 62(1), pp. 299–334. doi: 10.1146/annurev-arplant-042809-112256.

Inglese, P. (2010) *Cactus pear Opuntia ficus-indica L. (Mill.) for fruit production: an overview*, *Cactusnet Newsletter*. Edited by A. Nefzaoui, P. Inglese, and T. Belay. Improved utilization of cactus pear for food, feed, soil and water conservation and other products in Africa.

Ishihama, A. (1988) 'Promoter selectivity of prokaryotic RNA polymerases', *Trends in Genetics*, 4(10), pp. 282–286. doi: 10.1016/0168-9525(88)90170-9.

Jannoey, P. *et al.* (2015) 'Comparative proteomic analysis of two rice cultivars (Oryza sativa L.) contrasting in brown planthopper (BPH) stress resistance', *Plant OMICS*, 8(2), pp. 96–105.

Jia, F. *et al.* (2013) 'Genome-wide identification and characterisation of F-box family in maize', *Molecular Genetics and Genomics*, 288(11), pp. 559–577. doi: 10.1007/s00438-013-0769-1.

Jiang, Q. *et al.* (2014) 'Overexpression of GmDREB1 improves salt tolerance in transgenic wheat and leaf protein response to high salinity', *Crop Journal*. Elsevier B.V., 2(2–3), pp. 120–131. doi: 10.1016/j.cj.2014.02.003.

Kanamaru, K. and Tanaka, K. (2004) 'Roles of Chloroplast RNA Polymerase Sigma Factors in Chloroplast Development and Stress Response in Higher Plants', *Plant Cell*, 68(11), pp. 2215–2223.

Kiba, A. *et al.* (2016) 'Silencing of Nicotiana benthamiana SEC14 phospholipid transfer protein reduced jasmonic acid dependent defense against Pseudomonas syringae', *Plant Biotechnology*, 33(2), pp. 111–115. doi: 10.5511/plantbiotechnology.16.0503a.

Kim, H. S. and Delaney, T. P. (2002) 'Arabidopsis SON1 Is an F-Box Protein That Regulates a Novel Induced Defense Response Independent of Both Salicylic Acid and Systemic Acquired Resistance', *The Plant Cell*, 14(7), pp. 1469–1482. doi: 10.1105/tpc.001867.

Krishnan, A. *et al.* (2015) 'Virus-induced gene silencing (VIGS) for elucidation of pathogen defense role of serine / threonine protein kinase in the non-model', *Plant Cell, Tissue and Organ Culture (PCTOC)*. Springer Netherlands, pp. 269–283. doi: 10.1007/s11240-015-0764-9.

Kwon, Y. T., Kashina, a S. and Varshavsky, A. (1999) 'Alternative splicing results in differential expression, activity, and localization of the two forms of arginyl-tRNA-protein transferase, a component of the N-end rule pathway.', *Molecular and cellular biology*, 19(1), pp. 182–193. doi: 10.1128/MCB.19.1.182.

Laloi, M. *et al.* (2006) 'Insights into the Role of Specific Lipids in the Formation and Delivery of Lipid Microdomains to the Plasma Membrane of Plant Cells', *Plant Physiology*, 143(1), pp. 461–472. doi: 10.1104/pp.106.091496.

Lamb, R. S., Citarelli, M. and Teotia, S. (2012) 'Functions of the poly(ADP-ribose) polymerase superfamily in plants', *Cellular and Molecular Life Sciences*, 69(2), pp. 175–189. doi: 10.1007/s00018-011-0793-4.

Lefebvre, B. *et al.* (2007) 'Characterization of Lipid Rafts from Medicago truncatula Root Plasma Membranes: A Proteomic Study Reveals the Presence of a Raft-Associated Redox System', *Plant Physiology*, 144(1), pp. 402–418. doi: 10.1104/pp.106.094102.

Lefebvre, B. *et al.* (2010) 'A remorin protein interacts with symbiotic receptors and regulates bacterial infection', *Proceedings of the National Academy of Sciences*, 107(5), pp. 2343–2348. doi: 10.1073/pnas.0913320107.

Lerbs-Mache, S. (2011) 'Function of plastid sigma factors in higher plants: Regulation of gene expression or just preservation of constitutive transcription?', *Plant Molecular Biology*, 76(3–5), pp. 235–249. doi: 10.1007/s11103-010-9714-4.

Li, D. *et al.* (2008) 'OsBIRH1, a DEAD-box RNA helicase with functions in modulating defence responses against pathogen infection and oxidative stress', *Journal of Experimental Botany*, 59(8), pp. 2133–2146. doi: 10.1093/jxb/ern072.

Li, Q. *et al.* (2018) 'Wheat F-Box Protein Gene TaFBA1 Is Involved in Plant Tolerance to Heat Stress', *Frontiers in Plant Science*, 9(April), pp. 1–15. doi: 10.3389/fpls.2018.00521.

Li, S. *et al.* (2013) 'Molecular cloning and functional analysis of the Populus deltoides remorin gene PdREM', *Tree Physiology*, 33(10), pp. 1111–1121. doi: 10.1093/treephys/tpt072.

Liang, X. and Zhou, J.-M. (2018) 'Receptor-Like Cytoplasmic Kinases: Central Players in Plant Receptor Kinase–Mediated Signaling', *Annual Review of Plant Biology*, 69(1), pp. 267–299. doi: 10.1146/annurev-arplant-042817-040540.

Lin, W. *et al.* (2013) 'Big Roles of Small Kinases: The Complex Functions of Receptor-Like Cytoplasmic Kinases in Plant Immunity and Development', *Journal of Integrated Plant Biology*, 55(12), pp. 1188–1197. doi: 10.1111/jipb.12071.Big.

Liu, J. *et al.* (2009) 'RIN4 functions with plasma membrane H+-ATPases to regulate stomatal apertures during pathogen attack', *PLoS Biology*, 7(6). doi: 10.1371/journal.pbio.1000139.

Liu, Q., Luo, L. and Zheng, L. (2018) 'Lignins: Biosynthesis and biological functions in plants', *International Journal of Molecular Sciences*, 19(2). doi: 10.3390/ijms19020335.

Lohse, M. *et al.* (2014) 'Mercator: A fast and simple web server for genome scale functional annotation of plant sequence data', *Plant, Cell and Environment*, 37(5), pp. 1250–1258. doi: 10.1111/pce.12231.

Lonetto, M., Gribskov, M. and Gross, C. (1992) 'The σ7O Family : Sequence Conservation and Evolutionary Relationships', *Journal of Bacteriology*, 174(12), pp. 3843–3849. doi: 10.1128/jb.174.12.3843-3849.1992.

Longo, S. and Rapisarda, C. (2001) 'Pragas da Palma Forrageira', in *Agroecologia, cultivos e usos da palma forrageira*. João Pessoa: SEBRAE-PB, p. 241.

Lopes, E. B. (2007) *Palma forrageira: cultivo, uso atual e perspectivas de utilização no semiárido nordestino, Empresa estadual de Pesquisa Agropecuária da Paraíba S.A.*

Lopes, E. B. *et al.* (2010) 'Seleção de genótipos de palma forrageira (Opuntia spp.) e (Nopalea spp.) resistentes à cochonilha-do-carmim (Dactylopius opuntiae Cockerell, 1929) na

Paraíba, Brasil', *Engenharia Ambiental*, 7, pp. 204–215.

Luo, F. *et al.* (2018) 'Identification of phosphorylation proteins in response to water deficit during wheat flag leaf and grain development', *Botanical Studies*. Springer Berlin Heidelberg. doi: 10.1186/s40529-018-0245-7.

Mao, K. *et al.* (2017) 'Genome Wide Identification and Characterization of Apple bHLH Transcription Factors and Expression Analysis in Response to Drought and Salt Stress', *Frontiers in Plant Science*, 8(April). doi: 10.3389/fpls.2017.00480.

Marín, M. and Ott, T. (2012) 'Phosphorylation of Intrinsically Disordered Regions in Remorin Proteins', *Frontiers in Plant Science*, 3(May), pp. 1–6. doi: 10.3389/fpls.2012.00086.

Massari, M. E. and Murre, C. (2000) 'Helix-Loop-Helix Proteins: Regulators of Transcription in Eucaryotic Organisms', *Molecular and Cellular Biology*, 20(2), pp. 429–440. doi: 10.1128/MCB.20.2.429-440.2000.

Mazzucotelli, E. *et al.* (2006) 'The E3 Ubiquitin Ligase Gene Family in Plants: Regulation by Degradation', *Current Genomics*, 7(8), pp. 509–522. doi: 10.2174/138920206779315728.

Miedes, E. *et al.* (2014) 'The role of the secondary cell wall in plant resistance to pathogens', *Frontiers in Plant Science*, 5(August), pp. 1–13. doi: 10.3389/fpls.2014.00358.

Mongrand, S. *et al.* (2004) 'Lipid rafts in higher plant cells: Purification and characterization of triton X-100-insoluble microdomains from tobacco plasma membrane', *Journal of Biological Chemistry*, 279(35), pp. 36277–36286. doi: 10.1074/jbc.M403440200.

Morita, S. *et al.* (2015) 'Expression of a rice glutaredoxin in aleurone layers of developing and mature seeds: subcellular localization and possible functions in antioxidant defense', *Planta*. Springer Berlin Heidelberg, 242(5), pp. 1195–1206. doi: 10.1007/s00425-015-2354-9.

Moura, J. C. M. S. *et al.* (2010) 'Abiotic and biotic stresses and changes in the lignin content and composition in plants', *Journal of Integrative Plant Biology*, 52(4), pp. 360–376. doi: 10.1111/j.1744-7909.2010.00892.x.

Nabity, P. D., Zavala, J. A. and DeLucia, E. H. (2009) 'Indirect suppression of photosynthesis on individual leaves by arthropod herbivory', *Annals of Botany*, 103(4), pp. 655–663. doi: 10.1093/aob/mcn127.

