UNIVERSIDADE FEDERAL DO PIAUÍ
CAMPUS PROFESSORA CINOBELINA ELVAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS
DOUTORADO EM CIÊNCIAS AGRÁRIAS

GONÇALVES ALBINO DAUALA

PAPEL DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO NO METABOLISMO E DEFESA DA PALMA FORRAGEIRA (*Nopalea cochenillifera* Salm-Dyck) SOB DÉFICIT HÍDRICO

Bom Jesus – PI
2026

GONÇALVES ALBINO DAUALA

PAPEL DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO NO METABOLISMO E DEFESA DA PALMA FORRAGEIRA (*Nopalea cochenillifera* Salm-Dyck) SOB DÉFICIT HÍDRICO

Tese apresentada ao Programa de Pós-Graduação em Ciências Agrárias - PPGCA, Campus Professora Cinobelina Elvas - CPCE, da Universidade Federal do Piauí - UFPI, na área de concentração Produção Vegetal, como requisito para obtenção do título de Doutor em Ciências Agrárias.

Orientador: Prof. Dr. Rafael de Souza Miranda
Coorientadora: Dra. Stelamaris de Oliveira Paula Marinho

Bom Jesus – PI

2026

FICHA CATALOGRÁFICA

Universidade Federal do Piauí

Biblioteca Setorial Campus Professora Cinobelina Elvas

Setor de Processamento Técnico

D235p Dauala, Gonçalves Albino.

Papel de bactérias promotoras de crescimento no metabolismo e defesa da palma forrageira (*Nopalea cochenillifera* Salm-Dyck) sob déficit hídrico / Gonçalves Albino Dauala -- 2026.

133 f.

Tese (Doutorado) – Universidade Federal do Piauí. Programa de Pós-Graduação em Ciências Agrárias, Bom Jesus, Piauí, 2026.

"Orientação: Prof. Dr. Rafael de Souza Miranda".

"Coorientação: Profa. Dra. Stelamaris de Oliveira Paula Marinho".

1. Ciências agrárias. 2. Palma forrageira. 3. Inoculantes microbianos. I. Miranda, Rafael de Souza. II. Marinho, Stelamaris de Oliveira Paula. III. Título.

CDD 630

Bibliotecária: Nayra Fontinele Feijão-CRB3/1249

GONÇALVES ALBINO DAUALA

PAPEL DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO NO METABOLISMO E DEFESA DA PALMA FORRAGEIRA (*Nopalea cochenillifera* Salm-Dyck) SOB DÉFICIT HÍDRICO

Tese apresentada ao Programa de Pós-Graduação em Ciências Agrárias - PPGCA, do Campus Professora Cinobelina Elvas - CPCE, da Universidade Federal do Piauí - UFPI, como requisito à obtenção do título de Doutor em Ciências Agrárias.
Área de concentração: Produção Vegetal.
Linha de Pesquisa: Propagação de Plantas.
Orientador: Prof. Dr. Rafael de Souza Miranda.
Coorientadora: Dra. Stelamaris de Oliveira Paula Marinho.

Aprovada em: 31 de março de 2026.

BANCA EXAMINADORA

Documento assinado digitalmente
gov.br
RAFAEL DE SOUZA MIRANDA
Data: 24/05/2026 10:00:09-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Rafael de Souza Miranda (UFPI)
Orientador

Documento assinado digitalmente
gov.br
EVERALDO MOREIRA DA SILVA
Data: 25/05/2026 09:09:32-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Everaldo Moreira da Silva (UFPI)
Examinador Externo ao Programa

Documento assinado digitalmente
gov.br
STELAMARIS DE OLIVEIRA PAULA MARINHO
Data: 24/05/2026 18:11:05-0300
Verifique em https://validar.iti.gov.br

Dra. Stelamaris O. Paula Marinho (UFC)
Coorientadora

Documento assinado digitalmente
gov.br
WAGNER LUIZ ARAUJO
Data: 25/05/2026 17:48:34-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Wagner Luiz Araújo (UFV)
Examinador Externo ao Programa

Documento assinado digitalmente
gov.br
ELAINE MARTINS DA COSTA
Data: 24/05/2026 20:54:55-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. Elaine Martins da Costa (UFPI)
Examinadora Interna ao Programa

Documento assinado digitalmente
gov.br
EDIVAN RODRIGUES DE SOUZA
Data: 25/05/2026 13:44:34-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Edivan Rodrigues de Souza (UFRPE)
Examinador Externo ao Programa

À minha mãe, com carinho e amor.

Dedico.

AGRADECIMENTOS

A Deus, pela vida.

À Universidade Federal do Piauí (UFPI) e ao Programa de Pós-Graduação em Ciências Agrárias (PPGCA), pela oportunidade de realização deste curso, e à Direção do Campus Professora Cinobelina Elvas (CPCE/UFPI), pela criação de condições para o ensino e pesquisa.

À Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro ao desenvolvimento desta pesquisa, no âmbito do Edital nº 04/2021 – Apoio ao Desenvolvimento da Região Semiárida Brasileira. À CAPES, pela concessão da bolsa de doutorado, que possibilitou minha permanência no Brasil e dedicação aos estudos.

Ao meu orientador, Prof. Dr. Rafael de Souza Miranda, e à minha coorientadora, Dra. Stelamaris de Oliveira Paula Marinho, pela excelente orientação, apoio científico, atenção, disponibilidade, paciência e confiança. Ao grupo de pesquisa Bioplant, ao qual estou vinculado, e a todos(as) colegas pelo auxílio e suporte durante a condução dos experimentos, coletas e análises laboratoriais, em especial a Bruno Sousa Figueiredo de Fonseca e Renato Oliveira de Sousa, bem como aos professores, pós-doutorandos e bolsistas vinculados ao projeto.

Aos professores do PPGCA, pelos ensinamentos; aos técnicos de laboratório, pela colaboração na realização das análises experimentais; e à comunidade acadêmica do CPCE. Aos membros da comissão examinadora, por terem aceitado o convite.

À minha esposa, Júlia Chizingo; às minhas filhas, Laurina Dauala e Nazira Dauala; ao meu filho, Maxwell Dauala; à minha mãe, Laurina Mavelenge; e aos meus irmãos, Alberto Dauala e Afonso Dauala, pelo apoio, incentivo e compreensão.

Por fim, a todos que, direta ou indiretamente, contribuíram para o desenvolvimento deste trabalho.

“A educação é a arma mais poderosa que você pode usar para mudar o mundo.”

Nelson Mandela

RESUMO

A restrição hídrica tem sido um cenário recorrente no globo terrestre, especialmente nas áreas produtivas das regiões áridas e semiáridas. Embora a palma forrageira (*Nopalea cochenillifera* Salm-Dyck) apresente elevada tolerância à limitação hídrica, seu potencial produtivo é reduzido sob déficit hídrico prolongado. Nesse contexto, as bactérias promotoras de crescimento de plantas (BPCPs) representam uma estratégia promissora para aumentar a tolerância ao déficit hídrico. Assim, este estudo foi desenvolvido para testar a hipótese de que estirpes de BPCPs dos gêneros *Bacillus* e *Paenibacillus*, associadas à adubação nitrogenada, aumentam a tolerância ao déficit hídrico em plantas de palma forrageira das variedades Doce Miúda e Baiana, por meio da modulação de metabólitos e processos bioquímicos relacionados à homeostase hídrica e redox. O capítulo 1 apresenta a fundamentação teórica do estudo. Os capítulos 2 e 3 contemplam os resultados dos ensaios em casa de vegetação, conduzidos com plantas da variedade Doce Miúda, em delineamento em blocos casualizados (DBC), em esquema fatorial 8 × 2. Os tratamentos consistiram em oito combinações de inoculação [três estirpes de BPCPs (*Paenibacillus* sp. IPACC38 e IPACC55, e *Bacillus subtilis* IPACC29) com e sem adubação nitrogenada (N), além de dois controles não inoculados (UI), com e sem N] e dois regimes hídricos [controle (50% da capacidade de campo do solo - CC) e déficit hídrico (25% da CC)]. As avaliações foram realizadas 150 dias após a imposição do déficit hídrico. O capítulo 2 demonstra que as estirpes de BPCPs, especialmente IPACC29 associada ao N, atenuam os danos do déficit hídrico, promovendo maior acúmulo de N e compostos orgânicos, incremento da biomassa seca radicular e maior tolerância relativa ao estresse. Já o capítulo 3 aborda os mecanismos antioxidantes, teores de solutos orgânicos e perfil metabólico. Sob déficit hídrico, plantas inoculadas com IPACC29 apresentaram redução nos danos oxidativos, uma resposta associada com menores teores de H_2O_2 e MDA nos cladódios, acionamento de antioxidantes enzimáticos (atividade da dismutase do superóxido e peroxidase do ascorbato) e não enzimáticos (prolina, proteínas solúveis e ácidos orgânicos), e maior acúmulo de metabólitos primários (como ácido málico, sacarose e frutose) e marcadores moleculares de estresse. As respostas indicaram maior eficiência do metabolismo CAM frente ao déficit hídrico. Por fim, o capítulo 4 apresenta os ensaios no campo, visando a validação dos resultados em casa de vegetação. Nessa etapa foram conduzidos dois ensaios paralelamente com as variedades Baiana e Doce Miúda, em DBC, esquema fatorial 3 × 2, envolvendo três níveis de inoculação (UI, IPACC29 e IPACC55, todos com 50% da dose de N) e dois regimes hídricos com lâmina fixa de 8,5 mm (controle - irrigação a cada 7 dias e déficit hídrico - irrigação a cada

30 dias). As avaliações foram realizadas aos 108 dias após a imposição do déficit hídrico. Os resultados demonstraram que a inoculação com IPACC29 favorece o acúmulo de água e biomassa de cladódios sob condição bem irrigada. Sob déficit hídrico, embora haja ativação de respostas de defesa sob inoculação com IPACC29, como acúmulo de pigmentos fotossintéticos e status hídrico, esses ajustes foram insuficientes para manter a produtividade de cladódios. Conclui-se que as BPCPs são promissoras para mitigar os danos do déficit hídrico na palma forrageira, promovendo ajustes bioquímicos, regulação metabólica e redução do estresse oxidativo. A estirpe IPACC29 se mostrou efetiva para maior acúmulo de água e biomassa sob disponibilidade hídrica adequada no campo. Assim, o uso de BPCPs configura-se como estratégia biotecnológica sustentável para o aumento da produção de biomassa em regiões semiáridas.

Palavras-chave: Inoculantes microbianos, Respostas de defesa, Restrição hídrica, Semiárido, Tecnologia sustentável.

ABSTRACT

Water restriction has been a recurring scenario globally, especially in the productive areas of arid and semi-arid regions. Although cactus pear (*Nopalea cochenillifera* Salm-Dyck) exhibits high tolerance to water limitation, its productive potential is reduced under prolonged water deficit. In this context, plant growth-promoting bacteria (PGPB) represent a promising strategy to enhance tolerance to water deficit. Thus, this study was developed to test the hypothesis that PGPB strains of the genera *Bacillus* and *Paenibacillus*, associated with nitrogen fertilization, increase tolerance to water deficit in cactus pear plants of the varieties Doce Miúda and Baiana, through the modulation of metabolites and biochemical processes related to water and redox homeostasis. Chapter 1 presents the theoretical basis of the study. Chapters 2 and 3 present the results of greenhouse trials conducted with Doce Miúda variety plants in a randomized block design (RBD) in an 8 × 2 factorial scheme. Treatments consisted of eight inoculation combinations [three strains of PGPB (*Paenibacillus* sp. IPACC38 and IPACC55 and *Bacillus subtilis* IPACC29) with and without nitrogen (N) fertilization, in addition to two uninoculated (UI) controls, with and without N] and two water regimes [control (50% of soil field capacity - FC) and water deficit (25% FC)]. Evaluations were performed 150 days after the imposition of water deficit. Chapter 2 demonstrates that PGPB strains, especially IPACC29 associated with N, mitigate water deficit damage by promoting greater accumulation of N and organic compounds, increased root dry biomass, and higher relative stress tolerance. Chapter 3 addresses antioxidant mechanisms, organic solute contents, and metabolic profile. Under water deficit, plants inoculated with IPACC29 showed reduction in oxidative damage, a response associated with lower H_2O_2 and MDA levels in the cladodes, activation of enzymatic antioxidants (superoxide dismutase and ascorbate peroxidase activity) and non-enzymatic antioxidants (proline, soluble proteins, and organic acids), as well as greater accumulation of primary metabolites (such as malic acid, sucrose, and fructose) and molecular stress markers. The responses indicated greater efficiency of CAM metabolism in the face of water deficit. Finally, Chapter 4 presents field trials aimed at validating the results in greenhouse. In this stage, two parallel trials were conducted with the Baiana and Doce Miúda varieties, in a RBD, in a 3 × 2 factorial scheme, involving three inoculation levels (UI, IPACC29, and IPACC55, all with 50% of the N dose) and two water regimes with a fixed irrigation depth of 8.5 mm (control - irrigation every 7 days and water deficit - irrigation every 30 days). Evaluations were performed 108 days after the imposition of water deficit. The results demonstrated that inoculation with IPACC29 favors water accumulation and cladode biomass under well-irrigated

conditions. Under water deficit, although cactus pear plants activate defense responses under inoculation with IPACC29, such as accumulation of photosynthetic pigments and water status, these adjustments were insufficient to maintain cladode productivity. In conclusion, the PGPB are promising in mitigating the damage caused by water deficit in cactus pear, promoting biochemical adjustments, metabolic regulation, and reduction of oxidative stress. The IPACC29 strain proved effective for greater water and biomass accumulation under adequate water availability in the field. The use of PGPB is configured as a sustainable biotechnological strategy to increase biomass production in semi-arid regions.