Nagashima, A. *et al.* (2004) 'The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the psbD Blue Light-Responsive Promoter (BLRP) in Arabidopsis thaliana', *Plant and Cell Physiology*, 45(4), pp. 357–368. doi: 10.1093/pcp/pch050.

Ndamukong, I. *et al.* (2007) 'SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription', *The Plant Journal*, 50, pp. 128–139. doi: 10.1111/j.1365-313X.2007.03039.x.

Owttrim, G. W. (2006) 'RNA helicases and abiotic stress', *Nucleic Acids Research*, 34(11), pp. 3220–3230. doi: 10.1093/nar/gkl408.

Pandey, S. P. *et al.* (2017) 'Simulated herbivory in chickpea causes rapid changes in defense pathways and hormonal transcription networks of JA/ethylene/GA/auxin within minutes of wounding', *Scientific Reports*. Nature Publishing Group, 7(March), pp. 1–14. doi: 10.1038/srep44729.

Park, H. L. *et al.* (2017) 'Biochemical and Expression Analyses of the Rice Cinnamoyl-CoA

Reductase Gene Family', *Frontiers in Plant Science*, 8(December), pp. 1–14. doi: 10.3389/fpls.2017.02099.

Parry, M. A. J. *et al.* (2002) 'Rubisco activity: Effects of drought stress', *Annals of Botany*, 89(SPEC. ISS.), pp. 833–839. doi: 10.1093/aob/mcf103.

Raffaele, S. *et al.* (2009) 'Remorin, a Solanaceae Protein Resident in Membrane Rafts and Plasmodesmata, Impairs Potato virus X Movement', *the Plant Cell Online*, 21(5), pp. 1541–1555. doi: 10.1105/tpc.108.064279.

Rissel, D. *et al.* (2017) 'No Silver Bullet – Canonical Poly(ADP-Ribose) Polymerases (PARPs) Are No Universal Factors of Abiotic and Biotic Stress Resistance of Arabidopsis thaliana', *Frontiers in Plant Science*, 08(February), pp. 1–14. doi: 10.3389/fpls.2017.00059.

Rodriguez-Saona, C. R. *et al.* (2010) 'Molecular, Biochemical, and Organismal Analyses of Tomato Plants Simultaneously Attacked by Herbivores from Two Feeding Guilds', *Journal of Chemical Ecology*, 36(10), pp. 1043–1057. doi: 10.1007/s10886-010-9854-7.

Romeis, T. (2001) 'Protein kinases in the plant defence response', *Current Opinion in Plant Biology: Cell signalling and gene regulation*, (4), pp. 407–414.

Sáenz, C. *et al.* (2013) 'Opuntias as a natural resource', in *Agro-industrial utilization of cactus pear*, p. 168. Available at: www.fao.org/publications.

Saito, K., Tautz, L. and Mustelin, T. (2007) 'The lipid-binding SEC14 domain', *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1771(6), pp. 719–726. doi: 10.1016/j.bbalip.2007.02.010.

Sanan-Mishra, N. *et al.* (2005) 'Pea DNA helicase 45 overexpression in tobacco confers high salinity tolerance without affecting yield', *Proceedings of the National Academy of Sciences*, 102(2), pp. 509–514. doi: 10.1073/pnas.0406485102.

Santiago, R., Barros-Rios, J. and Malvar, R. A. (2013) 'Impact of cell wall composition on maize resistance to pests and diseases', *International Journal of Molecular Sciences*, 14(4), pp. 6960–6980. doi: 10.3390/ijms14046960.

Sha, S., Minakuchi, K. and Higaki, N. (1997) 'Purification and characterization of glutaredoxin (thioltransferase) from rice (Oryza sativa L.)', *Journal of Biochemistry*, 848, pp. 842–848. Available at: http://jb.oxfordjournals.org/content/121/5/842.short.

Sharma, R., Priya, P. and Jain, M. (2013) 'Modified expression of an auxin-responsive rice CC-type glutaredoxin gene affects multiple abiotic stress responses', *Planta*, 238(5), pp. 871–884. doi: 10.1007/s00425-013-1940-y.

Sheibani-Tezerji, R. *et al.* (2015) 'Transcriptome profiling of the endophyte burkholderia phytofirmans psjn indicates sensing of the plant environment and drought stress', *mBio*, 6(5), pp. 1–11. doi: 10.1128/mBio.00621-15.

Song, D. *et al.* (2011) 'Characterization of the plasma membrane proteins and receptor-like kinases associated with secondary vascular differentiation in poplar', *Plant Molecular Biology*, 76(1–2), pp. 97–115. doi: 10.1007/s11103-011-9771-3.

Song, J. *et al.* (2015) 'PARP2 Is the Predominant Poly(ADP-Ribose) Polymerase in Arabidopsis DNA Damage and Immune Responses', *PLoS Genetics*, 11(5), pp. 1–24. doi: 10.1371/journal.pgen.1005200.

Srivastava, S. *et al.* (2015) 'Abiotic stress induces change in Cinnamoyl CoA Reductase

(CCR) protein abundance and lignin deposition in developing seedlings of Leucaena leucocephala', *Physiology and Molecular Biology of Plants*, 21(2), pp. 197–205. doi: 10.1007/s12298-015-0289-z.

Sun, X. *et al.* (2013) 'GsSRK , a G-type lectin S-receptor-like serine / threonine protein kinase , is a positive regulator of plant tolerance to salt stress', *Journal of Plant Physiology*. Elsevier GmbH., 170(5), pp. 505–515. doi: 10.1016/j.jplph.2012.11.017.

Vanderauwera, S. *et al.* (2007) 'Silencing of poly(ADP-ribose) polymerase in plants alters abiotic stress signal transduction Sandy', *PNAS*, 104(38), pp. 15150–15155. doi: 10.1517/14656566.9.11.1829.

Vasconcelos, A. G. V. *et al.* (2009) 'Seleção de clones de palma forrageira resistentes à cochonilha-do-carmim (Dactylopius sp)', *Revista Brasileira de Zootecnia*, 38(5), pp. 827–831. doi: 10.1590/S1516-35982009000500007.

Vashisht, A. A. and Tuteja, N. (2006) 'Stress responsive DEAD-box helicases: A new pathway to engineer plant stress tolerance', *Journal of Photochemistry and Photobiology B: Biology*, 84(2), pp. 150–160. doi: 10.1016/j.jphotobiol.2006.02.010.

Vaudel, M. *et al.* (2011) 'SearchGUI: An open-source graphical user interface for simultaneous OMSSA and X!Tandem searches', *Proteomics*, 11(5), pp. 996–999. doi: 10.1002/pmic.201000595.

Vaudel, M. *et al.* (2015) 'PeptideShaker enables reanalysis of MS-derived proteomics data sets: To the editor', *Nature Biotechnology*, 33(1), pp. 22–24. doi: 10.1038/nbt.3109.

Vij, S. *et al.* (2008) 'The receptor-like cytoplasmic kinase (OsRLCK) gene family in rice: Organization, phylogenetic relationship, and expression during development and stress', *Molecular Plant*. The Authors 2008., 1(5), pp. 732–750. doi: 10.1093/mp/ssn047.

Wang, R. *et al.* (2018) 'Genome-wide identification and characterization of the Potato bHLH Transcription factor family', *Genes*, 9(1). doi: 10.3390/genes9010054.

Wang, X. *et al.* (2016) 'Drought-Responsive Mechanisms in Plant Leaves Revealed by Proteomics', *International Journal of Molecular Sciences*, 17, pp. 1–30. doi: 10.3390/ijms17101706.

Wang, Y. *et al.* (2016) 'Differential transcriptome analysis of leaves of tea plant (Camellia sinensis) provides comprehensive insights into the defense responses to Ectropis oblique attack using', *Functional & Integrative Genomics*. Functional & Integrative Genomics, pp. 383–398. doi: 10.1007/s10142-016-0491-2.

Wang, Y. *et al.* (2017) 'CmMYB19 over-expression improves aphid tolerance in chrysanthemum by promoting lignin synthesis', *International Journal of Molecular Sciences*, 18(3). doi: 10.3390/ijms18030619.

Warumby, J. F., Arruda Filho, G. P. and Cavalcanti, V. A. L. B. (2005) 'Pragas da palma', in Menezes, R. S. C., Simões, D. A., and Sampaio, E. V. S. B. (eds) *A palma no Nordeste do Brasil*, pp. 65–80.

Webster, J. and Oxley, D. (2005) 'Peptide mass fingerprinting: protein identification using MALDI-TOF mass spectrometry', *Methods Mol Biol.*, 310, pp. 227–240.

Widjaja, I. *et al.* (2009) 'Combining subproteome enrichment and Rubisco depletion enables identification of low abundance proteins differentially regulated during plant defense',

Proteomics, 9(1), pp. 138–147. doi: 10.1002/pmic.200800293.

Wu, X. *et al.* (2014) 'Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis', *Nature Protocols*, 9(2), pp. 362–374. doi: 10.1038/nprot.2014.022.

Yan, Y. S. *et al.* (2011) 'Overexpression of an F-box protein gene reduces abiotic stress tolerance and promotes root growth in rice', *Molecular Plant*, 4(1), pp. 190–197. doi: 10.1093/mp/ssq066.

Yang, T. *et al.* (2004) 'Calcium/calmodulin up-regulates a cytoplasmic receptor-like kinase in plants', *Journal of Biological Chemistry*, 279(41), pp. 42552–42559. doi: 10.1074/jbc.M402830200.

Yao, Y. *et al.* (2011) 'Comparative proteome analysis of metabolic changes by low phosphorus stress in two Brassica napus genotypes', *Planta*, 233(3), pp. 523–537. doi: 10.1007/s00425-010-1311-x.

Ye, Z. H. *et al.* (2001) 'Caffeoyl coenzyme A O-methyltransferase and lignin biosynthesis', *Phytochemistry*, 57(7), pp. 1177–1185. doi: 10.1016/S0031-9422(01)00051-6.

Yu, X. *et al.* (2016) 'A comparative proteomic study of drought-tolerant and drought-sensitive soybean seedlings under drought stress', *Crop and Pasture Science*, 67(5), pp. 528–540. doi: 10.1071/CP15314.

Zhang, T. *et al.* (2018) 'Genome-wide analysis of the basic Helix-Loop-Helix (bHLH) transcription factor family in maize', *BMC Plant Biology*. BMC Plant Biology, 18(1), pp. 1–14. doi: 10.1186/s12870-018-1441-z.

Zhao, Q. and Dixon, R. A. (2014) 'Altering the Cell Wall and Its Impact on Plant Disease: From Forage to Bioenergy', *Annual Review of Phytopathology*, 52(1), pp. 69–91. doi: 10.1146/annurev-phyto-082712-102237.

Zhou, S. *et al.* (2015) 'Alteration of plant primary metabolism in response to insect herbivory', *Plant Physiology*, 169(November), p. pp.01405.2015. doi: 10.1104/pp.15.01405.

Zörb, C., Schmitt, S. and Mühling, K. H. (2010) 'Proteomic changes in maize roots after short-term adjustment to saline growth conditions', *Proteomics*, 10(24), pp. 4441–4449. doi: 10.1002/pmic.201000231.

Zoschke, R. *et al.* (2010) 'An organellar maturase associates with multiple group II introns', *Proceedings of the National Academy of Sciences*, 107(7), pp. 3245–3250. doi: 10.1073/pnas.0909400107.

5 CONSIDERAÇÕES FINAIS

A tolerância a estresses bióticos causados por insetos vem sendo bastante estudada, no entanto, os organismos envolvidos nesta interação revelam respostas específicas. Em se tratando principalmente de herbívoros especialistas, como a cochonilha-de-escama, que coevoluíram conforme características das espécies hospedeiras, há respostas específicas da planta. Desta maneira, estudos particularizados sobre a resposta molecular da palma ao inseto viabilizam a compreensão dos mecanismos moleculares envolvidos na regulação de vias durante infestação.

Este estudo promove o entendimento pioneiro em um nível inicial da interação entre palma forrageira e cochonilha-de-escama, proporcionando a busca por variedades tolerantes à praga de forma mais direcionada, através dos biomarcadores candidatos encontrados, com maior probabilidade de resultados positivos e confiáveis, associando diferentes regimes de irrigação.

Avanços nas pesquisas a respeito desta interação, considerando aspectos como elicitores do inseto, compostos voláteis da planta, vias hormonais envolvidas e resposta precoce da planta complementarão a compreensão e a busca por tolerância desta praga.

REFERÊNCIAS

AEBERSOLD, R.; MANN, M. Mass-spectrometric exploration of proteome structure and function. **Nature**, v. 537, n. 7620, p. 347–355, 2016.

AHMED, N. Z.; AHMED-FARID, O. A. Assessment of in-vitro and in-vivo bioactivities potential of some egyptian wild plants as anti-ulcerogenic effect on male albino rats. **The Pharmaceutical and Chemical Journal**, v. 4, n. 6, p. 68–86, 2017.

ALBUQUERQUE, S. G. Cultivo da Palma Forrageira no Sertão do São Francisco. **Comunicado Técnico da Embrapa Semi-Árido**, p. 1–6, 2000.

AMORIM, P. L. de. **Caracterização morfológica e produtiva de variedades de palma forrageira**. 2011. Universidade Federal de Alagoas, 2011.

AMOUROUX, P. et al. Genetic diversity of armored scales (Hemiptera : Diaspididae) and soft scales (Hemiptera: Coccidae) in Chile. **Scientific Reports**, v. 7, p. 1–12, 2017. Disponível em: <http://dx.doi.org/10.1038/s41598-017-01997-6>.

ANDERSON, E. F. Classification of cacti. In: **The cactus family**. Portland: Timber Press, 2001. p. 484–526.

ANDERSON, L.; SEILHAMER, J. A comparison of selected mRNA and protein abundances in human liver. **Electrophoresis**, v. 18, n. 3–4, p. 533–537, 1997.

APEL, K.; HIRT, H. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. **Annual Review of Plant Biology**, v. 55, n. 1, p. 373–399, 2004. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.arplant.55.031903.141701>.

APG IV. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. **Botanical Journal of the Linnean Society**, v. 181, p. 1–20, 2016.

ARBA, M. et al. Biology, flowering and fruiting of the cactus Opuntia spp.: A review and some observations on three varieties in Morocco. **Brazilian Archives of Biology and Technology**, v. 60, p. 1–11, 2017.

ARIAS-MORENO, D. M. et al. Effects of catalase on chloroplast arrangement in Opuntia streptacantha chlorenchyma cells under salt stress. **Scientific Reports**, v. 7, n. 1, p. 1–14, 2017.

ARORA, R.; SANDHU, S. Insect-Plant Interrelationships. In: ARORA, R.; SANDHU, S. (Ed.). **Breeding Insect Resistant Crops for Sustainable Agriculture**. 1. ed. Ludhiana: Springer Singapore, 2017. p. 1–421.

ARRUDA FILHO, G. P.; ARRUDA, G. P. Manejo integrado da cochonilha Diaspis echinocacti praga da palma forrageira em Brasil. **Manejo Integrado de Plagas y Agroecología**, p. i–iv, 2002.

ARRUDA, G. P. **Aspectos etológicos da cochonilha da palma forrageira Diaspis echinocacti (Bouche, 1833) (Homoptera, Diaspididae)**. 1983. Universidade Federal Rural de Pernambuco, 1983.

BADOWIEC, A.; WEIDNER, S. Proteomic changes in the roots of germinating Phaseolus vulgaris seeds in response to chilling stress and post-stress recovery. **Journal of Plant Physiology**, v. 171, n. 6, p. 389–398, 2014. Disponível em: <http://dx.doi.org/10.1016/j.jplph.2013.10.020>.

BALEN, B. et al. Determination of Mammillaria gracillis N-glycan Patterns by ESI Q-TOF Mass Spectrometry. **Croatica Chemica Acta**, v. 78, n. 3, p. 463–477, 2005.

BALEN, B. et al. Glycoproteomic survey of Mammillaria gracillis tissues grown in vitro. **Journal of Proteome Research**, v. 5, n. 7, p. 1658–1666, 2006.

BALLHORN, D. J.; KAUTZ, S.; LIEBEREI, R. Comparing responses of generalist and specialist herbivores to various cyanogenic plant features. **Entomologia Experimentalis et Applicata**, v. 134, n. 3, p. 245–259, 2010.

BARBERA, G. História e Importância Econômica e Agroecológica. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 1–11.

BARBERA, G.; CARIMI, F.; INGLESE, P. The reflowering of prickly pear Opuntia ficusindica (L.) Miller: Influence of removal time and cladode load on yield and fruit ripening. **Advances in Horticultural Science**, v. 5, p. 77–80, 1991.

BARREIROS, A. L. B. S.; DAVID, J. P. Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo. **Quimica Nova**, v. 29, n. 1, p. 113–123, 2006.

BEARDSLEY, J. W.; GONZALEZ, R. H. The Biology and Ecology of Armored Scales. **Annual Review of Entomology**, v. 20, n. 2, p. 47–73, 1975.

BELTRAN, P. M. J. et al. Proteomics and integrative omic approaches for understanding host–pathogen interactions and infectious diseases. **Molecular Systems Biology**, v. 13, n. 3, p. 922, 2017. Disponível em: <http://msb.embopress.org/lookup/doi/10.15252/msb.20167062>.

BERGMÜLLER, E.; BAGINSKY, S.; GRUISSEM, W. Mass spectrometry-based proteomics: ientifying plant. In: AGRAWAL, G. K.; RAKWAL, R. (Ed.). **Plant Proteomics: technologies, strategies, and applications**. [s.l.] John Wiley & Sons, 2008. p. 33–46.

BHASKARA, G. B.; YANG, T.; VERSLUES, P. E. Dynamic proline metabolism : importance and regulation in water. **Frontiers in Plant Science**, v. 6, n. June, p. 1–7, 2015.

BHATTACHARJEE, S. Sites of generation and physicochemical basis of formation of reactive oxygen species in plant cell. In: GUPTA, S. D. (Ed.). **Reactive Oxygen Species and Antioxidants in Higher Plants**. Enfield: Science Publishers, 2010. p. 1–30.

BLOCK, A. et al. Herbivore-derived fatty-acid amides elicit reactive oxygen species burst in plants. **Journal of Experimental Botany**, v. 69, n. 5, p. 1235–1245, 2017.

BLOKHINA, O.; VIROLAINEN, E.; FAGERSTEDT, K. V. Antioxidants , Oxidative Damage and Oxygen Deprivation Stress : a Review. **Annals of Botany**, v. 91, p. 179–194, 2003.

BORN, F. S. et al. Alternative and biological control of Diaspis echinocacti (Bouché, 1833) (Hemiptera, Sternorrhyncha: Diaspididae) in prickly pear. **Acta Horticulturae**, v. 811, p. 223–226, 2009.

BOTHA, A. M. et al. Is photosynthetic transcriptional regulation in Triticum aestivum L. cv. “TugelaDN” a contributing factor for tolerance to Diuraphis noxia (Homoptera: Aphididae)? **Plant Cell Reports**, v. 25, n. 1, p. 41–54, 2006.

BOWERS, J. E. More flowers or new cladodes ? environmental correlates and

biological consequences of sexual reproduction in a Sonoran Desert prickly pear cactus, Opuntia engelmannii. **Bulletin of the Torrey Botanical Club**, v. 123, n. 1, p. 34–40, 1996.

BRADSHAW, R. A. An introduction to proteomics: applications to plant biology. In: AGRAWAL, G. K.; RAKWAL, R. (Ed.). **Plant Proteomics: technologies, strategies, and applications**. [s.l.] John Wiley & Sons, 2008. p. 1–10.

BRAHIM, S. et al. Leaf proteome responses of Arabidopsis thaliana exposed to mild cadmium stress. **Journal of Plant Physiology**, v. 167, n. 4, p. 247–254, 2010.