Keywords: Microbial inoculants, Defense responses, Water restriction, Semi-arid, Sustainable technology.

LISTA DE FIGURAS

Figure 1 - Relative humidity and maximum, minimum and average air temperatures recorded in the greenhouse during the cultivation of cactus pear plants under different treatments..... 54

Figure 2 - Clustering analysis of growth and biomass parameters of cactus pear plants exposed to different inoculation and drought treatments..... 57

Figure 3 - Phenotypic appearance of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, 150 days after drought imposition..... 58

Figure 4 - Root morphology of cactus pear plants exposed to different inoculation and drought treatments……... 59

Figure 5 - Cladode succulence, relative tolerance to drought, and cladode temperature of cactus pear plants exposed to different inoculation and drought treatments……. 60

Figura 6 - Content and accumulation of nitrogen in cladodes and roots of cactus pear plants exposed different inoculation and drought treatments..... 61

Figure 7 - Content of chlorophyll *a*, chlorophyll *b*, chlorophyll total and carotenoids of cactus pear plants exposed different inoculation and drought treatments..... 62

Figure 8 - Content of soluble carbohydrates and free aminoacids of cactus pear plants exposed different inoculation and drought treatments..... 63

Figure 9 - Principal Component Analysis (PCA) for water regimes (control and drought) and treatments uninoculated (UI) or inoculation with *Bacillus subtilis* (IC29) and *Paenibacillus* sp. (IC38 and IC55) strains, without and with 50% in cactus pear plants…… 64

Figura 10 - Correlation network for bacterial strains and uninoculation (UI) treatments in cactus pear plants……..... 66

Figure 11 - Dry mass of root and cladode, and total dry mass of cactus pear (variety Doce Miúda) subjected to inoculation and drought treatments…….. 82

Figure 12 - Content of soluble carbohydrates in roots and cladodes, and organic acids malic, citric, lactic and succinic in cladodes of cactus pear plants exposed different inoculation and drought treatments..... 83

Figure 13 - Content of nitrate (NO_3^-) and ammonium (NH_4^+) in roots and cladodes, and activity of nitrate reductase in roots of cactus pear plants exposed different inoculation and drought treatments..... 85
Figura 14 - Content of proline in roots and cladodes, and malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in cladodes of cactus pear plants exposed different inoculation and drought treatments..... 86
Figure 15 - Contents of soluble protein and activity of ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT) in cladodes of cactus pear plants exposed different inoculation and drought treatments..... 87
Figure 16 - Principal Component Analysis (PCA) and dendrogram of growth and biochemical parameters in cactus pear (variety Doce Miúda) subjected to inoculation and drought treatments…….. 88
Figure 17 - Partial least squares discriminant analysis (PLS-DA) of the metabolic profiles and loading plot of metabolites in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation and drought treatments…….. 89
Figura 18 - VIP scores plot of metabolites in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation and drought treatments……..... 90
Figura 19 - Heatmap representation of the relative abundance of 52 detected metabolites in cladodes of cactus pear (var. Doce Miúda) subjected to inoculation and drought treatments…… 92
Figure 20 - Dynamics of maximum, minimum, average temperature and air relative humidity, during the drought imposition in the field experiment with cactus pear varieties and inoculation treatments……..…….. 104
Figure 21 - Timeline of main activities conducted during the field experiment with cactus pear varieties under different irrigation intervals and inoculation treatments…... 105
Figure 22 - Monthly accumulated irrigation and rainfall, irrigation depth applied during the drought imposition, and total accumulated depth in the field experiment with cactus pear varieties subjected to different irrigation intervals and inoculation treatments……..……... 107
Figura 23 - Plant height, number of cladodes per plant, total cladode area, thickness, length, and width of the primary cladode of cactus pear (Baiana and Doce Miúda varieties) exposed to different treatments in field……..…..... 110

Figure 24 - Production, yield of fresh and dry biomass, and water use efficiency of cactus pear (Baiana and Doce Miúda varieties) exposed to different treatments in field. 111
Figure 25 - Cladode succulence, cladode temperature, relative tolerance to drought, and plant accumulation water of cactus pear (Baiana and Doce Miúda varieties) exposed to different treatments in field…… 112
Figure 26 - Content of chlorophyll *a*, chlorophyll *b*, chlorophyll total and carotenoids of cactus pear (Baiana and Doce Miúda varieties) exposed to different treatments in field…….. 113
Figura 27 - Principal Component Analysis (PCA) and dendrogram of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation and drought treatments in field…….. 115

LISTA DE ABREVIATURAS

ABA	Ácido Abscísico
ACC	1-Aminociclopropano-1-Carboxilato
APX	Peroxidase do Ascorbato
BPCPs/PGPB	Bactérias Promotoras de Crescimento de Plantas
CAM	Metabolismo Ácido das Crassuláceas
CAT	Catalase
CC/FC	Capacidade de Campo
DBC	Delineamento em Blocos Casualizados
EROs/ROS	Espécies Reativas de Oxigênio
ETc	Evapotranspiração da Cultura
ETo	Evapotranspiração de Referência
FBN	Fixação Biológica de Nitrogênio
H_2O_2	Peróxido de Hidrogênio
MDA	Malondialdeído
N	Nitrogênio
NH_4^+	Amônio
NO_3^-	Nitrato
PEP-case	Fosfoenolpiruvato Carboxilase
Rubisco	Ribulose-1,5-Bisfosfato Carboxilase/Oxigenase
SAB	Semiárido brasileiro
SOD	Dismutase do Superóxido
UI	Não inocula
AIA	Ácido indol-3-acético
AG	Ácido giberélico
AS	Ácido salicílico
FDN	Fibra em detergente neutro
FDA	Fibra em detergente ácido
EPS	Exopolissacarídeos
COVs	Compostos orgânicos voláteis

SUMÁRIO

1	**INTRODUÇÃO GERAL**	**19**
2	**ESTRATÉGIA EXPERIMENTAL**	**21**
3	**CAPÍTULO 1: REFERENCIAL TEÓRICO**	**22**
3.1	**Impacto do déficit hídrico na fisiologia das plantas**	**22**
3.2	**Mecanismos de defesa das plantas ao déficit hídrico**	**23**
3.3	**Bactérias promotoras de crescimento de plantas**	**27**
3.3.1	Mecanismos de tolerância ao déficit hídrico induzidos por BPCPs	28
3.4	**Cultura da palma forrageira**	**31**
3.4.1	Caracterização e condições de cultivo	31
3.4.2	Metabolismo fotossintético	34
3.4.3	Importância alimentar e nutricional	35
3.4.4	Desempenho da palma sob déficit hídrico	36
3.4.5	Estratégias de manejo da irrigação	37
	REFERÊNCIAS	**39**
4	**CAPÍTULO 2: PLANT GROWTH-PROMOTING BACTERIA MODULATE METABOLISM AND NITROGEN ACCUMULATION TO COUNTERACT DROUGHT DAMAGE IN CACTUS PEAR PLANTS**	**50**
	ABSTRACT	**50**
4.1	**Introduction**	**51**
4.2	**Material and Methods**	**52**
4.2.1	Study site	52
4.2.2	Growing conditions, experimental design and treatments	52
4.2.3	Irrigation management	53
4.2.4	Growth parameters and relative tolerance to drought	54
4.2.5	Cladode succulence and temperature	55
4.2.6	Photosynthetic pigments	55
4.2.7	Nitrogen, soluble carbohydrates and free amino acids	55
4.2.8	Statistical analysis	56
4.3	**Results**	**56**
4.3.1	Plant growth, phenotypic appearance and root morphology	56
4.3.2	Water status and drought tolerance	59

4.3.3 Nitrogen accumulation..... 60
4.3.4 Photosynthetic pigments and organic compounds……..... 61
4.3.5 Principal Component Analysis…..... 63
4.4 Discussion..... 65
4.4.1 Cactus pear resistance against drought can be improved by plant growth-promoting bacteria…….. 65
4.4.2 Superior performance under drought induced by plant growth-promoting bacteria is associated with metabolic adjustments…… 67
4.4.3 Multivariate assays may explain how PGPB modulate cactus pear performance under water restriction…… 68
4.5 Conclusion..... 69
REFERENCES..... 69
5 CAPÍTULO 3: BIOCHEMICAL AND METABOLIC ADJUSTMENTS UNDERLYING *Bacillus subtilis*-INDUCED DROUGHT TOLERANCE IN CACTUS PEAR PLANTS……..... 74
ABSTRACT..... 74
5.1 Introduction..... 74
5.2 Material and Methods..... 76
5.2.1 Study site and environmental conditions 76
5.2.2 Growing conditions, experimental design and treatments…….. 76
5.2.3 Irrigation management..... 77
5.2.4 Plant harvest and dry biomass..... 78
5.2.5 Carbohydrates and organic acids..... 78
5.2.6 Inorganic ions and proline..... 78
5.2.7 Nitrate reductase activity……..... 79
5.2.8 Lipid peroxidation and hydrogen peroxide..... 79
5.2.9 Protein content and antioxidant enzymes..... 79
5.2.10 Metabolic profile..... 80
5.2.11 Statistical analysis……..... 80
5.3 Results..... 81
5.3.1 Plant growth responses……... 81
5.3.2 Accumulation of organic solutes…… 81
5.3.3 NO_3^- and NH_4^+ contents and NR activity…… 83

5.3.4 Proline, hydrogen peroxide and lipid peroxidation……. 84
5.3.5 Protein content and antioxidant enzymatic system……. 84
5.3.6 PCA of growth and biochemical parameters……... 87
5.3.7 Metabolomic profiling…… 89
5.4 Discussion..... 91
5.4.1 The PGPB inoculation boosts organic compounds accumulation and intensify antioxidant defense in cactus pear plants under drought……. 91
5.4.2 Metabolic profiling reveals key primary metabolites induced by IPAC-C29 strain to counteract drought damage in cactus pear cladodes…… 94
5.5 Conclusion..... 95
REFERENCES..... 95
6 CAPÍTULO 4: MICROBIAL INOCULANTS BOOST WATER AND BIOMASS ACCUMULATION IN CACTUS PEAR UNDER CONTRASTING WATER LEVELS IN FIELD-PRODUCTION SYSTEM.... 101
ABSTRACT..... 101
6.1 Introduction..... 102
6.2 Material and Methods..... 103
6.2.1 Study site and environmental conditions 103
6.2.2 Growing conditions, experimental design and treatments…….. 104
6.2.3 Irrigation management..... 106
6.2.4 Plant harvest, growth and biomass parameters……..... 106
6.2.5 Relative tolerance to drought, water use efficiency and plant accumulated water..... 107
6.2.6 Cladode succulence and temperature..... 108
6.2.7 Photosynthetic pigments..... 108
6.2.8 Statistical analysis……..... 109
6.3 Results..... 109
6.3.1 Growth parameters……..……... 109
6.3.2 Biomass parameters and water use efficiency……. 109
6.3.3 Water status and drought tolerance……..…… 111
6.3.4 Photosynthetic pigments…… 112
6.3.5 Principal Component Analysis……... 114
6.4 Discussion..... 116

6.4.1 PGPB inoculation enhances biomass accumulation and water storage in cactus pear under well-watered conditions……..……. 116
6.4.2 Microbial inoculants activate physiological responses but are insufficient to overcome severe water restriction in cactus pear……..……..... 117
6.5 Conclusion..... 118
REFERENCES..... 119
7 CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS..... 124
APÊNDICES 126

1 INTRODUÇÃO GERAL

O Semiárido brasileiro (SAB) apresenta características climáticas marcantes, como a distribuição irregular das chuvas ao longo do ano, com níveis inferiores a 800 mm ano^{-1}, elevadas temperaturas do ar e baixa umidade relativa (Santos *et al.,* 2017; Alves *et al.,* 2022). Essas condições reduzem a disponibilidade de água no sistema solo-planta-atmosfera em diferentes períodos do ano e promovem alterações na homeostase osmótica das células vegetais, comprometendo processos fisiológicos, bioquímicos e metabólicos. Todas essas limitações comprometem o crescimento e desenvolvimento de espécies forrageiras nativas e cultivadas, como gramíneas e leguminosas (Edvan *et al.,* 2020; IIyas *et al.,* 2021; Singh *et al.,* 2024).