BRASIL. MINISTÉRIO DA AGRICULTURA PECUÁRIA E ABASTECIMENTO (MAPA). **Cultivares Registradas**. Disponível em: <http://sistemas.agricultura.gov.br/snpc/cultivarweb/cultivares_registradas.php>. Acesso em: 10 fev. 2018.

BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. **Biochemistry & molecular biology of plants**. 2. ed. Rockville, Md.: American Society of Plant Physiologists, Willey Blackwell, 2015.

CABALLERO, A.; RAMOS-PORTILLA, A. A.; KONDO, T. Scale insects (Hemiptera: Coccomorpha) on sugarcane in Colombia, with description of a new species of Tillancoccus Ben-Dov (Coccidae). **Zootaxa**, v. 4258, n. 5, p. 490–500, 2017.

CALDANA, C. et al. High-density kinetic analysis of the metabolomic and transcriptomic response of Arabidopsis to eight environmental conditions. **The Plant Journal**, v. 67, p. 869–884, 2011.

CARVALHO, H. M. C. de. Drought stress and reactive oxygen species. **Plant Signaling & Behavior**, v. 3, n. 3, p. 156–165, 2008.

ČERNÝ, M. et al. Role of the proteome in phytohormonal signaling. **Biochimica et Biophysica Acta - Proteins and Proteomics**, v. 1864, n. 8, p. 1003–1015, 2016.

CEUSTERS, J.; BORLAND, A. M.; PROFT, M. P. De. Drought adaptation in plants with crassulacean acid metabolism involves the flexible use of different storage carbohydrate pools. **Plant Signaling & Behavior**, v. 4, n. 3, p. 212–214, 2009.

CHEN, S.; HARMON, A. C. Advances in plant proteomics. **Proteomics**, p. 5504–5516, 2006.

CHIOZZA, M. V; NEAL, M. E. O. Constitutive and Induced Differential Accumulation of Amino Acid in Leaves of Susceptible and Resistant Soybean Plants in Response to the Soybean Aphid (Hemiptera: Aphididae). **Plant-Insect Interactions**, 2010.

CLAPS, L. E.; HARO, M. E. de. Coccoidea (Insecta: Hemiptera) associated with Cactaceae in Argentina. **Journal of the Professional Association for Cactus Development**, v. 4, p. 77–83, 2001.

CLAPS, L. E.; ZAMUDIO, P.; BRIZ, L. D. Las Dactylopiidae y Diaspididae (Hemiptera, Coccoidea) de la Colección Kenneth Hayward, Tucumán, Argentina. **Revista Brasileira de Entomologia**, v. 50, n. 1, p. 33–38, 2006.

COPOLOVICI, L. et al. Volatile organic compound emissions from Alnus glutinosa under interacting drought and herbivory stresses. **Environmental and Experimental Botany**, v. 100, p. 55–63, 2014. Disponível em: <https://www.sciencedirect.com/science/article/pii/S0098847213002220#fig0005>.

COPPOLA, M. et al. Trichoderma harzianum enhances tomato indirect defense against aphids. **Insect Science**, v. 24, n. 6, p. 1025–1033, 2017.

CUSHMAN, J. C. Crassulacean Acid Metabolism. A Plastic Photosynthetic Adaptation to Arid Environments. **American Society of Plant Biologists**, v. 127, n. 3, p. 1439–1448, 2001. Disponível em: <http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.1990.tb00407.x/full>.

DAHAL, D. et al. Analysis of cell wall proteins regulated in stem of susceptible and resistant tomato species after inoculation with Ralstonia solanacearum: A proteomic approach. **Plant Molecular Biology**, v. 73, n. 6, p. 643–658, 2010.

DAS, A. et al. Leaf Proteome Analysis Reveals Prospective Drought and Heat Stress Response Mechanisms in Soybean. **BioMed research international**, v. 2016, p. 6021047, 2016. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/27034942%255Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4808539>.

DE CLERCQ, I. et al. The Membrane-Bound NAC Transcription Factor ANAC013 Functions in Mitochondrial Retrograde Regulation of the Oxidative Stress Response in Arabidopsis. **The Plant Cell**, v. 25, n. 9, p. 3472–3490, 2013. Disponível em: <http://www.plantcell.org/cgi/doi/10.1105/tpc.113.117168>.

DE LUCIA, E. H. et al. Climate Change: Resetting Plant-Insect Interactions. **Update on Climate Change**, v. 160, n. December, p. 1677–1685, 2012.

DIAS, M. S. C.; COSTA, A. C. F.; JESUS, A. M. de. Manejo fitossanitário da palma forrageira. **Informe Agropecuário**, v. 38, n. 296, p. 90–96, 2017.

DIÁZ, M. del S. S. et al. Opuntia spp.: Characterization and Benefits in Chronic Diseases. **Oxidative Medicine and Cellular Longevity**, v. 2017, 2017.

DITA, M. A. et al. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. **Euphytica**, v. 147, p. 1–24, 2006.

DUBEUX JUNIOR, J. C. B.; BEN SALEM, H.; NEFZAOUI, A. F. Forage production and supply for animal nutrition. In: INGLESE, P. et al. (Ed.). **Crop ecology. cultivation and uses of cactus pear: CAM crops for a hotter and drier world.** Rome: Food and Agriculture Organization of the United Nations (FAO) and International Center for Agricultural Research in the Dry Areas (ICARDA), 2017. p. 74–91.

FANG, X. et al. Proteomic dissection of plant responses to various pathogens. **Proteomics**, v. 15, n. 9, p. 1525–1543, 2015.

FEUSSNER, I.; POLLE, A. ScienceDirect What the transcriptome does not tell — proteomics and metabolomics are closer to the plants ’ patho-phenotype. **Current Opinion in Plant Biology**, v. 26, p. 26–31, 2015. Disponível em: <http://dx.doi.org/10.1016/j.pbi.2015.05.023>.

FLORENCIO-ORTIZ, V. et al. Changes in the free amino acid composition of Capsicum annuum (pepper) leaves in response to Myzus persicae (green peach aphid) infestation. A comparison with water stress. **PLoS ONE**, v. 13, n. 6, p. 1–19, 2018.

FLORES-HERNÁNDEZ, A. et al. Yield and physiological traits of prickly pear cactus “nopal” (Opuntia spp.) cultivars under drip irrigation. **Agricultural Water Management**, v. 70, n. 2, p. 97–107, 2004.

FLORES, R. G. R. et al. La Hormiga Argentina Linepithema humile : Primer Registro en Opuntia spp . para el Estado de San Luis. **Southwestern Enthomologist**, v. 43,

n. 3, p. 780–785, 2018.

FOYER, C. H. Redox Homeostasis and Antioxidant Signaling: A Metabolic Interface between Stress Perception and Physiological Responses. **The Plant Cell Online**, v. 17, n. 7, p. 1866–1875, 2005. Disponível em: <http://www.plantcell.org/cgi/doi/10.1105/tpc.105.033589>.

FOYER, C. H. et al. Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation. **Journal of Experimental Botany**, v. 67, n. 7, p. 2025–2037, 2016. Disponível em: <https://academic.oup.com/jxb/article-lookup/doi/10.1093/jxb/erw079>.

FOYER, C. H.; LELANDAIS, M.; KUNERT, K. J. Mechanism of walnut induced anaphylaxis-like shock reaction in mice. **Physiologia Plantarum**, n. 92, p. 696–717, 1994.

FRAIRE-VELAZQUEZ, S.; RODRIGUEZ-GUERRA, R.; SANCHEZ-CALDERO, L. Abiotic and Biotic Stress Response Crosstalk in Plants. In: SHANKER, A. K.; VENKATESWARLU, B. (Ed.). **Abiotic Stress Response in Plants - Physiological, Biochemical and Genetic Perspectives**. Rijeka: InTech, 2011. p. 3–26.

FRANCO-SALAZAR, V. A.; VÉLIZ, J. A. Respuestas de la tuna [Opuntia ficus-indica (l.) Mill.] al NaCl. **Interciência**, v. 32, n. 2, 2007.

GADJEV, I.; STONE, J. M.; GECHEV, T. S. Programmed Cell Death in Plants. New Insights into Redox Regulation and the Role of Hydrogen Peroxide. **International Review of Cell and Molecular Biology**, v. 270, p. 87–144, 2008. Disponível em: <http://dx.doi.org/10.1016/S1937-6448(08)01403-2>.

GARCÍA MORALES, M. et al. ScaleNet: A literature-based model of scale insect biology and systematics. **Database**, p. 1–5, 2016. Disponível em: <http://scalenet.info/catalogue/Diaspis echinocacti/>.

GATEHOUSE, A. M. R.; FERRY, N.; ROMAAN, J. M. R. The case of the monarch butterfly: a verdict is returned. **Trends in Genetic**, v. 18, n. 5, p. 249–251, 2002.

GERSON, U.; ZOR, Y. The armoured scale insects (Homoptera: Diaspididae) of avocado trees in Israel. **Journal of Natural History**, v. 7, n. 5, p. 513–533, 2007.

GHOSH, D. et al. Editorial: How Plants Deal with Stress: Exploration through Proteome Investigation. **Frontiers in Plant Science**, v. 8, n. June, p. 1–2, 2017. Disponível em: <http://journal.frontiersin.org/article/10.3389/fpls.2017.01176/full>.

GILL, S. S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12, p. 909–930, 2010.

GRANT, M.; LAMB, C. Systemic immunity. **Current Opinion in Plant Biology**, v. 9, n. 4, p. 414–420, 2006.

GREENBAUM, D. et al. Comparing protein abundance and mRNA expression levels on a genomic scale. **Genome Biology**, v. 4, n. 9, 2003.

GUAN, W. et al. Proteomic analysis shows that stress response proteins are significantly up-regulated in resistant diploid wheat (Triticum monococcum) in response to attack by the grain aphid (Sitobion avenae). **Molecular Breeding**, v. 35, n. 2, p. 1–22, 2015.

GUTSCHE, A. et al. Gene expression profiling of tolerant barley in response to diuraphis noxia (Hemiptera: Aphididae) feeding. **Bulletin of Entomological**

Research, v. 99, n. 2, p. 163–173, 2009.

GYGI, S. P. et al. Correlation between Protein and mRNA Abundance in Yeast. **Molecular and Cellular Biology**, v. 19, n. 3, p. 1720–1730, 1999. Disponível em: <http://mcb.asm.org/lookup/doi/10.1128/MCB.19.3.1720>.

HABIBI, G.; HAJIBOLAND, R. Comparison of water stress and UV radiation effects on induction of CAM and antioxidative defense in the succulent rosularia elymaitica (Crassulaceae). **Acta Biologica Cracoviensia Series Botanica**, v. 53, n. 2, p. 15–24, 2011.

HABIBI, G.; HAJIBOLAND, R. Comparison of photosynthesis and antioxidative protection in Sedum album and Sedum stoloniferum (Crassulaceae) under water stress. **Photosynthetica**, v. 50, n. 4, p. 508–518, 2012.

HALITSCHKE, R.; HAMILTON, J. G.; KESSLER, A. Herbivore-specific elicitation of photosynthesis by mirid bug salivary secretions in the wild tobacco nicotiana attenuata. **New Phytologist**, v. 191, n. 2, p. 528–535, 2011.

HAO, P. et al. An integrative proteome analysis of different seedling organs in tolerant and sensitive wheat cultivars under drought stress and recovery. **Proteomics**, v. 15, n. 9, p. 1544–1563, 2015.

HEAZLEWOOD, J. L.; MILLAR, A. H. Plant proteomics: challenges and resources. **Annual Plant Reviews**, v. 28, p. 1–31, 2006.

HENZEL, W. J.; WATANABE, C.; STULTS, J. T. Protein identification: The origins of peptide mass fingerprinting. **Journal of the American Society for Mass Spectrometry**, v. 14, n. 9, p. 931–942, 2003.

HERIOT, A. The renewal and replacemant of the stylets of sucking insects during each stadium and the metohd of penetration. **Canadian Journal of Research**, v. 11, n. 5, p. 602–612, 1934.

HERNÁNDEZ, C. S. Fabricação de alimentos e obtenção de subprodutos. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 140–146.

HERRERA, A. Crassulacean acid metabolism and fitness under water deficit stress: if not for carbon gain, what is facultative CAM good for? **Annals of Botany**, v. 103, p. 645–653, 2009.

HERRERA, A.; FERNÁNDEZ, M. D.; TAISMA, M. A. Effects of Drought on CAM and Water Relations in Plants of Peperomia carnevalii. **Annals of Botany**, v. 86, n. 3, p. 511–517, 2000. Disponível em: <https://academic.oup.com/aob/article-lookup/doi/10.1006/anbo.2000.1210>.

HILLS, F. S. Anatomia e Morfologia. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. [s.l: s.n.]p. 28–35.

HOFFMAN, W. Etnobotânica. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 12–19.

HOLLIS-BRAVO, H. **Las Cactaceas de México**. Cidade do México: Univ. Nac. Aut. México, 1978.

HOWE, G. A.; JANDER, G. Plant Immunity to Insect Herbivores. **Annual Review of Plant Biology**, v. 59, n. 1, p. 41–66, 2008. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.arplant.59.032607.092825>.

HSIAO, T. C. Plant Responses To. **Ann Rev. Plant Pysiol**, v. 24, p. 519–570, 1973.

HUA, Q. et al. Proteomic analysis of Hylocereus polyrhizus reveals metabolic pathway changes. **International Journal of Molecular Sciences**, v. 17, n. 10, p. 1–13, 2016.

HUBERTY, A. F.; DENNO, R. F. Plant water stress and its consequences for herbivorous insects: A new synthesis. **Ecology**, v. 85, n. 5, p. 1383–1398, 2004.

HULTINE, K. R.; CUSHMAN, J. C.; WILLIAMS, D. G. New perspectives on crassulacean acid metabolism biology. **Extra Botany**, v. 70, n. 22, p. 6489–6493, 2019.

HURKMAN, W. J.; TANAKA, C. K. High-Resolution Two-Dimensional Gel Electrophoresis: A Cornerstone of Plant Proteomics. In: ŠAMAJ, J.; THELEN, J. J. (Ed.). **Plant Proteomics**. Verlag Berlin Heidelberg New York: Springer, 2007. p. 42–56.

HUSEYNOVA, I. M.; ALIYEVA, D. R.; ALIYEV, J. A. Subcellular localization and responses of superoxide dismutase isoforms in local wheat varieties subjected to continuous soil drought. **Plant Physiology and Biochemistry**, v. 81, p. 54–60, 2014. Disponível em: <http://dx.doi.org/10.1016/j.plaphy.2014.01.018>.

INGLESE, P. **Cactus pear Opuntia ficus-indica L. (Mill.) for fruit production: an overview**. Mekelle (Ethiopia): Cactus Net Newsletter, 2010.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA- IBGE. **Cartograma - Palma forrageira do Brasil por Área colhida**. Disponível em: <https://censoagro2017.ibge.gov.br/templates/censo_>.

ISSAQ, H. J.; VEENSTRA, T. D. Two-dimensional difference in gel electrophoresis for biomarker discovery. In: **Proteomic and Metabolomic Approaches to Biomarker Discovery**. 2. ed. San Diego: Elsevier Inc., 2013. p. 191–195.

ITIS. **Diaspis echinocacti**. Disponível em: <https://www.itis.gov/>. Acesso em: 15 maio. 2018.

IZUEGBUNA, O.; OTUNOLA, G.; GRAEME, B. inflammatory , and cytotoxic activities of Opuntia stricta cladodes. **PLoS ONE**, v. 14, n. 1, p. 1–27, 2019.

JAOUANNET, M. et al. Plant immunity in plant aphid interactions. **Frontiers in Plant Science**, v. 5, n. December, p. 1–10, 2014. Disponível em: <http://journal.frontiersin.org/article/10.3389/fpls.2014.00663/abstract>.

JAYA, S.; KUMAR, B. Hypoglycemic efficacy of Catharanthus roseus and Opuntia ficus-indica in mice diabetic model. **International Journal of Current Microbiology and Applied Sciences**, v. 6, n. 7, p. 1193–1209, 2017.

JI, W. et al. Comparative Proteomic Analysis of Soybean Leaves and Roots by iTRAQ Provides Insights into Response Mechanisms to Short-Term Salt Stress. **Frontiers in Plant Science**, v. 7, n. April, p. 1–15, 2016. Disponível em: <http://journal.frontiersin.org/Article/10.3389/fpls.2016.00573/abstract>.

JORRIN-NOVO, J. V et al. Gel electrophoresis-based plant proteomics: Past, present, and future. Happy 10th anniversary Journal of Proteomics! **Journal of Proteomics**, v. 198, n. Abril, p. 1–10, 2019. Disponível em: <https://doi.org/10.1016/j.jprot.2018.08.016>.

JUNGBLUT, P. R. et al. The speciation of the proteome. **Chemistry Central Journal**, v. 2, n. 1, p. 1–10, 2008.

KARUPPANAPANDIAN, T. et al. Reactive oxygen species in plants: Their generation, signal transduction, and scavenging mechanisms. **Australian Journal of Crop Science**, v. 5, n. 6, p. 709–725, 2011.

KERCHEV, P. I. et al. Plant responses to insect herbivory: Interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. **Plant, Cell and Environment**, v. 35, n. 2, p. 441–453, 2012.

KOCH, K. G. et al. Plant Tolerance: A Unique Approach to Control Hemipteran Pests. **Frontiers in Plant Science**, v. 7, 2016. Disponível em: <http://journal.frontiersin.org/Article/10.3389/fpls.2016.01363/abstract>.

KOTCHONI, S. O.; GACHOMO, E. W. The reactive oxygen species network pathways: An essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. **Journal of Biosciences**, v. 31, n. 3, p. 389–404, 2006.

KULOYO, O. O. et al. Opuntia ficus-indica cladodes as feedstock for ethanol production by Kluyveromyces marxianus and Saccharomyces cerevisiae. **World Journal of Microbiology and Biotechnology**, v. 30, n. 12, p. 3173–3183, 2014.

KUMAR, S. et al. Genome-wide transcriptomic and proteomic analyses of bollworm-infested developing cotton bolls revealed the genes and pathways involved in the insect pest defence mechanism. **Plant Biotechnology Journal**, v. 14, n. 6, p. 1438–1455, 2016.

KWAK, J. M.; NGUYEN, V.; SCHROEDER, J. I. Quantum Booth's array multiplier. **Plant Physiology**, v. 141, n. June, p. 323–329, 2006. Disponível em: <http://arxiv.org/abs/quant-ph/0611007>.

LABBUDA, M. **Lipid peroxidation as a biochemical marker for oxidative stress during drought. An effective tool for plant breeding**. 2013. Warsal University of Life Sciences, 2013.

LASCANO, H. . et al. Antioxidant system response of different wheat cultivars under drought: field and invitro studies. **Aust. J. Plant Physiol.**, v. 28, n. September 2017, p. 1095–1102, 2001.

LEE, J. C. et al. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. **Journal of Agricultural and Food Chemistry**, v. 50, n. 22, p. 6490–6496, 2002.

LENZI, M.; ORTH, A. I. Mixed reproduction systems in Opuntia monacantha (Cactaceae) in Southern Brazil. **Brazilian Journal of Botany**, v. 35, n. 1, p. 49–58, 2012. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-84042012000100006&lng=en&nrm=iso&tlng=en>.

LI, B. et al. Transcriptional regulation of pattern-triggered immunity in plants. **Cell Host Microbe**, v. 59, n. 3, p. 641–650, 2016.

LIEBLER, D. C. **Introduction to Proteomics Tools for the New Biology**. Totowa: Human Press, 2002.

LIMA, N. C. de. **Avaliação de Unidades Demonstrativas de Palma Forrageira (Nopalea e Opuntia) no Estado de Pernambuco**. 2013. Universidade Federal Rural de Pernambuco, 2013.