A pecuária constitui uma das principais atividades econômicas desenvolvidas pela agricultura familiar no SAB, tendo a vegetação nativa como principal fonte de alimento para os rebanhos (Queiroz *et al.,* 2020). Entretanto, essa vegetação apresenta limitações de crescimento, especialmente durante os períodos de estiagem, ocasionando redução da biomassa e capacidade de suporte das pastagens (Queiroz *et al.,* 2020; Nascimento *et al.,* 2024). Dessa forma, torna-se necessário o cultivo de espécies forrageiras mais adaptadas às condições climáticas do SAB, a fim de garantir a oferta de alimento ao longo do ano. Nesse contexto, o cultivo da palma forrageira tem sido amplamente adotado como alternativa estratégica para a sustentabilidade da pecuária no SAB (Edvan *et al.,* 2020; Araújo Júnior *et al.,* 2024).

A palma forrageira destaca-se como importante fonte de alimento, água e energia para ruminantes e outros animais, apresentando boa palatabilidade, alta digestibilidade e elevada capacidade de armazenamento de água nos tecidos, caracterizando-se como reserva hídrica durante o período seco (Dubeux Jr *et al.,* 2021; Araújo Júnior *et al*., 2023). Além disso, devido ao Metabolismo Ácido das Crassuláceas (CAM), essa cactácea apresenta alta eficiência no uso da água e produção satisfatória de biomassa quando comparada a gramíneas e leguminosas, sobretudo em períodos de estiagem prolongada (Dubeux Jr *et al.,* 2021; Queiroz *et al.,* 2021). Os principais gêneros cultivados no SAB são *Opuntia* e *Nopalea*, destacando-se *Nopalea cochenillifera* Salm-Dyck, devido sua resistência à cochonilha-do-carmim (*Dactylopius opuntiae*), considerada a principal praga da cultura (Edvan; Carneiro, 2019). As variedades Doce Miúda e Baiana são amplamente cultivadas na região (Edvan *et al.,* 2020).

Apesar da reconhecida adaptação às condições semiáridas, o potencial produtivo da palma forrageira ainda permanece limitado sob déficit hídrico prolongado, cenário comum no SAB. Diversos estudos relatam efeitos negativos do déficit hídrico prolongado sobre a produtividade, crescimento, parâmetros fisiológicos e bioquímicos da cultura (Scalisi *et al.,*

2016; Lahbouki *et al.,* 2022, 2023). Dentre os principais efeitos, destacam-se a redução da condutância estomática noturna, alterações no acúmulo de ácido málico, diminuição do teor de clorofila e teor relativo de água, além da redução da espessura e número de cladódios por planta e do acúmulo de biomassa em raízes e cladódios (Scalisi *et al.,* 2016; Lahbouki *et al.,* 2023). Ademais, a deficiência hídrica nos primeiros meses após o plantio pode comprometer o estabelecimento das plantas, resultando em mortalidade e perdas produtivas em condições de sequeiro (Edvan *et al.,* 2023).

Diante desse cenário, a adoção de tecnologias sustentáveis e de baixo custo torna-se essencial para aliviar os danos causados pelo déficit hídrico na cultura. Nesse contexto, o uso de microrganismos, como as bactérias promotoras de crescimento de plantas (BPCPs), destaca-se como estratégia promissora, capaz de aumentar a resiliência da palma forrageira e otimizar a produção de biomassa sob déficit hídrico. Além disso essa abordagem contribui para sustentabilidade agrícola ao reduzir a dependência de fertilizantes químicos e promover maior eficiência no uso de nutrientes (Gupta *et al.,* 2022; Giller *et al.,* 2025).

As BPCPs são microrganismos que habitam a rizosfera e estimulam o crescimento vegetal por meio de diversos mecanismos (Bittencourt *et al.,* 2023). Essas bactérias podem conferir tolerância ao déficit hídrico por mecanismos diretos e indiretos, incluindo a produção de fitohormônios, síntese de compostos orgânicos voláteis, produção de exopolissacarídeos e formação de biofilmes, modificações no sistema radicular, síntese da enzima 1-aminociclopropano-1-carboxilato (ACC) desaminase, produção e acúmulo de osmólitos, ativação do sistema de defesa antioxidante, produção de sideróforos, além da fixação biológica de nitrogênio e solubilização de nutrientes (Vurukonda *et al.,* 2016; Chieb; Gachomo, 2023; Singh *et al.,* 2024; Khan *et al.,* 2026). Embora estirpes de BPCPs dos gêneros *Bacillus* e *Paenibacillus* sejam consideradas alternativas promissoras para mitigação dos danos de déficit hídrico em diversas culturas (Liu *et al.,* 2019; Patani *et al.,* 2024; Tariq *et al.,* 2025), seu papel na palma forrageira ainda é pouco conhecido.

Diante do exposto, a hipótese de trabalho foi que estirpes de BPCPs, dos gêneros *Bacillus* e *Paenibacillus*, associadas à adubação nitrogenada, aumentam a tolerância ao déficit hídrico em plantas das variedades Doce Miúda e Baiana de palma forrageira (*Nopalea cochenillifera* Salm-Dyck), por meio da modulação de metabólitos e processos bioquímicos relacionados à homeostase hídrica e redox. O objetivo foi investigar se estirpes de BPCPs são capazes de ativar respostas fisiológicas, bioquímicas e metabólicas de defesa, bem como melhorar o desempenho produtivo de variedades de palma forrageira sob déficit hídrico.

2 ESTRATÉGIA EXPERIMENTAL

Para testar a hipótese levantada, o presente trabalho foi desenvolvido em duas etapas dependentes e consecutivas, sendo a primeira conduzida em casa de vegetação e a segunda sob condições de campo.

Na primeira etapa, os experimentos foram conduzidos com plantas de palma forrageira da variedade Doce Miúda, em delineamento em blocos casualizados (DBC), esquema fatorial 8 × 2. O primeiro fator consistiu na combinação da inoculação de três estirpes de BPCPs [IPACC38 e IPACC55 (*Paenibacillus* sp.) e IPACC29 (*Bacillus subtilis)*] e dois controles não inoculados (UI), associados ou não à adubação nitrogenada (N). O segundo fator compreendeu dois níveis de disponibilidade hídrica [50% (controle bem irrigado) e 25% (déficit hídrico) da capacidade de campo do solo]. Inicialmente, investigou-se o papel das estirpes de BPCPs no desempenho de plantas da variedade Doce Miúda sob déficit hídrico, por meio da avaliação de parâmetros de crescimento, status hídrico, e indicadores fisiológicos e bioquímicos. A descrição detalhada da metodologia e dos resultados encontra-se no **capítulo 2** (Apêndice 3). Os resultados indicaram que a estirpe IPACC29, associada à adubação nitrogenada, apresentou maior eficiência na ativação de mecanismos de defesa, promovendo respostas de defesa e maior acúmulo de biomassa seca sob déficit hídrico. Posteriormente, foram realizadas análises bioquímicas e metabólicas mais detalhadas, incluindo a atividade de enzimas antioxidantes (SOD, APX e CAT), indicadores de danos oxidativos (H_2O_2 e MDA), atividade da nitrato redutase, teores de solutos orgânicos (proteínas solúveis e ácidos orgânicos), prolina, nitrato (NO_3^-) e amônio (NH_4^+), além do perfil metabólico das plantas. A metodologia e resultados dessa etapa estão detalhados no **capítulo 3** (Apêndices 4, 5, 6).

Na segunda etapa, foram conduzidos dois experimentos paralelos e independentes em campo, cada um com uma variedade de palma forrageira (Doce Miúda e Baiana). O delineamento foi em DBC, esquema fatorial 3 × 2, avaliando três níveis de inoculação [UI, IPACC29 e IPACC55] associados à adubação com N, e dois intervalos de irrigação (7 dias - bem irrigado; e 30 dias - déficit hídrico) com lâmina fixa de 8,5 mm. O objetivo foi validar, em campo, os resultados obtidos em casa de vegetação, por meio da avalição de crescimento, status hídrico, parâmetros fisiológicos e produtividade. Os procedimentos metodológicos e resultados encontram-se detalhados no **capítulo 4** (Apêndice 7).

3 CAPÍTULO 1

REFERÊNCIAL TEÓRICO

3.1 Impacto do déficit hídrico na fisiologia das plantas

A água constitui um fator essencial para o crescimento e desenvolvimento das plantas, sendo, simultaneamente, o principal elemento limitante em ecossistemas áridos e semiáridos, devido à baixa pluviosidade e às elevadas temperaturas do ar ao longo do ano (Seleiman *et al.,* 2021; Araújo Júnior *et al.,* 2024). Nessas condições, as plantas podem enfrentar déficit hídrico devido a limitada disponibilidade de água no solo ou quando a taxa evapotranspiratória se torna excessiva, resultando em alterações morfológicas, fisiológicas, bioquímicas, metabólicas e moleculares. Tais alterações variam conforme a intensidade e duração do estresse, bem como do estágio de desenvolvimento, genótipo e espécie (Soares *et al.,* 2019; IIyas *et al.,* 2021).

O déficit hídrico provoca desequilíbrio no balanço hídrico vegetal, pois a redução do potencial hídrico do solo implica diminuição do potencial hídrico da planta (Farooq *et al.,* 2009; IIyas *et al.,* 2021). Em resposta, ocorre fechamento parcial ou total dos estômatos, mediado pelo ácido abscísico (ABA), como mecanismo para reduzir as perdas de água por transpiração (Sah; Reddy; Li, 2016; Kuromori *et al.,* 2018). Entretanto, esse mecanismo limita a fixação de CO_2 nas folhas, mediada pelas enzimas ribulose-1,5-bisfosfato carboxilase/oxigenase (Rubisco) e fosfoenolpiruvato carboxilase (PEP-case), resultando na redução da taxa fotossintética (Liguori *et al.,* 2013; Siddiqui *et al.,* 2015). Como consequência, podem ocorrer limitações na biossíntese e acúmulo de pigmentos fotossintéticos, degradação da clorofila, redução da atividade enzimática da Rubisco e PEP-case, alterações na integridade dos cloroplastos e danos ao fotossistema II (PSII) (Batra; Sharma; Kumari, 2014; Zhang *et al.,* 2019). Esse conjunto de alterações está associado ao desequilíbrio energético entre a geração e utilização de elétrons na cadeia transportadora dos tilacoides, decorrente da absorção excessiva da energia luminosa, o que intensifica a produção de espécies reativas de oxigênio (EROs) (Batra; Sharma; Kumari, 2014; Zhang *et al.,* 2019). Em concentrações elevadas, as EROs podem causar danos oxidativos a ácidos nucleicos, proteínas e lipídios de membrana, podendo, em situações mais severas, desencadear morte celular programada (Sharma *et al.,* 2012; Wang *et al.,* 2024).

Os principais tipos de EROs incluem o radical superóxido ($^{\bullet}O_2^-$), o radical hidroxila ($^{\bullet}OH$), o peróxido de hidrogênio (H_2O_2) e o oxigênio singleto ($^1O_2$) (Sharma *et al.,* 2012; Wang

et al., 2024). O ânion superóxido é produzido principalmente nos cloroplastos, mitocôndrias e membranas celulares; o H_2O_2 é gerado nos peroxissomos, cloroplastos e citosol; o radical hidroxila pode ser formado na parede celular e em praticamente todos os compartimentos celulares; enquanto o oxigênio singleto é produzido predominantemente no fotossistema II e nos cloroplastos (Sharma *et al.,* 2012; Sachdev *et al.,* 2021; Wang *et al.,* 2024). De modo geral, a produção de EROs é avaliada por meio da quantificação dos teores de malondialdeído (MDA) e das espécies como H_2O_2 (Liu *et al.,* 2021). O MDA é um subproduto da peroxidação lipídica e, portanto, níveis reduzidos desse composto têm sido associados a menor dano oxidativo em diferentes espécies vegetais submetidas ao déficit hídrico (Liu *et al.,* 2021). Ressalta-se, contudo, que, em baixas concentrações, as EROs desempenham papel fisiológico, atuando como moléculas sinalizadoras do estresse, participando na regulação da cadeia transportadora de elétrons e contribuindo para a manutenção da homeostase redox celular (Ren *et al.,* 2016; Wang *et al.,* 2024).

Em nível molecular, as plantas produzem e acumulam diversos compostos bioquímicos, incluindo metabólitos primários e secundários, além de modularem a expressão gênica em resposta ao déficit hídrico. Essas alterações desempenham papel fundamental no crescimento e desenvolvimento vegetal, atuando na manutenção da integridade celular, sinalização celular, formação e estabilidade das membranas, e no armazenamento e fornecimento de energia (Wen *et al.,* 2015; Kang *et al.,* 2019).

Adicionalmente, o déficit hídrico altera o status hídrico das plantas por meio da redução da pressão de turgor e volume celular, promovendo alterações nos potenciais osmótico e hídrico (Farooq *et al.,* 2009; IIyas *et al.,* 2021). Essas alterações afetam negativamente parâmetros de crescimento, como a altura das plantas, área foliar, número de folhas, espessura de cladódios e acúmulo de biomassa fresca e seca (Scalisi *et al.,* 2016; Batool *et al.,* 2020). O teor relativo de água (TRA), suculência foliar (SF) e potencial osmótico (Ψs) são indicadores amplamente utilizados para inferir o status hídrico das plantas sob déficit hídrico, estando diretamente associados ao acúmulo de solutos orgânicos, também denominados solutos compatíveis ou osmólitos (Kasim *et al.,* 2013; Leite *et al.,* 2023).

3.2 Mecanismos de defesa das plantas ao déficit hídrico

As plantas desenvolvem diversos mecanismos de defesa em resposta ao déficit hídrico, desde alterações morfológicas a modificações fisiológicas, bioquímicas, metabólicas e moleculares, as quais contribuem para a tolerância em ambientes com baixa disponibilidade

hídrica (Farooq *et al.,* 2009; Blum, 2017; Nemeskéri; Helyes, 2019; IIyas *et al.,* 2021). De modo geral, a aclimatação ao déficit hídrico envolve, principalmente, ativação de dois mecanismos centrais: (i) ajustamento osmótico, associado ao acúmulo de solutos compatíveis, e (ii) fortalecimento do sistema de defesa antioxidante, enzimático e não enzimático (Farooq *et al.,* 2009; Blum, 2017; Nemeskéri; Helyes, 2019). Tais mecanismos devem atuar de modo integrado com respostas hormonais, morfológicas, retardamento da desidratação, ajustes hidráulicos e regulação molecular. A nível molecular, a modulação da expressão de síntese de proteínas relacionadas ao estresse, e ativação de vias de sinalização são essenciais para manter a turgescência celular e mitigar os danos decorrentes do estresse oxidativo (Farooq *et al.,* 2009; IIyas *et al.,* 2021; Seleiman *et al.,* 2021).

O ajustamento osmótico constitui um dos principais mecanismos de tolerância das plantas à seca e envolve o acúmulo de compostos de baixo peso molecular e altamente solúveis, conhecidos como solutos compatíveis. A biossíntese de muitos desses solutos requer elevado suprimento de grupos metila, oriundos das vias precursoras da serina/glicina, entre outras (Serraj; Sinclair, 2002; Singh *et al.,* 2022). O acúmulo desses compostos representa uma estratégia amplamente utilizada pelas plantas para preservar a integridade das membranas celulares, bem como prevenir a desidratação sob condições de estresse osmótico induzido pelo déficit hídrico (Serraj; Sinclair, 2002; Blum, 2017). Nesse contexto, o ajustamento osmótico permite a manutenção da pressão de turgor e volume celular mesmo sob baixo potencial hídrico, por meio da redução do potencial osmótico celular, favorecendo a absorção de água pelas raízes e, consequentemente, a manutenção do status hídrico dos tecidos e preservação das membranas celulares, condição essencial para continuidade das funções metabólicas (Wu *et al.,* 2014; Blum, 2017). Além disso, esse mecanismo contribui para a redução do estresse oxidativo, ao favorecer a desintoxicação de EROs, preservando as atividades fisiológicas e facilitando a recuperação metabólica após o alívio do estresse (Blum, 2017).

Diversos tipos de solutos são modulados durante o ajustamento osmótico, incluindo açúcares solúveis, proteínas, prolina, glicina betaína, ácidos orgânicos, flavonoides, poliaminas, compostos fenólicos, álcoois de açúcares (como manitol, sorbitol e trealose), além de íons inorgânicos, como cloreto, cálcio e potássio, entre outros (Wu *et al.,* 2014; Nemeskéri; Helyes, 2019). De modo geral, observa-se aumento nos teores desses osmólitos em resposta ao déficit hídrico, auxiliando na retenção de água e manutenção da pressão de turgor celular (Wu *et al.,* 2014; Blum, 2017). A síntese e acúmulo de osmólitos orgânicos ocorrem predominantemente no citoplasma, enquanto os osmólitos inorgânicos são, em sua maioria, compartimentalizados no vacúolo (Singh *et al.,* 2022).

O acúmulo de prolina tem sido amplamente relatado em plantas submetidas ao déficit hídrico. Esse osmólito contribui para a manutenção do potencial hídrico celular, permitindo a estabilização de proteínas e membranas, e eliminação de radicais livres. Ademais, participa da remoção de EROs, protege os fotossistemas contra a peroxidação lipídica e atua na sinalização celular e regulação da expressão gênica, desempenhando papel central na tolerância das plantas ao déficit hídrico (Benitez *et al.,* 2016; IIyas *et al.,* 2021; Singh *et al.,* 2022). Outro osmólito amplamente descrito é a glicina betaína, que exerce funções semelhantes às da prolina, estando envolvida na proteção estrutural de proteínas, manutenção da integridade das membranas celulares e preservação do aparato fotossintético. Além disso, contribui para a desintoxicação de EROs e ativação de mecanismos de defesa antioxidante (IIyas *et al.,* 2021).

Em plantas de feijão-caupi, cultivar Xique-xique, o ajustamento osmótico, decorrente do acúmulo de prolina e carboidratos solúveis, contribuiu para a manutenção do teor relativo da água e pigmentos fotossintéticos sob déficit hídrico (Miranda *et al.,* 2023a). Respostas semelhantes foram observadas em plantas de trigo, nas quais o déficit hídrico promoveu maior acúmulo, no perfil metabólico foliar, de aminoácidos, ácidos orgânicos, açúcares solúveis, álcoois de açúcares e álcoois graxos (Kang *et al.,* 2019).

Para prevenir ou mitigar os danos celulares decorrentes do acúmulo excessivo de EROs, as plantas também ativam mecanismos do sistema de defesa antioxidante enzimático e não enzimático. Entre os mecanismos enzimáticos, destacam-se a dismutase do superóxido (SOD), a catalase (CAT), a peroxidase do ascorbato (APX) e a peroxidase do guaiacol (GPOX). Paralelamente, o sistema antioxidante não enzimático inclui carotenoides, ácido ascórbico, compostos fenólicos, flavonoides, poliaminas, glutationa, α-tocoferol, além de osmólitos como prolina e glicina betaína (Sharma *et al.,* 2012; Wang *et al.,* 2024). As enzimas e os compostos atuam de forma integrada na eliminação das EROs, controlando reações oxidativas excessivas e preservando a integridade celular (Wang *et al.,* 2024).

No sistema antioxidante enzimático, a SOD constitui a primeira linha de defesa contra as EROs, catalisando a dismutação do radical superóxido ($^{\bullet}O_2^-$) em H_2O_2 e oxigênio molecular (O_2) nos cloroplastos, mitocôndrias e citosol. O H_2O_2 gerado é subsequentemente convertido em água (H_2O) e O_2 pela CAT, principalmente nos peroxissomos e mitocôndrias, prevenindo a formação do radical hidroxila ($^{\bullet}OH$). A APX, enzima chave do ciclo ascorbato (Asc)-glutationa (GSH), também atua na redução do H_2O_2 a H_2O, utilizando Asc como doador de elétrons, com ação predominante no citosol e nos cloroplastos (Sharma *et al.,* 2012; Sachdev *et al.,* 2021; Wang *et al.,* 2024). A atuação coordenada dessas enzimas, juntamente com outras do ciclo Asc–GSH, como a redutase do desidroascorbato (DHAR), a redutase do monodesidroascorbato

(MDHAR) e a redutase da glutationa (GR), além da GPOX e peroxidase da glutationa (GPX), é fundamental para a manutenção do equilíbrio redox celular (Sharma *et al.,* 2012; Sachdev *et al.,* 2021; Wang *et al.,* 2024). Esses antioxidantes encontram-se distribuídos em praticamente todos os compartimentos celulares, evidenciando a importância da desintoxicação das EROs quando produzidas em altas concentrações (Wang *et al.,* 2024).

As enzimas SOD, CAT e APX são amplamente utilizadas como indicadores da eficiência do sistema antioxidante vegetal, pois mantêm baixos os níveis de EROs nas células. Elevadas atividades dessas enzimas têm sido consistentemente associadas à maior tolerância de plantas ao déficit hídrico (Abdelaal *et al.,* 2021). Segundo Wang *et al.* (2024) a tolerância ao déficit hídrico está frequentemente relacionada ao aprimoramento do sistema de defesa antioxidante.

Entre os antioxidantes não enzimáticos, os carotenoides, como licopeno, β-caroteno, α-caroteno, zeaxantina e outras xantofilas, exercem função essencial na fotoproteção, promovendo a dissipação do excesso de energia luminosa na forma de calor e reduzindo a produção de EROs. Além disso, atuam na desativação do oxigênio singleto ($^1O_2$) no fotossistema II (PSII), prevenindo danos foto-oxidativos à clorofila e às proteínas do aparato fotossintético (Dhami; Cazzonelli, 2020; Sachdev *et al.,* 2021).

O ácido ascórbico constitui outro componente relevante do sistema antioxidante não enzimático, estando amplamente distribuído em compartimentos celulares como cloroplastos, citosol, mitocôndrias, peroxissomos e vacúolos. Sua atuação envolve neutralização de EROs e participação no ciclo Asc-GSH, contribuindo significativamente para proteção do aparato fotossintético e estabilidade metabólica sob déficit hídrico (Dumanović *et al.,* 2021; Wang *et al.,* 2024).

Os compostos fenólicos, como ácidos fenólicos, flavonoides e antocianinas, representam outro grupo relevante de metabólitos secundários com elevada capacidade antioxidante. Esses compostos atuam na captura de radicais livres por meio da doação de elétrons e neutralização de EROs, protegendo proteínas estruturais, enzimas e membranas lipídicas contra a peroxidação (Dumanović *et al.,* 2021; Singh *et al.,* 2022). Além da função antioxidante, os flavonoides podem contribuir para o ajustamento osmótico, reforçando a integridade celular sob déficit hídrico (Wang *et al.,* 2024).

Adicionalmente, a prolina desempenha múltiplas funções fisiológicas em plantas submetidas à seca. Além de atuar no ajustamento osmótico, participa da eliminação de EROs e contribui para o fortalecimento do sistema de defesa antioxidante, auxiliando na mitigação do estresse oxidativo induzido pelo déficit hídrico (Benitez *et al.,* 2016; Singh *et al.,* 2022). Em

conjunto, esses mecanismos antioxidantes e osmoprotetores são fundamentais para manutenção da integridade estrutural e funcional das células sob condições de déficit hídrico.

3.3 Bactérias promotoras de crescimento de plantas

O termo rizobactérias promotoras do crescimento de plantas, RPCP em português ou PGPR em inglês, foi utilizado pela primeira vez por Kloepper e colaboradores no final da década de 1970. Nesse grupo, os autores incorporaram microrganismos benéficos capazes de colonizar a rizosfera/endo-rizosfera, promovendo o crescimento vegetal (Vessey, 2003; Mendonça *et al.,* 2020). Posteriormente, passou-se a adotar a denominação bactérias promotoras do crescimento de plantas (BPCPs em português, ou PGPB em inglês). De modo geral, as BPCPs são microrganismos que habitam a rizosfera, estimulam crescimento vegetal e protegem as plantas contra estresses abióticos e bióticos **(**Hungria *et al.,* 2010; Ali; Khan, 2021). Segundo Gupta *et al*. (2022), essas bactérias representam uma alternativa promissora para aumento da produtividade agrícola, pois contribuem para a sustentabilidade dos sistemas de produção, reduzindo a dependência de fertilizantes químicos.