LIMA, I. M. M.; GAMA, N. S. Registro de Plantas Hospedeiras (Cactaceae) e de Nova Forma de Disseminação de Diaspis echinocacti (Bouché) nos Estados de

Pernambuco e Alagoas. **Neotropical Entomology**, v. 30, n. 3, p. 479–481, 2001.

LIRA, M. de A. et al. Utilização Da Palma Forrageira Na Pecuária Leiteira Do Semi-Árido. **Anais da Academia Pernambucana de Ciência Agronômica**, v. 2, p. 107–120, 2005.

LIRA, M. de A. **Cadernos do Semiárido: riquezas & oportunidades**. 2. ed. Recife: Editora do IPA, 2017. v. 7

LISAR, S. Y. S. et al. Water Stress in Plants: Causes, Effects and Responses. In: RAHMAN, I. M. M.; HASEGAWA, H. (Ed.). **Water stress**. Rijeka, Croatia: IntechOpen, 2012. p. 310.

LLEDÍAS, F. et al. A Rapid and Reliable Method for Total Protein Extraction from Succulent Plants for Proteomic Analysis. **The Protein Journal**, v. 36, n. 4, p. 308–321, 2017. Disponível em: <http://link.springer.com/10.1007/s10930-017-9720-3>.

LOCATO, V. et al. Reactive oxygen species and ascorbateglutathione interplay in signaling and stress responses. In: GUPTA, S. D. (Ed.). **Reactive oxygen species and antioxidants in higher plants**. Enfield: Science Publishers, 2010. p. 45–64.

LOIK, M. E.; NOBEL, P. S. Water Relations and Mucopolysaccharide Increases for a Winter Hardy Cactus during Acclimation to Subzero Temperatures. **Oecologia**, v. 88, n. 3, p. 340–346, 1991.

LONGO, S.; RAPISARDA, C. Pragas da Palma Forrageira. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivos e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 241.

LOO, S. et al. Bleogens: Cactus-Derived Anti-Candida Cysteine-Rich Peptides with Three Different Precursor Arrangements. **Frontiers in Plant Science**, v. 8, n. December, p. 1–13, 2017. Disponível em: <http://journal.frontiersin.org/article/10.3389/fpls.2017.02162/full>.

LOPES, E. B. **Palma forrageira: cultivo, uso atual e perspectivas de utilização no semiárido nordestino**. João Pessoa: EMEPA/FAEPA, 2007. v. 1

LOPES, E. B. et al. Seleção de genótipos de palma forrageira (Opuntia spp.) e (Nopalea spp.) resistentes à cochonilha-do-carmim (Dactylopius opuntiae Cockerell, 1929) na Paraíba, Brasil. **Engenharia Ambiental**, v. 7, p. 204–215, 2010.

LU, J. et al. Induced Jasmonate Signaling Leads to Contrasting Effects on Root Damage and Herbivore Performance. **Plant Physiology**, v. 167, n. 3, p. 1100–1116, 2015. Disponível em: <http://www.plantphysiol.org/lookup/doi/10.1104/pp.114.252700>.

MADLUNG, A.; COMAI, L. The Effect of Stress on Genome Regulation and Structure. **Annals of Botany**, v. 94, p. 481–495, 2004.

MALAININE, M. E. et al. Structure and morphology of cladodes and spines of Opuntia ficus-indica. Cellulose extraction and characterisation. **Carbohydrate Polymers**, v. 51, n. 1, p. 77–83, 2003.

MANDUJANO, C. et al. The relative contributions of sexual reproduction and in Opuntia clonal propagation rastrera from two habitats in the Chihuahuan Desert. **Journal of Ecology**, v. 86, n. 6, p. 911–921, 1998.

MANGOLIN, C. A.; OTTOBONI, L. M. M.; MACHADO, M. F. P. S. Two-dimensional electrophoresis of Cereus peruvianus (Cactaceae) callus tissue proteins. **Electrophoresis**, v. 20, n. 3, p. 626–629, 1999.

MANN, J. **Cactus-feeding insects and mites**. [s.l.] US Natn. Mus., 1969. v. 256

MARCHI, M. N. G. **Aspectos fisiológicos, anatômicos e moleculares da propagação e conservação in vitro de espécies de cactos endêmicos da Bahia**. 2016. Universidade Estadual de Feira de Santana, 2016.

MARTÍNEZ-GONZÁLEZ, C. R. et al. Improved method for genomic DNA extraction for Opuntia Mill. (Cactaceae). **Plant Methods**, v. 13, n. 1, p. 1–10, 2017.

MEHTA, A. et al. Plant-pathogen interactions: What is proteomics telling us? **FEBS Journal**, v. 275, n. 15, p. 3731–3746, 2008.

MELGAR, B. et al. Extensive profiling of three varieties of Opuntia spp. fruit for innovative food ingredients. **Food Research International**, v. 101, n. June, p. 259–265, 2017. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.09.024>.

MILLER, D. R.; DAVIDSON, J. . **Armored Scale Insect Pests of Trees and Shrubs**. NY: Cornell Univ. Press Ithaca, 2005.

MILLER, D. R.; KOSZTARAB, M. Recent Advances in the Study of Scale Insects. **Annual Review Entomol**, p. 1–27, 1979.

MITTLER, R. Abiotic stress, the field environment and stress combination. **Trends in Plant Science**, v. 11, n. 1, p. 15–19, 2006.

MOCHIDA, K.; SHINOZAKI, K. Advances in omics and bioinformatics tools for systems analyses of plant functions. **Plant and Cell Physiology**, v. 52, n. 12, p. 2017–2038, 2011.

MONDRAGÓN-JACOBO, C.; PIMIENTA-BARRIOS, E. Propagação. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivos e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 241.

MORALES, M. G. et al. ScaleNet: A literature-based model of scale insect biology and systematics. **Database**, v. 2016, n. 1, p. 1–5, 2016.

MORTIMORE, M. **Dryland Opportunities - A new paradigm for people, ecosystems and development**. Gland, Switzerland: International Union for Conservation of Nature, 2009.

MOURA, M. S. B. de et al. Aptidão do Nordeste Brasileiro ao Cultivo da Palma Forrageira sob Cenários de Mudanças Climáticas. **Simpósio de Mudanças Climáticas e Desertificação no Semiárido Brasileiro**, p. 6, 2011. Disponível em: <http://www.alice.cnptia.embrapa.br/alice/handle/doc/911518>.

MULLAN, D. M.; PIETRAGALLA, J. Leaf relative water content. In: PASK, A. . et al. (Ed.). **Physiological breeding II: A field guide to wheat genotyping**. Mexico: CIMMYT, 2012. p. 25–27.

MYLONA, P. V.; POLIDOROS, A. N. ROS regulation of antioxidant genes. In: GUPTA, S. (Ed.). **Reactive oxygen species and antioxidants in higher plants**. [s.l.] Enfields, 2010. p. 101–128.

NELSON, E. A.; SAGE, T. L.; SAGE, R. F. Functional leaf anatomy of plants with crassulacean acid metabolism. **Functional Plant Biology**, v. 32, n. 5, p. 409–419, 2005.

NEPOMUCENO, A. L. et al. Tolerância à seca em plantas. **Biotecnologia Ciência & Desenvolvimento**, n. 23, p. 12–18, 2001. Disponível em: <https://www.mendeley.com/viewer/?fileId=6c27ceaf-4455-6697-05b9-

77853789cb10&documentId=c9dbc51f-3e82-3ced-8147-b815e0404c59>.

NERD, A.; MIZRAHI, Y. Reproductive Biology of Cactus Fruit Crops. **Horticultural Reviews**, v. 18, 1997.

NERY, J. F. et al. Potential of Opuntia stricta Haw (Mexican elephant ear) in removing cyanobacteria in surface water. **African Journal of Biotechnology**, v. 18, n. 29, p. 875–881, 2019.

NIELSEN, U. N.; BALL, B. A. Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. **Global Change Biology**, v. 21, p. 1407–1421, 2015.

NOBEL, P. S. et al. Historical aspects and net CO2 uptake for cultivated Crassulacean acid metabolism plants in Mexico. **Annals of Applied Biology**, v. 140, n. 2, p. 133–142, 2002. Disponível em: <http://doi.wiley.com/10.1111/j.1744-7348.2002.tb00165.x>.

NULTSCH, W. **Botânica Geral**. 10. ed. Porto Alegre: Artmed, 2000.

NUNES, C. S. Usos e aplicações da palma forrageira como uma grande fonte de economia para o semiárido. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 6, n. 1, p. 58–66, 2011.

OETTING, R. D. Biology of the cactus scale, Diaspis echinocacti (Bouche) (Homoptera: Diaspididae). **Ann. Entomol. Soc. Am.**, v. 77, p. 88–92, 1984.

OLIVEIRA, F. T. de et al. Palma Forrageira: Adaptação e Importância para os Ecossistemas Áridos e Semiáridos. **Revista Verde**, v. 5, n. 4, p. 27–37, 2010.

OLVERA, C. de la P. P.; ROMERO, J. C. **Atlas de anatomía vegetal**. 1. ed. [s.l: s.n.]

ONAGA, G.; WYDRA, K. Advances in Plant Tolerance to Biotic Stresses. In: **Plant Genomics**. [s.l: s.n.]3p. 229–272.

OSORIO-ESQUIVEL, O. et al. Phenolics, betacyanins and antioxidant activity in Opuntia joconostle fruits. **Food Research International**, v. 44, n. 7, p. 2160–2168, 2011. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2011.02.011>.

PANDEY, P. et al. Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-morphological Traits. **Frontiers in Plant Science**, v. 8, n. April, p. 1–15, 2017.

PASTORIZA, R. J. G. **Biologia floral da palma forrageira: ocorrência de mecanismos reprodutivos e causa do abortamento de frutos**. 2016. 2016.

PEREZ, I. B.; BROWN, P. J. The role of ROS signaling in cross-tolerance: from model to crop. **Frontiers in Plant Science**, v. 5, n. December, p. 1–6, 2014. Disponível em: <http://journal.frontiersin.org/article/10.3389/fpls.2014.00754/abstract>.