As BPCPs podem promover o crescimento vegetal por meio de um ou vários mecanismos de ação; na maioria dos casos, um mesmo isolado apresenta mecanismos combinados de promoção do crescimento. Esses mecanismos são classificados em diretos e indiretos (Glick, 2012). Os mecanismos diretos envolvem a produção de substâncias promotoras de crescimento e aumento da disponibilidade de nutrientes às plantas, enquanto os mecanismos indiretos estão associados, principalmente, ao incremento da tolerância das culturas aos estresses abióticos e bióticos (Glick, 2012; Bittencourt *et al.,* 2023).

Entre os mecanismos de ação direta, destacam-se aqueles relacionados à atuação como biofertilizantes, tais como a fixação biológica de nitrogênio (FBN), a solubilização de nutrientes minerais (como fósforo e potássio) e produção de sideróforos, que aumentam a disponibilidade de nutrientes para as plantas (Glick, 2012; Bittencourt *et al.,* 2023). Além disso, essas bactérias são capazes de sintetizar fitohormônios, como auxinas, principalmente o ácido indol-3-acético (AIA), ácido abscísico (ABA), ácido giberélico (AG), ácido salicílico (AS), citoquininas e etileno (Yang; Kloepper; Ryu, 2009; Glick, 2012; Ali; Khan, 2021). Já os mecanismos de ação indireta incluem a produção de antibióticos, que reduzem os efeitos inibitórios de diversos fitopatógenos sobre o desenvolvimento das culturas, bem como a síntese de enzimas e osmólitos envolvidos na mitigação dos danos causados por estresses abióticos nas plantas (Glick, 2012; Vurukonda *et al.,* 2016; Bittencourt *et al.,* 2023).

Diversos gêneros de BPCPs apresentam associação com espécies de importância agrícola, incluindo *Azospirillum*, *Bacillus*, *Paenibacillus*, *Rhizobium*, *Pseudomonas*, *Bradyrhizobium*, *Arthrobacter*, *Azotobacter*, *Burkholderia*, *Clostridium*, *Gluconacetobacter*, *Herbaspirillum* e *Streptomyces*, entre outros (Saharan; Nehra, 2011; Liu *et al.,* 2019; Patani *et al.,* 2024). Estudos relatam que estirpes de BPCPs, quando associadas à adubação nitrogenada, tendem a aumentar a eficiência de uso do N, reduzindo a necessidade de fertilizantes nitrogenados e, consequentemente, o impacto ambiental (Aquino *et al.,* 2021, 2022; Hungria *et al.,* 2022).

As estirpes utilizadas no presente estudo pertencem aos gêneros *Bacillus* e *Paenibacillus*, sendo *Bacillus subtilis* (IPACC29) e *Paenibacillus* sp. (IPACC38 e IPACC55), isoladas de tecidos de cana-de-açúcar e identificadas por Antunes *et al*. (2017). Essas estirpes apresentam características associadas à promoção do crescimento vegetal, incluindo a produção de fitohormônios, como AIA, e a capacidade de FBN, evidenciada pela atividade redutora de acetileno (ARA), conforme relatado por Aquino *et al*. (2021). Estudos demonstram que a inoculação dessas estirpes associada à aplicação de 50% da dose recomendada de nitrogênio melhora o metabolismo do N e crescimento do milho e sorgo em condições casa de vegetação (Aquino *et al.,* 2019, 2021) e em campo (Aquino *et al.,* 2022). Respostas semelhantes foram observadas em plantas das variedades Baiana e Doce Miúda de palma forrageira cultivadas em casa de vegetação (Sousa *et al.,* 2025).

Apesar dos avanços observados, o uso de BPCPs no cultivo da palma forrageira e seus efeitos sobre o crescimento, a produtividade e tolerância ao déficit hídrico ainda são pouco explorados na literatura. Esse cenário evidencia a necessidade de novos estudos que aprofundem a compreensão desses mecanismos e ampliem suas aplicações em sistemas agrícolas do semiárido.

3.3.1 Mecanismos de tolerância ao déficit hídrico induzidos por BPCPs

As BPCPs apresentam diversos mecanismos capazes de aumentar a tolerância ao déficit hídrico nas plantas, principalmente por meio de suas interações com o sistema radicular e de sua capacidade de adaptação a ambientes com limitação hídrica (Glick, 2012; Abdelaal *et al.,* 2021). Sob déficit hídrico, inúmeros estudos têm reportado os efeitos benéficos dessas bactérias sobre os processos fisiológicos, bioquímicos e moleculares das plantas, por meio de mecanismos que envolvem inúmeros eventos: produção e modulação de fitohormônios, como AIA, ABA, AG, AS, ácido jasmônico, citocininas e etileno; alterações na arquitetura do

sistema radicular; síntese e liberação de compostos orgânicos voláteis (COVs); produção de exopolissacarídeos (EPS) e formação de biofilmes; síntese da enzima 1-aminociclopropano-1-carboxilato (ACC) desaminase (Yang; Kloepper; Ryu, 2009; Glick, 2012; Vurukonda *et al.,* 2016; Ali; Khan, 2021); modulação do sistema de defesa antioxidante; produção e acúmulo de osmólitos compatíveis; síntese de enzimas relacionadas à resistência sistêmica; regulação da expressão gênica, incluindo genes responsivos ao estresse; FBN; produção de sideróforos e solubilização de nutrientes, entre outros processos (Vurukonda *et al.,* 2016; Gupta *et al.,* 2022; Chieb; Gachomo, 2023; Singh *et al.,* 2024). Coletivamente, esses mecanismos contribuem para o aumento da tolerância ao déficit hídrico, mitigando os danos causados pelo estresse oxidativo aos componentes celulares (Gupta *et al.,* 2022).

Entre os principais mecanismos envolvidos na resposta das plantas à inoculação com BPCPs sob déficit hídrico destaca-se a modulação de fitohormônios, especialmente a auxina, representada principalmente pelo AIA. Esse fitohormônio favorece o desenvolvimento da arquitetura do sistema radicular por meio da proliferação de raízes laterais e aumento da formação de pelos radiculares, resultando em maior área de superfície radicular e, consequentemente, maior capacidade de absorção de água e nutrientes. Esses efeitos refletem-se no aumento da biomassa radicular e parte aérea (Kavamura *et al.,* 2013; Andrade *et al.,* 2023; Giller *et al.,* 2025). Alterações no desenvolvimento radicular também contribuem para maior estabilidade das membranas celulares, melhor condutância estomática, ajuste da sinalização hormonal e incremento da atividade de antioxidante enzimático e não enzimático (Vurukonda *et al.,* 2016; Andrade *et al;* 2023).

Sob déficit hídrico, ocorre acúmulo de ABA nas plantas, hormônio diretamente associado ao fechamento estomático (Sah; Reddy; Li, 2016). Nesse contexto, as BPCPs desempenham papel relevante na modulação do equilíbrio hormonal, contribuindo para a manutenção do crescimento vegetal mesmo sob baixa disponibilidade de água (Gupta *et al.,* 2022; Bittencourt *et al.,* 2023). A rede de sinalização de antagonismo entre AIA e ABA atua de forma integrada, estando associada aos mecanismos de osmorregulação e ao sistema de defesa antioxidante, não devendo ser considerada de forma isolada (Bittencourt *et al.,* 2023).

Em condições de déficit hídrico, as plantas também apresentam aumento na produção de etileno, hormônio que, em concentrações elevadas, pode inibir o crescimento vegetal (Dubois; Van Den Broeck; Inzé, 2018). BPCPs produtoras da enzima ACC desaminase desempenham papel fundamental na redução dos níveis desse hormônio, pois essa enzima cliva o ACC, precursor imediato do etileno, em amônia e α-cetobutirato, compostos que podem ser utilizados como fonte metabólica pelos microrganismos. Como consequência, ocorre redução

da biossíntese de etileno nas plantas, favorecendo o crescimento e atenuando os danos do déficit hídrico (Zarei *et al.,* 2020; Danish *et al.,* 2021; Gupta *et al.,* 2022).

A produção EPS pelas BPCPs contribui significativamente para a melhoria das propriedades biológicas e físico-químicas do solo na rizosfera, promovendo a formação de macro agregados e aumentando a retenção de umidade ao redor das raízes. Esse processo mantém o potencial hídrico mais elevado na rizosfera, ampliando a disponibilidade de água e, consequentemente, a tolerância das plantas ao déficit hídrico (Sandhya *et al.,* 2010; Vurukonda *et al.,* 2016; Carezzano *et al.,* 2023). Além disso, os EPS participam na formação e manutenção de biofilmes, protegem células bacterianas contra a dessecação e auxiliam na FBN ao reduzir a concentração de oxigênio no microambiente, favorecendo a atividade da nitrogenase (Jha; Saraf, 2015; Ali; Khan, 2021; Carezzano *et al.,* 2023).

Os COVs produzidos pelas BPCPs também desempenham papel relevante na tolerância ao déficit hídrico, atuando como moléculas sinalizadoras na rizosfera, regulando a formação de biofilmes induzindo resistência sistêmica nas plantas (Naseem; Bano, 2014; Vurukonda *et al.,* 2016). Esses compostos contribuem para a redução do extravasamento de eletrólitos e dos níveis de MDA, além de estimularem a síntese de pigmentos fotossintéticos, prolina e fitohormônios (Yasmin *et al.,* 2021). Entre os principais COVs destacam-se aldeídos alifáticos de cadeia curta, álcoois, cetonas, ésteres, éteres, compostos sulfurados e hidrocarbonetos (Vurukonda *et al.,* 2016; Ali; Khan, 2021).

As BPCPs também induzem tolerância ao déficit hídrico por meio da modulação do sistema antioxidante enzimático e não enzimático. Estudos demonstram que a inoculação com essas bactérias altera significativamente a atividade de enzimas antioxidantes em plantas sob déficit hídrico, configurando mecanismo eficiente de tolerância sistêmica induzida (Sandhya *et al.,* 2010; Abdelaal *et al.,* 2021; Fadiji *et al.,* 2022). Buqori *et al*. (2025) observaram que plantas de cana-de-açúcar sob déficit hídrico inoculadas com estirpes de *Bacillus* apresentaram menores teores de H_2O_2 e MDA, bem como menor extravasamento de eletrólitos, associados ao aumento da atividade de SOD, CAT e APX nas folhas. Resultados semelhantes foram observados em trigo inoculado com *Paenibacillus* (Pishchik *et al.,* 2024).

Além disso, essas bactérias produzem e acumulam solutos compatíveis que atuam sinergicamente com os osmólitos sintetizados pelas plantas, promovendo ajustamento osmótico e contribuindo para a manutenção do equilíbrio hídrico celular e integridade estrutural de proteínas e membranas (Sandhya *et al.,* 2010; Vurukonda *et al.,* 2016; Ali; Khan, 2021). Entre os principais osmólitos modulados destacam-se prolina, glicina betaína, açúcares solúveis, colina, flavonoides, compostos fenólicos, ácidos orgânicos, álcoois de açúcares (manitol,

sorbitol e trealose), poliaminas e aminoácidos (Vurukonda *et al.,* 2016; Fadiji *et al.,* 2022). A trealose, por exemplo, confere maior estabilidade a proteínas e membranas sob seca, enquanto a colina atua como precursora da glicina betaína (Yang; Kloepper; Ryu, 2009; Vilchez *et al.,* 2016). Batool *et al*. (2020) verificaram que plantas de batata (*Solanum tuberosum* L.) sob déficit hídrico inoculadas com *Bacillus subtilis* apresentaram menores níveis de H_2O_2 e MDA, devido aumento da atividade das enzimas antioxidantes e acúmulo de açúcares solúveis, prolina e proteínas solúveis. Em plantas de grão-de-bico a inoculação com diferentes espécies de *Bacillus* promoveu alterações metabólicas significativas, evidenciadas pelo maior acúmulo de succinato, leucina, dissacarídeos, ácido glicérico, malonato, 5-oxo-L-prolina e trans-cinamato, entre outros metabólitos (Khan; Bano; Babar, 2019).

As BPCPs aumentam a disponibilidade de nutrientes por meio da solubilização de fósforo e potássio, FBN e produção de sideróforos (Glick, 2012; Saharan; Nehra, 2011; Chieb; Gachomo, 2023). O ferro quelatizado por sideróforos torna-se mais acessível às plantas, favorecendo processos como síntese de clorofila e fotossíntese. O aumento da disponibilidade de nutrientes contribui para a manutenção da integridade das membranas e redução dos danos oxidativos (Saharan; Nehra, 2011; Chieb; Gachomo, 2023). Nitrogênio e fósforo influenciam diretamente a biossíntese de aminoácidos, proteínas, carboidratos solúveis e metabólitos antioxidantes associados à tolerância à seca (Ibrahim *et al.,* 2012; Stincone *et al.,* 2015).

Em relação ao metabolismo do N, a fixação desse elemento nas plantas tem aumentado a atividade da nitrato redutase, bem como os teores de nitrato, amônio, aminoácidos, proteínas e carboidratos solúveis (Lee *et al.,* 2020; Aquino *et al.,* 2021; Pataczek *et al.,* 2024). Em conjunto, esses processos contribuem significativamente para a mitigação dos danos causados pelo déficit hídrico (Patel *et al.,* 2023; Patani *et al.,* 2024). Por fim, a modulação da expressão gênica desempenha papel central na interação planta-BPCPs, envolvendo complexa rede de respostas moleculares responsável pelo estabelecimento e manutenção dessas interações benéficas (Vurukonda *et al.,* 2016; Fadiji *et al.,* 2022; Salem *et al.,* 2024).

3.4 Cultura da palma forrageira

3.4.1 Caracterização e condições de cultivo

A palma forrageira é originária do México, porém apresenta ampla distribuição geográfica, estando presente em todos os continentes, exceto nas regiões polares (Marques *et al.,* 2017). Essa forrageira pertence à divisão *Embryophyta,* subdivisão *Angiospermae*, classe

Dicotyledoneae, subclasse *Archichlamydeae,* ordem *Opuntiales* e família *Cactaceae* (Silva; Santos, 2006; Oliveira *et al.,* 2010). A família Cactaceae compreende aproximadamente 178 gêneros e cerca de 2.000 espécies conhecidas. Dentre esses, os gêneros *Opuntia* e *Nopalea* destacam-se pela sua importância na produção de forragem no semiárido brasileiro (Edvan; Carneiro, 2019; Edvan *et al.,* 2020).

No gênero *Opuntia*, incluem-se as variedades Gigante, Redonda e IPA-20 (*Opuntia ficus-indica* Mill.), além da Orelha de Elefante Mexicana (*Opuntia stricta* (Haw.) Haw.). No gênero *Nopalea*, destacam-se as variedades Doce Miúda e Baiana (*Nopalea cochenillifera* Salm-Dyck) (Edvan; Carneiro, 2019; Edvan *et al.,* 2020). De modo geral, as espécies do gênero *Nopalea* apresentam maior exigência quanto às condições edafoclimáticas quando comparadas às espécies do gênero *Opuntia*, além de apresentarem maior número de cladódios, porém de menor tamanho (Edvan; Carneiro, 2019; Ramos *et al.,* 2021; Miranda *et al.,* 2023b). Atualmente, as variedades Orelha de Elefante Mexicana, Doce Miúda e Baiana são amplamente utilizadas na alimentação animal no Nordeste brasileiro, principalmente devido à resistência à cochonilha do carmim (*Dactylopius opuntiae*, Cockerell, 1896, Hemiptera: Dactylopidae), considerada a principal praga da palma forrageira (Edvan; Carneiro, 2019; Araújo Júnior *et al.,* 2021; Nascimento *et al.,* 2024).

As variedades utilizadas no presente estudo foram Doce Miúda e Baiana. Embora ambas pertençam à mesma espécie e ao mesmo gênero (*Nopalea*), apresentam diferenças morfológicas relevantes. A variedade Baiana caracteriza-se pela maior espessura dos cladódios, enquanto a variedade Doce Miúda se destaca pela produção de cladódios menores, porém em maior número, podendo tais características variar conforme as condições de cultivo **(**Silva *et al.,* 2016; Edvan *et al.,* 2020). As principais variáveis de crescimento da palma forrageira são o número de cladódios e produção de biomassa da planta, pois ambas são correlacionadas e indicativas do potencial forrageiro para alimentação animal (Cavalcante *et al.,* 2014; Edvan *et al.,* 2023).

O Brasil detém a maior área cultivada com palma forrageira no mundo, com mais de 600 mil hectares plantados, concentrados principalmente na região Nordeste, sendo o estado da Bahia o maior produtor (Fonseca *et al.,* 2023; Sousa *et al.,* 2024). A produtividade anual pode variar entre 20 e 250 t ha^{-1} de biomassa verde, dependendo de fatores como condições climáticas (temperatura, umidade relativa do ar e precipitação), manejo do solo (correção e adubação), sistema de cultivo (tradicional ou intensivo) e práticas culturais, incluindo o controle de pragas, doenças e plantas daninhas (Edvan; Carneiro, 2019).

A rusticidade é uma das principais características da palma forrageira, permitindo seu desenvolvimento em regiões com baixos índices pluviométricos. Contudo, temperatura e

umidade relativa do ar, tanto no período diurno quanto noturno, são determinantes para a produção da cultura (Rocha, 2012; Lucena *et al.,* 2016). A máxima capacidade produtiva ocorre em regiões com temperatura média entre 16,1 ºC e 25,4 ºC, temperaturas máximas entre 28,5 ºC e 31,5 ºC e mínimas entre 8,6 ºC e 20,4 ºC. A umidade relativa ideal situa-se entre 37,3% e 63,1%, recomendando-se que permaneça acima de 40%, enquanto a precipitação anual ideal varia entre 368 mm e 812 mm (Souza *et al*., 2008). Em regiões com noites quentes e secas, pode ocorrer maior perda de água por transpiração, comprometendo o acúmulo de biomassa e podendo levar à morte das plantas quando a temperatura noturna é superior a 15 ºC associada à umidade relativa inferior a 40% (Oliveira *et al.,* 2010; Edvan; Carneiro, 2019).

Quanto ao solo, recomendam-se solos argilo-arenosos, com boa fertilidade e adequada drenagem. Solos com pH entre 5,5 e 7,5 são considerados aptos ao cultivo. A cultura responde positivamente à adubação mineral, orgânica e organomineral (Santos *et al.,* 2006; Nefazoui, 2018; Dubeux Jr *et al.,* 2021).

A adubação nitrogenada difere daquela utilizada em culturas convencionais, sendo as doses ajustadas conforme o nível tecnológico adotado (médio, alto ou sistema irrigado) e a produtividade esperada de biomassa verde. O nitrogênio deve ser aplicado de forma parcelada, com a primeira dose aos 30 dias após o plantio dos cladódios. No presente estudo, utilizou-se a dose de 200 kg ha^{-1} de N, correspondente ao nível tecnológico alto e expectativa de produtividade entre 100 e 250 t ha^{-1} de biomassa verde (Edvan; Carneiro, 2019).

Para o plantio, recomenda-se o uso de cladódios sadios, livres de pragas e doenças, com tamanho e peso uniformes, evitando-se materiais muito jovens ou envelhecidos. Após o corte, os cladódios devem permanecer à sombra, em local ventilado e seco, por 10 a 15 dias, para adequada cicatrização. No plantio, recomenda-se enterrar pelo menos dois terços do comprimento do cladódio, a fim de evitar tombamento e favorecer o desenvolvimento vegetativo. O posicionamento também é importante, devendo as faces dos cladódios estar orientadas no sentido nascente-poente, para melhor aproveitamento da radiação solar (Santos *et al.,* 2006; Edvan; Carneiro, 2019; Ramos *et al.*, 2021).

Em sistemas de sequeiro, deve-se evitar o plantio durante a estação seca, prevenindo a mortalidade dos cladódios matriz por déficit hídrico. Da mesma forma, o plantio não é recomendado em períodos de elevada umidade do solo, pois favorece a incidência de podridão mole, causada pela bactéria *Erwinia carotovora*, além de doenças fúngicas que promovem o tombamento, apodrecimento e, consequentemente, a morte das plantas (Santos *et al.,* 2006; Edvan; Carneiro, 2019; Ramos *et al*., 2021; Edvan *et al.* 2023).

Por fim, durante a colheita, tanto em sistemas de sequeiro quanto irrigados, recomenda-se preservar os cladódios matriz e primários, garantindo a continuidade e longevidade do palmal. Em cultivos de sequeiro, a colheita é geralmente realizada a cada dois anos, enquanto em sistemas irrigados pode ocorrer entre seis e doze meses (Edvan; Carneiro, 2019; Ramos *et al*., 2021). As condições climáticas e as práticas agronômicas adotadas ao longo do ciclo podem acelerar ou retardar as fases de desenvolvimento da cultura, influenciando o momento da colheita (Amorim *et al.,* 2017). O método de colheita mais utilizado é o manual.

3.4.2 Metabolismo fotossintético

A palma forrageira é amplamente adaptada às condições semiáridas. Essa adaptação está relacionada à sua fisiologia, especialmente ao processo fotossintético denominado Metabolismo Ácido das Crassuláceas (CAM) (Nobel, 2001; Liguori *et al.,* 2013). Por se tratar de uma planta com metabolismo CAM, mantém os estômatos fechados durante o dia e os abre no período noturno, quando ocorre a fixação de CO_2 por meio da enzima PEP-case. O CO_2 absorvido à noite é fixado na forma de ácido málico e armazenado nos vacúolos até o período diurno. Durante o dia, esse ácido é descarboxilado, liberando CO_2, que é então assimilado no ciclo de Calvin-Benson para a síntese de carboidratos (Nobel, 2001; Taiz *et al.*, 2017). Esse mecanismo reduz significativamente as perdas de água por transpiração, contribuindo para a manutenção da hidratação dos tecidos por períodos mais prolongados e favorecendo a tolerância ao déficit hídrico em ambientes semiáridos (Nobel, 2001; Scalisi *et al.,* 2016; Ojeda-Pérez *et al.,* 2017).

Esse sistema fotossintético diferenciado foi decisivo para a adaptação da palma forrageira às condições semiáridas conferindo-lhe elevada eficiência no uso da água, expressa como a relação entre a quantidade de água consumida e a matéria seca produzida. Essa eficiência é superior à observada em espécies com metabolismo C_3 e C_4, como leguminosas e gramíneas, respectivamente (Nobel, 2001; Santos *et al.,* 2006; Queiroz *et al.,* 2021). Sampaio *et al*. (2020) relatam que a palma forrageira apresenta uma relação aproximada de 50:1, ou seja, são necessários cerca de 50 kg de água para a produção de 1 kg de biomassa seca. Em contraste, plantas C_3 e C_4 apresentam relações médias em torno de 1000:1 e 500:1, respectivamente, tornando a palma forrageira aproximadamente 20 vezes mais eficiente que espécies C_3 e 10 vezes mais eficiente que espécies C_4.

Além da adaptação fisiológica associada à assimilação de CO_2, a palma forrageira apresenta características morfológicas nos cladódios que reforçam sua tolerância a condições

adversas. Destaca-se a presença de cutícula espessa, cerosa e relativamente impermeável, que reduz a perda de água; de grandes vacúolos, que favorecem o armazenamento de água; e de estômatos em menor densidade, que contribuem para a minimização da transpiração (Hassan *et al*., 2019; Sampaio *et al*., 2020; Ramos *et al*., 2021). Adicionalmente, a espécie possui sistema radicular composto por diferentes tipos de raízes adaptadas ao semiárido, o que potencializa sua capacidade de absorção de água mesmo sob condições de baixa disponibilidade hídrica (Hassan *et al*., 2019; Sampaio *et al*., 2020).

3.4.3 Importância alimentar e nutricional

A palma forrageira apresenta ampla diversidade de usos e aplicações. Embora possua potencial para alimentação humana, utilização na indústria cosmética, produção de biocombustíveis e conservação do solo, entre outras finalidades, no Brasil sua principal destinação é a produção de cladódios para alimentação animal. Esses podem ser incluídos na dieta de ruminantes na forma de farelo desidratado ou fornecidos picados diretamente no cocho (Edvan; Carneiro, 2019; Dubeux Jr *et al.,* 2021).

A cultura da palma constitui importante fonte de energia e água para ruminantes e outros animais, especialmente durante o período seco, atuando como reserva forrageira estratégica e contribuindo para a segurança alimentar nas regiões semiáridas (Silva; Santos, 2006; Dubeux Jr *et al.,* 2021). Embora os cladódios apresentem baixos teores de matéria seca, proteína bruta, fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA), destacam-se pelos elevados teores de carboidratos solúveis, alta palatabilidade, elevado coeficiente de digestibilidade da matéria seca, alto teor de nutrientes digestíveis totais e grande capacidade de armazenamento de água. Essas características tornam a palma forrageira uma alternativa estratégica para a alimentação animal no Nordeste do Brasil, sobretudo em períodos de estiagem, quando a oferta de pastagens provenientes da vegetação nativa ou de outras espécies forrageiras é reduzida (Silva; Santos, 2006; Araújo Júnior *et al.,* 2021). Além disso, a espécie é considerada fonte relevante de vitaminas, minerais e aminoácidos, entre outros compostos.