PERGANDE, M.; COLOGNA, S. Isoelectric Point Separations of Peptides and Proteins. **Proteomes**, v. 5, n. 1, p. 4, 2017. Disponível em: <http://www.mdpi.com/2227-7382/5/1/4>.

PI, E. et al. Mechanisms of Soybean Roots' Tolerances to Salinity Revealed by Proteomic and Phosphoproteomic Comparisons Between Two Cultivars. **Molecular & Cellular Proteomics**, v. 15, n. 1, p. 266–288, 2016. Disponível em: <http://www.mcponline.org/lookup/doi/10.1074/mcp.M115.051961>.

PICHEREAUX, C. et al. Comparative shotgun proteomic analysis of wild and

domesticated Opuntia spp. species shows a metabolic adaptation through domestication. **Journal of Proteomics**, v. 143, p. 353–364, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jprot.2016.04.003>.

PIMIENTA-BARRIOS, E. et al. Young daughter cladodes affect CO2 uptake by mother cladodes of Opuntia ficus-indica. **Annals of Botany**, v. 95, n. 2, p. 363–369, 2005.

PIMIENTA-BARRIOS, E.; CASTILLO, R. F. del. Reproductive Biology. In: NOBEL, P. . . (Ed.). **Cacti Biology and Uses**. Berkeley, Los Angeles, London: University of California Press, 2002. p. 75–90.

PIMIENTA-BARRIOS, E.; LOZA, S. C.; CASTILLO-ARANDA, M. E. G. Del. Caracterización anatómica y conductividad hidráulica teórica de los elementos de vaso de variedades silvestres y cultivadas de nopal tunero (Opuntia). **Acta Botanica Mexicana**, n. 62, p. 21–30, 2003.

PIMIENTA-BARRIOS, E.; MUÑOZ-URIAS, A. Domesticação das opuntias e variedades cultivadas. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e utilizações da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 51–64.

QI, J. et al. Oral secretions from Mythimna separata insects specifically induce defence responses in maize as revealed by high-dimensional biological data. **Plant Cell and Environment**, v. 39, n. 8, p. 1749–1766, 2016.

RAMOS, S. R. R. et al. Germoplasma vegetal conservado no Nordeste Brasileiro : situação atual , prioridades e perspectivas. **Magistra**, v. 20, n. 3, p. 205–217, 2008.

RANA, V.; RAM, S.; NEHRA, K. Review Proline Biosynthesis and its Role in Abiotic Stress. **International Journal of Agriculture Innovations and Research**, v. 6, n. 3, p. 473–478, 2017.

REINDERS, J. et al. Challenges in mass spectrometry-based proteomics. **Proteomics**, p. 3686–3703, 2004.

REIS, M. B. de A. **Análise comparativa de proteínas expressadas sob estresse hídrico em palma forrageira (Opuntia cochenillifera)**. 2009. Universidade Católica de Brasília, 2009.

RESENDE, M.; SALGADO, S.; CHAVES, Z. Espécies Ativas de Oxigênio na Resposta de Defesa de Plantas a Patógenos. **Fitopatologia Brasileira**, v. 28, n. 2, p. 123–130, 2003.

REYES-AGÜERO, J. A.; AGUIRRE R., J. R.; VALIENTE-BANUET, A. Reproductive biology of Opuntia: A review. **Journal of Arid Environments**, v. 64, n. 4, p. 549–585, 2006.

REYES-AGÜERO, J. A.; RIVERA, J. R. R.; FLORES, J. L. F. Variación morfológica de opuntia (cactaceae) en relación con su domesticación en la altiplanicie meridional de México. **Interciencia**, v. 30, n. 8, p. 476–484, 2005.

RIGHETTI, P. G. et al. Gel-based proteomics. In: AGRAWAL, G. K.; RAKWAL, R. (Ed.). **Plant Proteomics: technologies, strategies, and applications**. [s.l.] John Wiley & Sons, 2008. p. 11–32.

RIZHSKY, L. et al. When Defense Pathways Collide. The Response of Arabidopsis to a Combination of Drought and Heat Stress. **Plant Physiology**, v. 134, n. April, p. 1683–1696, 2004.

ROCHA, J. E. da S. **Palma Forrageira no Nordeste do Brasil: Estado da Arte**. Sobral: Embrapa Caprinos e Ovinos, 2012.

ROGIĆ, T. et al. Proteomic analysis of Mammillaria gracilis Pfeiff. in vitro-grown cultures exposed to iso-osmotic NaCl and mannitol. **Plant Cell, Tissue and Organ Culture**, v. 122, n. 1, p. 127–146, 2015.

ROSAS-CÁRDENAS, F. F.; PAREDES-LÓPEZ, O.; CRUZ-HERNÁNDEZ, A. Proteomic analysis of prickly pear. **Acta Horticulturae**, v. 929, n. January 2016, 2012.

SÁENZ, C. Opuntias as a natural resource. In: SAENZ, C. et al. (Ed.). **Agro-industrial utilization of cactus pear**. Rome: FAO, 2013a. p. 168.

SÁENZ, C. Chemical composition and characteristics of Opuntia spp. In: SAENZ, C. et al. (Ed.). **Agro-industrial utilization of cactus pear**. Rome: FAO, 2013b. p. 168.

SAMPAIO, E. V. S. . Caracterização do Bioma Caatinga - características e potencialidades. In: GARIGLIO, M. A. et al. (Ed.). **Uso sustentável e conservação dos recursos florestais da caatinga**. Brasília: Serviço Florestal Brasileiro, 2010. p. 368.

SÁNCHEZ-HERRERA, R. et al. Secondary metabolites from Cactaceae with antifungal effect. In: MÉNDEZ-VILAS, A. (Ed.). **Antimicrobial research: Novel bioknowledge and educational programs**. Badajoz: Formatex Research Center, 2017. p. 155–165.

SANGHA, J. S. et al. Proteome analysis of rice (Oryza sativa L.) mutants reveals differentially induced proteins during brown planthopper (Nilaparvata lugens) infestation. **International Journal of Molecular Sciences**, v. 14, n. 2, p. 3921–3945, 2013.

SANGHERA, G. S. et al. Designing crop plants for biotic stresses using transgenic approach. **Vegetos**, v. 24, n. 1, p. 1–25, 2011.

SANTA-CECÍLIA, L. V. C.; PRADO, E.; OLIVEIRA, M. S. Sobre o condicionamento alimentar na cochonilha-branca, Planococcus citri (Risso) (Hemiptera: Pseudococcidae). **Revista Brasileira de Fruticultura**, v. 35, n. 1, p. 86–92, 2013.

SANTAMARIA, M. E. et al. Understanding plant defence responses against herbivore attacks: An essential first step towards the development of sustainable resistance against pests. **Transgenic Research**, v. 22, n. 4, p. 697–708, 2013.

SANTOS, A. B. R.; ARRUDA, E. C.; ARRUDA, G. P. Comportamento da cochonilha Diaspis echinocacti (Homoptera, Diaspididae) em diferentes espécies de “palma forrageira”. In: SOCIEDADE ENTOMOLÓGICA DO BRASIL (Ed.). **XIII Congresso Brasileiro de Entomologia**. Recife: UFRPE, 1991. IIp. 601.

SANTOS, D. C. et al. Estratégias para Uso de Cactáceas em Zonas Semiáridas: Novas Cultivares e Uso Sustentável das Espécies Nativas. **Rev. Cient. Prod. Anim.**, v. 15, n. 2, p. 111–121, 2013.

SANTOS, D. C. do. et al. **Manejo e Utilização da Palma Forrageira (Opuntia e Nopalea) em Pernambuco**. Recife: IPA, 2006.

SANTOS, D. C. dos et al. Estimativa de parâmetros genéticos em clones de palma forrageira Opuntia ficus-indica Mill e Nopalea Cochenillifera Salm-Dick. **Pesquisa Agropecuária Brasileira**, v. 29, n. 12, p. 1947–1957, 1994.

SANTOS, D. C. dos et al. Programa de melhoramento e coleção de palma forrageira.

In: QUEIROZ, M. A. DE; GOEDERT, C. O.; RAMOS, S. R. R. (Ed.). **Recursos genéticos e melhoramento de plantas para o Nordeste brasileiro**. Petrolina; Brasília: Embrapa Semi-Árido; Embrapa Recursos Genéticos e Biotecnologia, 1999.

SANTOS, T. do N. et al. Potential for biofuels from the biomass of prickly pear cladodes: Challenges for bioethanol and biogas production in dry areas. **Biomass and Bioenergy**, v. 85, p. 215–222, 2016.

SARASWATHY, N.; RAMALINGAM, P. **Concepts and Techniques in Genomics and Proteomics**. Oxford: Biohealthcare, 2011.

SCALISI, A. et al. Cladode growth dynamics in Opuntia ficus-indica under drought. **Environmental and Experimental Botany**, v. 122, p. 158–167, 2016. Disponível em: <http://dx.doi.org/10.1016/j.envexpbot.2015.10.003>.

SCHEINVAR, L. Taxonomia das Opuntias utilizadas. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 20–27.

SCHWARZLÄNDER, M. et al. The impact of impaired mitochondrial function on retrograde signalling: A meta-analysis of transcriptomic responses. **Journal of Experimental Botany**, v. 63, n. 4, p. 1735–1750, 2012.

SERGEANT, K.; RENAUT, J. Plant Biotic Stress and Proteomics. **Current Proteomics**, v. 7, n. 4, p. 275–297, 2010. Disponível em: <http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1570-1646&volume=7&issue=4&spage=275>.

SILVA, D. S.; ANDRADE, A. P. A. A Caatinga. In: REIS, R. A.; BERNARDES, T. F.; SIQUEIRA, G. R. (Ed.). **Forragicultura: ciência, tecnologia e gestão dos recursos forrageiros**. Jabotical: FUNEP, 2013. p. 187–199.

SILVA, T. G. F. da et al. Crescimento e produtividade de clones de palma forrageira no semiárido e relações com variáveis meteorológicas. **Revista Caatinga**, v. 28, n. 2, p. 10–18, 2015. Disponível em: <http://periodicos.ufersa.edu.br/revistas/index.php/sistema>.