Em virtude do metabolismo CAM, a palma forrageira contribui na redução da sazonalidade da produção de forragem ao longo do ano. Além de suprir parte das exigências nutricionais dos animais, a cultura também influencia a ingestão hídrica, diminuindo a necessidade de fornecimento adicional de água, aspecto particularmente relevante em regiões semiáridas onde esse recurso é escasso (Silva; Santos, 2006; Soares, 2017). Adicionalmente,

destaca-se sua contribuição na redução dos custos com rações concentradas na alimentação de ruminantes (Frota *et al.,* 2015; Soares, 2017).

Entretanto, apesar da boa aceitabilidade pelos animais, a palma forrageira não deve ser utilizada como única fonte alimentar, pois seus teores de fibra e proteína são insuficientes para garantir desempenho animal adequado. Assim, recomenda-se sua associação com outras fontes de nutrientes, a fim de promover dietas balanceadas e melhores resultados produtivos (Silva; Santos, 2006; Frota *et al.,* 2015; Edvan; Carneiro, 2019).

3.4.4 Desempenho da palma sob déficit hídrico

A palma forrageira apresenta adaptações morfofisiológicas que possibilitam seu crescimento e cultivo em áreas áridas e semiáridas. No entanto, as condições climáticas, especialmente a quantidade e distribuição das chuvas, afetam significativamente o potencial produtivo dessa cultura (Oliveira *et al.,* 2010). Estudos demonstram que, quando submetida a longos períodos d déficit hídrico, a palma forrageira apresenta alterações fisiológicas, como a redução da fotossíntese nos cladódios, associada à diminuição do teor de água, da espessura do parênquima, do teor de clorofila, da condutância e densidade estomática, resultando em menor acúmulo de ácido málico nos vacúolos. Como consequência, observam-se reduções nos parâmetros de crescimento, incluindo tamanho, número e área de cladódios por planta, e acúmulo de biomassa fresca e seca (Scalisi *et al.,* 2016; Araújo Júnior *et al.,* 2021; Lahbouki *et al.,* 2021, 2023).

Paralelamente, alterações bioquímicas e metabólicas também são amplamente registradas. Lahbouki *et al*. (2021) demonstraram que plantas de palma forrageira não irrigadas apresentaram redução significativa nos teores de proteínas solúveis e concentração total de ácidos nos cladódios, em comparação às plantas bem irrigadas. Além disso, o déficit hídrico prolongado intensificou o estresse oxidativo, evidenciado pelo aumento dos níveis de MDA e H_2O_2. Em resposta a esse estresse, observou-se maior acúmulo de prolina, carboidratos solúveis totais, aminoácidos livres totais, fenóis e flavonoides totais, bem como incremento da atividade das enzimas SOD, CAT e APX, evidenciando ativação de mecanismos de defesa associados ao fortalecimento dos sistemas antioxidantes não enzimático e enzimático, respectivamente. De acordo com Dubeux Jr *et al*. (2021) os cladódios são ricos em compostos fenólicos, os quais desempenham papel fundamental na proteção contra EROs.

Respostas semelhantes foram observadas em plantas da palma submetidas à condição de sequeiro e sem inoculação com fungos micorrízicos arbusculares (FMA), quando

comparadas às plantas inoculadas. Nesse estudo, além dos parâmetros bioquímicos anteriormente mencionados, verificou-se maior acúmulo de antocianinas e ácido ascórbico, bem como aumento da atividade das enzimas peroxidase (POX) e polifenol oxidase (PPO), indicando intensificação das respostas antioxidantes em plantas sob déficit hídrico na ausência dos FMA (Lahbouki *et al.,* 2023).

Estudos prévios relatam ainda que, sob déficit hídrico prolongado, plantas de palma forrageira podem reduzir a espessura dos cladódios e suprimir a emissão de novos cladódios mecanismo para minimizar a perda de água (Scalisi *et al.,* 2016; Campos *et al.,* 2021). Ademais, trabalhos anteriores indicam que cladódios recém-formados (cladódios-filhos) tendem a apresentar metabolismo fotossintético do tipo C_3, com abertura estomática durante o dia, favorecendo a importação de água a partir dos cladódios-mãe (Pimenta-Barrios *et al.,* 2005; Scalisi *et al.,* 2016). Pimenta-Barrios *et al*. (2007) observaram que, sob déficit hídrico prolongado, as plantas tendem a armazenar água nos cladódios-filhos, possivelmente como estratégia de sobrevivência e suprimento hídrico aos órgãos mais fotossinteticamente ativos. Entretanto, o desenvolvimento desses cladódios sob limitação hídrica prolongada pode aumentar a perda de água, reduzindo as vantagens ecológicas associadas à suculência da palma forrageira (Pimenta-Barrios *et al.,* 2005). Adicionalmente, Edvan *et al*. (2023) destacam o risco de mortalidade das plantas em decorrência do déficit hídrico nos primeiros meses após o plantio dos cladódios em sistemas de produção sem irrigação suplementar.

Os impactos negativos supramencionados resultam na redução da disponibilidade e qualidade nutricional da forragem, aumentando a vulnerabilidade da produção agrícola e pecuária em regiões áridas e semiáridas. Nesse contexto, a adoção de estratégias inovadoras que promovam a melhoria do desempenho produtivo da palma forrageira sob déficit hídrico prolongado torna-se relevante. O uso de microrganismos benéficos, como BPCPs, configura-se como alternativa promissora para mitigar os danos causados pelo déficit hídrico e favorecer sistemas agrícolas mais resilientes e sustentáveis.

3.4.5 Estratégias de manejo da irrigação

A irrigação da palma forrageira assume papel estratégico em regiões onde a umidade relativa do ar é inferior a 40%, devendo ser realizada preferencialmente no período noturno, a fim de minimizar as perdas de água por evapotranspiração (Edvan; Carneiro, 2019; Araújo Júnior *et al.,* 2024). Um dos principais fatores que justificam a irrigação dessa cultura é sua elevada eficiência no uso da água (Edvan; Carneiro, 2019; Queiroz *et al.,* 2021).

Diversos estudos demonstram que o fornecimento estratégico de água suplementar em sistemas de produção de palma forrageira no semiárido brasileiro constitui alternativa viável, pois promove melhorias no crescimento, desenvolvimento e na produtividade da cultura (Scalisi *et al.,* 2016; Araújo Júnior *et al.,* 2021, 2024). Nesse contexto, o manejo da irrigação baseado em estratégias de déficit hídrico controlado, fundamentado na evapotranspiração da cultura (ETc), evapotranspiração de referência (ETo), capacidade de campo (CC), potencial matricial do solo e na adoção de lâminas fixas associadas à frequência de irrigação, surge como alternativa eficiente para evitar o desperdício de água, recurso escasso na região semiárida (Amorim *et al*., 2017; Araújo Júnior *et al.,* 2021, 2024). O déficit hídrico controlado visa aumentar a produtividade da água por meio da aplicação de lâminas inferiores à demanda hídrica da cultura, sem causar prejuízos significativos ao desempenho produtivo (Kogler; Soffker, 2017).

Corroborando essa abordagem, Araújo Júnior *et al.* (2021) verificaram que o cultivo irrigado com lâminas correspondentes a até 40% da ETc para a variedade Orelha de Elefante Mexicana (OEM) e até 80% da ETc para as variedades Baiana e Doce Miúda antecipou o tempo de colheita, possibilitando o fornecimento de alimento ao rebanho em menor intervalo quando comparado ao cultivo em sequeiro. Os autores destacam ainda que a redução controlada da lâmina de irrigação pode ser adotada sem impactos negativos sobre a dinâmica de crescimento, a fenologia e o tempo de corte em ambiente semiárido.

De forma complementar, Pereira *et al*. (2021) observaram que lâminas de irrigação de até 77% da ETo aumenta a produtividade de forragem, sendo possível adotar frequências de irrigação variando de 7 a 28 dias para a variedade OEM. Miranda *et al*. (2023b), ao avaliarem variáveis fisiológicas de variedades do gênero *Opuntia*, constataram que plantas submetidas à lâmina de 8,74 mm a cada 7 dias apresentaram maior concentração interna de CO_2, taxa de assimilação de CO_2, eficiência instantânea no uso da água e eficiência instantânea de carboxilação quando comparadas à frequência de irrigação de 28 dias. Por sua vez, Pereira *et al*. (2015) verificaram que diferentes condições de disponibilidade hídrica (7,5 mm a cada 7, 14 e 28 dias) influenciaram poucas variáveis de crescimento das variedades Baiana, Doce Miúda e OEM. Adicionalmente, Campos *et al*. (2021) verificaram que o manejo da irrigação com potencial matricial igual ou superior a -3 atm (atmosfera), nas plantas de variedades Baiana, Doce Miúda, OEM e Gigante, resultou em aumento no número de cladódios por planta e incremento da produtividade de biomassa fresca e seca.

Diante do exposto, infere-se que o manejo adequado da irrigação, especialmente por meio de estratégias de déficit hídrico controlado, contribui significativamente para a melhoria do potencial produtivo da palma forrageira em condições semiáridas.

REFERÊNCIAS

ABDELAAL, K.; ALKAHTANI, M.; ATTIA, K.; HAFEZ, Y.; KIRÁLY, L.; KÜNSTLER, A. The role of plant growth-promoting bacteria in alleviating the adverse effects of drought on plants. **Biology**, v. 10, p. 520, 2021.

ALI, S.; KHAN, N. Delineation of mechanistic approaches employed by plant growth promoting microorganisms for improving drought stress tolerance in plants. **Microbiological Research**, v. 249, p. 126771, 2021.

ALVES, C. P.; JARDIM, A. M. R. F.; ARAÚJO JÚNIOR, G. N.; SOUZA, L. S. B.; ARAÚJO, G. G. L.; SOUZA, C. A.; SALVADOR, K. R. S.; LEITE, R. M. C.; PINHEIRO, A. G.; SILVA, T. G. F. How to enhance the agronomic performance of cactus-sorghum intercropped system: planting configurations, density and orientation. **Industrial Crops and Products**, v. 184, p. 115059, 2022.

AMORIM, D. M.; SILVA, T. G. F.; PEREIRA, P. C.; SOUZA, L. S. B.; MINUZZI, R. B. Phenophases and cutting time of forage cactus under irrigation and cropping systems. **Pesquisa Agropecuária Tropical**, v. 47, p. 62-71, 2017.

ANDRADE, L. A.; SANTOS, C. H. B.; FREZARIN, E. T.; SALES, L. R.; RIGOBELO, E. C. Plant growth-promoting rhizobacteria for sustainable agricultural production. **Microorganisms**, v. 11, p. 1088, 2023.

ANTUNES, J. E. L.; LYRA, M. C. C. P.; OLLERO, F. J.; FREITAS, A. S.; OLIVEIRA, L. M. S.; ARAÚJO, A. S. F.; FIGUEIREDO, M. V. B. Diversity of plant growth promoting bacteria associated with sugarcane. **Genetics and Molecular Research**, v. 16, p. 16029662, 2017.

AQUINO, J. P. A.; ANTUNES, J. E. L.; BONIFÁCIO, A.; ROCHA, S. M. B.; AMORIM, M. R.; ALCÂNTARA NETO, F.; ARAÚJO, A. S. F. Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. **Theoretical and Experimental Plant Physiology**, v. 33, p. 249-260, 2021.

AQUINO, J. P. A.; JUNIOR, F. B. M.; ANTUNES, J. E. L.; FIGUEIREDO, W. V. B.; NETO, F. A.; ARAÚJO, A. S. F. Plant growth-promoting endophytic bacteria on maize and sorghum. **Pesquisa Agropecuária Tropical**, v. 49, p. e56241, 2019.

AQUINO, J. P. A.; NETO, V. P. C.; ANDRADE, M. R. S.; OLIVEIRA, L. M. S.; ANTUNES, J. E. L.; FREITAS, A. S.; ALCÂNTARA NETO, F.; ARAÚJO, A. S. F. Plant growth-promoting bacteria increase the yield of green maize and sweet sorghum. **Journal of Plant Nutrition**, v. 46, p. 58-68, 2022.

ARAÚJO JÚNIOR, G. N.; LEITE, R. M. C.; MORAIS, J. E. F.; ALVES, C. P.; SOUZA, C. A. A.; ALMEIDA, A. C. S.; JARDIM, A. M. R. F.; SOUZA, L. S. B.; EUGENIO, S.; SILVA, T. G. F. Growth dynamic, productivity, evapotranspiration, and water-economic indices of forage cactus under different irrigation depths. **Agronomy**, v. 14, p. 691, 2024.