SILVA, F. et al. Plant proteomics and peptidomics in host-pathogen interactgions: the weapons used by each side. **Current Protein & Peptide Science**, v. 18, n. 4, p. 400–410, 2017. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/27455973%250Ahttp://www.ingentaconnect.com/content/ben/cpps/2017/00000018/00000004/art00010>.

SILVA, H.; ACEVEDO, E.; SILVA, P. Anatomia del tejido fotosintético de diez taxas de Opuntia establecidos en el secano árido mediterraneo de Chile. **Revista Chilena de Historia Natural**, v. 74, p. 341–351, 2001.

SILVA, M. M. de A.; SOUZA, L. M. de. Respuesta de plantas micropropagadas de Opuntia stricta Haw . en aclimatización y campo. **Biotecnologia Vegetal**, v. 17, n. 2, p. 91–97, 2017.

SILVA, P. et al. Caracterização do Semiárido brasileiro: fatores naturais e humanos. **Semiárido brasileiro: pesquisa, desenvolvimento e inovação**, p. 16–48, 2010.

SILVA, R. C. et al. Orelha de Elefante Mexicana (Opuntia stricta [Haw.] Haw.) spineless cactus as an option in crossbred dairy cattle diet. **South African Journal of Animal Science**, v. 48, n. 3, p. 516–525, 2018.

SILVA, B. V. et al. Proteínas quinases: características estruturais e inibidores

químicos. **Química Nova**, v. 32, n. 2, p. 453–462, 2009.

SINGH, S.; GUPTA, A. K.; KAUR, N. Differential Responses of Antioxidative Defence System to Long-Term Field Drought in Wheat (Triticum aestivum L.) Genotypes Differing in Drought Tolerance. **Journal of Agronomy and Crop Science**, v. 198, n. 3, p. 185–195, 2012.

SINGH, V. et al. TREHALOSE PHOSPHATE SYNTHASE11-dependent trehalose metabolism promotes Arabidopsis thaliana defense against the phloem-feeding insect Myzus persicae. **Plant Journal**, v. 67, n. 1, p. 94–104, 2011.

SISTEMA FAEMG. **EPAMIG implanta banco de germoplasma de palma forrageira no Norte de Minas**. Disponível em: <http://www.programasemeando.org.br/Noticia.aspx?Code=13040&Portal=1&PortalNews=1&ParentCode=139&ParentPath=None&ContentVersion=R&show=all%250A>.

SMITH, C. M. **Plant Resistance to Arthropods Molecular and Conventional Approaches**. Manhattan: Springer, 2005.

SMITH, C. M. et al. Aphid Feeding Activates Expression of a Transcriptome of Oxylipin-Based Defense Signals in Wheat Involved in Resistance to Herbivory. **J Chem Ecol**, v. 36, n. 3, 2010.

STAHL, E.; HILFIKER, O.; REYMOND, P. Plant–arthropod interactions: who is the winner? **Plant Journal**, v. 93, n. 4, p. 703–728, 2018.

SUN, C. X. et al. Metabolic response of maize plants to multi-factorial abiotic stresses. **Plant Biology**, v. 18, p. 120–129, 2016.

SUZUKI, N. et al. Abiotic and biotic stress combinations. **New Phytologist**, v. 203, n. 1, p. 32–43, 2014.

SYTYKIEWICZ, H. et al. Expression profiling of selected glutathione transferase genes in Zea mays (L.) seedlings infested with cereal aphids. **PLoS ONE**, v. 9, n. 11, 2014.

TAIZ, L. et al. **Fisiologia e Desenvolvimento Vegetal**. 6. ed. Porto Alegre: Artmed, 2017.

TAYLOR, N. P.; ZAPPI, D. C. **Cacti of Eastern Brazil**. [s.l.] Royal Botanic Gardens Kew, 2004.

TRUONG, D. H. et al. Proteomic analysis of Arabidopsis thaliana (L.) Heynh responses to a generalist sucking pest (Myzus persicae Sulzer). **Plant Biology**, v. 17, n. 6, p. 1210–1217, 2015.

VALDERRAMA-CHÁIREZ, M. L.; CRUZ-HERNÁNDEZ, A.; PAREDES-LÓPEZ, O. Isolation of functional RNA from cactus fruit. **Plant Molecular Biology Reporter**, v. 20, n. 3, p. 279–286, 2002.

VASCONCELOS, A. G. V. et al. Seleção de clones de palma forrageira resistentes à cochonilha-do-carmim (Dactylopius sp). **Revista Brasileira de Zootecnia**, v. 38, n. 5, p. 827–831, 2009.

VENTURA-AGUILAR, R. I. et al. Cactus stem (Opuntia ficus-indica Mill): anatomy, physiology and chemical composition with emphasis on its biofunctional properties. **Journal of the Science of Food and Agriculture**, v. 97, n. 15, p. 5065–5073, 2017.

VICKERS, L. **Aphid responses to drought: a combined physiological and**

transcriptomic approach. 2011. University of Birmingham, 2011. Disponível em: <http://etheses.bham.ac.uk/3453/2/Vickers_12_PhD.pdf>.

VIDAL, N. et al. Comparative study of transgenic and non-transgenic maize (Zea mays) flours commercialized in Brazil, focussing on proteomic analyses. **Food Chemistry**, v. 180, p. 288–294, 2015.

VILLALOBOS, V. Aplicação do cultivo de tecidos para a micropropagação de Opuntia sp. In: BARBERA, G.; INGLESE, P.; BARRIOS, E. P. (Ed.). **Agroecologia, cultivo e usos da palma forrageira**. João Pessoa: SEBRAE-PB/FAO, 2001. p. 72–78.

VINICIO, A. J.; JAIME, S. C. Plagas y enfermedades de la tuna Opuntia ficus indica L. en las condiciones ecológicas de la provincia de Loja. **Bosques Latitud Cero**, v. 7, n. 1, p. 1–17, 2017.

VOGEL, C.; MARCOTTE, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. **Nature Reviews Genetics**, v. 13, n. 4, p. 227–232, 2012. Disponível em: <http://dx.doi.org/10.1038/nrg3185>.

WAR, A. R. et al. Mechanisms of plant defense against insect herbivores. **Plant Signaling & Behavior**, v. 7, n. 10, p. 1306–1320, 2012.

WARUMBY, J. F.; ARRUDA FILHO, G. P.; CAVALCANTI, V. A. L. B. Pragas da palma. In: MENEZES, R. S. C.; SIMÕES, D. A.; SAMPAIO, E. V. S. B. (Ed.). **A palma no Nordeste do Brasil**. [s.l: s.n.]p. 65–80.

WASINGER, V. C. et al. Progress with gene-product mapping of the Mollicutes:Mycoplasma genitalium. **Electrophoresis**, v. 16, n. 1, p. 1090–1094, 1995. Disponível em: <http://doi.wiley.com/10.1002/elps.11501601185>.

WASTERNACK, C.; HAUSE, B. Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. **Annals of Botany**, v. 111, n. 6, p. 1021–1058, 2013.

WHITHAM, S. A. et al. Molecular Soybean-Pathogen Interactions. **Annual Review of Phytopathology**, v. 54, n. 1, p. 443–468, 2016. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev-phyto-080615-100156>.

WIJK, K. J. Van. Challenges and Prospects of Plant Proteomics. **Plant Physiology**, v. 126, n. 2, p. 501–508, 2001.

WILKINS, M.; GOOLEY, A. A. Protein Identification in Proteome Projects. In: WILKINS, M. et al. (Ed.). **Proteome Research: New Frontiers in Functional Genomics**. Verlag Berlin Heidelberg New York: Springer, 1997. p. 35–64.

WILSON, A. C. C.; STERNBERG, L. d. S. L.; HURLEY, K. B. Aphids alter host-plant nitrogen isotope fractionation. **Proceedings of the National Academy of Sciences**, v. 108, n. 25, p. 10220–10224, 2011. Disponível em: <http://www.pnas.org/cgi/doi/10.1073/pnas.1007065108>.

YANG, L. et al. Biomass characterization of Agave and Opuntia as potential biofuel feedstocks. **Biomass and Bioenergy**, v. 76, p. 43–53, 2015.

YOU, J.; CHAN, Z. ROS Regulation During Abiotic Stress Responses in Crop Plants. **Frontiers in Plant Science**, v. 6, n. December, p. 1–15, 2015.

ZADRAŽNIK, T. et al. Differential proteomic analysis of drought stress response in leaves of common bean (Phaseolus vulgaris L.). **Journal of Proteomics**, v. 78, p.

254–272, 2013.

ZANDALINASA, S. I. et al. Plant adaptations to the combination of drought and high temperatures. **Physiologia Plantarum**, v. 162, n. 2, p. 2–12, 2018.

ZAPPI, D. et al. **Plano de ação nacional para a conservação das Cactáceas**. Brasília: Instituto Chico Mendes de Conservação da Biodiversidade - ICMBIO, 2011.

ZENG, W. et al. Proteomic analysis by iTRAQ-MRM of soybean resistance to Lamprosema Indicate. **BMC Genomics**, v. 18, n. 1, p. 1–22, 2017.

ZHOU, S. et al. Alteration of plant primary metabolism in response to insect herbivory. **Plant Physiology**, v. 169, n. November, p. 1488–1498, 2015. Disponível em: <http://www.plantphysiol.org/lookup/doi/10.1104/pp.15.01405>.

ZIEGLER, J.; FACCHINI, P. J. Alkaloid Biosynthesis: Metabolism and Trafficking. **Annual Review of Plant Biology**, v. 59, n. 1, p. 735–769, 2008. Disponível em: <http://www.annualreviews.org/doi/10.1146/annurev.arplant.59.032607.092730>.

ZIMMERMANN, H. G.; MC FAYDEN, R. E.; ERB, H. F. Annotated list of some cactus-feeding insects of South America. **Acta Zoologica Lilloana.**, v. 33, p. 101–112, 1979.