ARAÚJO JÚNIOR, G. N.; MORAIS, J. E. F.; SOUZA, L. S. B.; NETO, A. J. S.; ARAÚJO, G. G. L.; SILVA, T. G. F. Use of lower quality water in irrigated agriculture and effects on forages with productive potential in semiarid regions: a review. **Environmental Processes**, v. 10, p. 44, 2023.

ARAÚJO JÚNIOR, G. N.; SILVA, T. G. F.; SOUZA, L. S. B.; ARAÚJO, G. G. L.; MOURA, M. S. B.; ALVES, C. P.; SALVADOR, K. R. S.; SOUZA, C. A.; MONTENEGRO, A. A. A.; SILVA, M. J. Phenophases, morphophysiological indices and cutting time in clones of the forage cacti under controlled water regimes in a semiarid environment. **Journal of Arid Environments**, v. 190, p. 104510, 2021.

BATOOL, T.; ALI, S.; SELEIMAN, M. F.; NAVEED, N. H.; ALI, A.; AHMED, K.; ABID, M.; RIZWAN, M.; SHAHID, M. R.; ALOTAIBI, M.; AL ASHKAR, I.; MUBUSHAR, M. Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. **Scientific Reports**, v. 10, p. 16975, 2020.

BATRA, N. G.; SHARMA, V.; KUMARI, N. Drought-induced changes in chlorophyll fluorescence, photosynthetic pigments, and thylakoid membrane proteins of *Vigna radiata*. **Journal of Plant Interactions**, v. 9, p. 712-721, 2014.

BENITEZ, L. C.; VIGHI, I. L.; AULER, P. A.; AMARAL, M. N.; MORAES, G. P.; RODRIGUES, G. S.; MAIA, L. C.; JUNIOR, A. M. M.; BRAGA, E. J. B. Correlation of proline content and gene expression involved in the metabolism of this amino acid under abiotic stress. **Acta Physiologiae Plantarum**, v. 38, p. 267, 2016.

BITTENCOURT, P. P.; ALVES, A. F.; FERREIRA, M. B.; SILVA, I. L. E. S.; PINTO, V. B.; OLIVARES, F. L. Mechanisms and applications of bacterial inoculants in plant drought stress tolerance. **Microorganisms**, v. 11, p. 502, 2023.

BLUM, A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. **Plant, Cell and Environment**, v. 40, p. 4-10, 2017.

BUQORI, D. M. A. I.; SUGIHARTO, B.; SUHERMAN; SISWOYO, T. A.; HARIYONO, K. Mitigating drought stress by application of drought-tolerant *Bacillus* spp. enhanced root architecture, growth, antioxidant and photosynthetic genes expression in sugarcane. **Scientific Reports**, v. 15, p. 5259, 2025.

CAMPOS, A. R. F.; SILVA, A. J. P.; VAN LIER, Q. J.; NASCIMENTO, F. A. L.; FERNANDES, R. M.; ALMEIDA, J. N.; SILVA PAZ, V. P. Yield and morphology of forage cactus cultivars under drip irrigation management based on soil water matric potential thresholds. **Journal of Arid Environments**, v. 193, p. 104564, 2021.

CAREZZANO, M. E.; ALVAREZ STRAZZI, F. B.; PÉREZ, V.; BOGINO, P.; GIORDANO, W. Exopolysaccharides synthesized by rhizospheric bacteria: a review focused on their roles in protecting plants against stress. **Applied Microbiology**, v. 3, p. 1249-1261, 2023.

CAVALCANTE, L. A.; SANTOS, G. R. A.; SILVA, L. M.; FAGUNDES, J. L.; SILVA, M. A. Respostas de genótipos de palma forrageira a diferentes densidades de cultivo. **Pesquisa Agropecuária Tropical**, v. 44, p. 424-433, 2014.

CHIEB, M.; GACHOMO, E. W. The role of plant growth promoting rhizobacteria in plant drought stress responses. **BMC Plant Biology**, v. 23, p. 407, 2023.

DANISH, S.; ZAFAR-UL-HY, M.; MOHSIN, F.; HUSSAIN, M. ACC-deaminase producing plant growth promoting rhizobacteria and biochar mitigate adverse effects of drought stress on maize growth. **PLoS One**, v. 15, p. e0230615, 2020.

DHAMI, N.; CAZZONELLI, C. I. Environmental impacts on carotenoid metabolism in leaves. **Plant Growth Regulation**, v. 92, p. 455-477, 2020.

DUBEUX JR., J. C. B.; SANTOS, M. V. F.; CUNHA, M. V.; SANTOS, D. C.; SOUZA, R. T. A.; MELLO, A. C. L.; SOUZA, T. C. C. Cactus (*Opuntia* and *Nopalea*) nutritive value: a review. **Animal Feed Science and Technology**, v. 275, p. 114890, 2021.

DUBOIS, M.; VAN DEN BROECK, L.; INZÉ, D. The pivotal role of ethylene in plant growth. **Trends in Plant Science**, v. 23, p. 311-323, 2018.

DUMANOVIĆ, J.; NEPOVIMOVA, E.; NATIĆ, M.; KUČA, K.; JAĆEVIĆ, V. The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. **Frontiers in Plant Science**, v. 11, p. 552969, 2021.

EDVAN, R. L.; BARROS, D. M.; NASCIMENTO, R. R.; ARAÚJO, M. J.; SOUSA, H. R.; NERIS, L. M. L.; BEZERRA, L. R.; SILVA-FILHO, E. C. Potential use of hydrogel from natural fiber in the initial growth of *Nopalea cochenillifera* under water stress: water source of slow release. **Communications in Soil Science and Plant Analysis**, v. 54, p. 3063-3075, 2023.

EDVAN, R. L.; CARNEIRO, M. S. S. **Palma forrageira: cultivo e uso na alimentação animal**. Appris: Curitiba, Brazil, 2019. 93 p.

EDVAN, R. L.; MOTA, R. R. M.; DIAS, S. T. P.; NASCIMENTO, R. R.; SOUSA, S. V.; SILVA, A. L.; ARAÚJO, J. S. Resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. **Scientific Reports**, v. 10, p. 10040, 2020.

FADIJI, A. E.; SANTOYO, G.; YADAV, A. N.; BABALOLA, O. O. Efforts towards overcoming drought stress in crops: revisiting the mechanisms employed by plant growth-promoting bacteria. **Frontiers in Microbiology**, v. 13, p. 962427, 2022.

FAROOQ, M.; WAHID, A.; KOBAYASHI, N.; FUJITA, D.; BASRA, S. M. Plant drought stress: effects, mechanisms and management. **Agronomy for Sustainable Development**, v. 29, p. 185-212, 2009.

FONSECA, V. A.; DONATO, S. L.; SANTOS, M. R.; SILVA, J. A.; OLIVEIRA, C. M.; BATISTA, R. S. Morphometry and yield of Gigante forage cactus pear under irrigation and different planting densities. **Revista Caatinga**, v. 36, p. 690-701, 2023.

FROTA, M. N. L.; CARNEIRO, M. S. S.; CARVALHO, G. M. C.; NETO, R. B. A. **Palma forrageira na alimentação animal**. Teresina: Embrapa Meio-Norte, 2015. 44 p. (Documentos 233).

GILLER, K. E.; JAMES, E. K.; ARDLEY, J.; UNKOVICH, M. J. Science losing its way: examples from the realm of microbial N_2-fixation in cereals and other non-legumes. **Plant and Soil**, v. 511, p. 1-24, 2025.

GLICK, B. R. Plant growth-promoting bacteria: mechanisms and applications. **Scientifica**, v. 2012, p. 963401, 2012.

GUPTA, A.; MISHRA, R.; RAI, S.; BANO, A.; PATHAK, N.; FUJITA, M.; HASANUZZAMAN, M. Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. **International Journal of Molecular Sciences**, v. 23, p. 3741, 2022.

HASSAN, S.; INGLESE, P.; GRISTINA, L.; LIGUORI, G.; NOVARA, A.; LOUHAICHI, M.; SORTINO, G. Root growth and soil carbon turnover in *Opuntia ficus-indica* as affected by soil volume availability. **European Journal of Agronomy**, v. 105, p. 104-110, 2019.

HUNGRIA, M.; BARBOSA, J. Z.; RONDINA, A. B. L.; NOGUEIRA, M. A. Improving maize sustainability with partial replacement of N fertilizers by inoculation with *Azospirillum brasilense*. **Agronomy Journal**, v. 114, p. 2969-2980, 2022.

HUNGRIA, M.; CAMPO, R. J.; SOUZA, E. M.; PEDROSA, F. O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. **Plant and Soil**, v. 331, p. 413-425, 2010.

IBRAHIM, M. H.; JAAFAR, H. Z.; RAHMAT, A.; RAHMAN, Z. A. Involvement of nitrogen on flavonoids, glutathione, anthocyanin, ascorbic acid and antioxidant activities of Malaysian medicinal plant *Labisia pumila* Blume (Kacip Fatimah). **International Journal of Molecular Sciences**, v. 13, p. 393-408, 2011.

ILLYAS, M.; NISAR, M.; KHAN, N.; HAZRAT, A.; KHAN, A. H.; HAYAT, K.; FAHAD, S.; KHAN, A.; ULLAH, A. Drought tolerance strategies in plants: a mechanistic approach. **Journal of Plant Growth Regulation**, v. 40, n. 3, p. 926-944, 2021.

JHA, C. K.; SARAF, M. Plant growth promoting rhizobacteria (PGPR): a review. **Journal of Agricultural Research and Development**, v. 52, p. 0108-0119, 2015.

KANG, Z.; BABAR, M. A.; KHAN, N.; GUO, J.; KHAN, J.; ISLAM; SHRESTHA, S.; SHAHI, D. Comparative metabolomic profiling in the roots and leaves in contrasting genotypes reveals complex mechanisms involved in post-anthesis drought tolerance in wheat. **PLoS One**, v. 14, p. e0213502, 2019.

KASIM, W. A.; OSMAN, M. E.; OMAR, M. N.; ABD EL-DAIM, I. A.; BEJAI, S.; MEIJER, J. Control of drought stress in wheat using plant-growth-promoting bacteria. **Journal of Plant Growth Regulation**, v. 32, p. 122-130, 2013.

KAVAMURA, V. N.; SANTOS, S. N.; SILVA, J. L.; PARMA, M. M.; ÁVILA, L. A.; VISCONTI, A.; ZUCCHI, T.; TAKETANI, R. G.; ANDREOTE, F.; MELO, I. S. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. **Microbiological Research**, v. 168, p. 183-191, 2013.

KHAN, I.; ULLAH, N.; MAQBOOL, H.; HASHMI, A.; KHAN, B.; KHALID, A.; NAZ, I.; PAKER, N. P.; MUNIS, M. F. H.; CHAUDHARY, H. J. Holistic review on *Paenibacillus*: the multifunctional genus in heavy metal detoxification, pesticide degradation, and abiotic stress mitigation for sustainable agriculture. **Plant and Soil**, v. 518, p. 1245-1280, 2026.

KHAN, N.; BANO, A.; BABAR, M. A. Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum L*. **PloS One**, v. 14, p. e0213040, 2019.

KHAN, N.; BANO, A. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. **PLoS One**, v. 14, p. e0222302, 2019.

KÖGLER, F.; SÖFFKER, D. Water (stress) models and deficit irrigation: system-theoretical description and causality mapping. **Ecological Modelling**, v. 361, p. 135-156, 2017.

KUROMORI, T.; SEO, M.; SHINOZAKI, K. ABA transport and plant water stress responses. **Trends in Plant Science**, v. 23, p. 513-522, 2018.

LAHBOUKI, S.; BEN-LAOUANE, R.; ANLI, M.; BOUTASKNIT, A.; AIT-RAHOU, Y.; AIT-EL-MOKHTAR, M.; EL GABARDI, S.; DOUIRA, A.; WAHBI, S.; OUTZOURHIT, A. Arbuscular mycorrhizal fungi and/or organic amendment enhance the tolerance of prickly pear (*Opuntia ficus-indica*) under drought stress. **Journal of Arid Environments**, v. 199, p. 104703, 2022.

LAHBOUKI, S.; ECH-CHATIR, L.; ER-RAKI, S.; OUTZOURHIT, A.; MEDDICH, A. Improving drought tolerance of *Opuntia ficus-indica* under field using subsurface water retention technology: changes in physiological and biochemical parameters. **Canadian Journal of Soil Science**, v. 102, p. 888-898, 2021.

LAHBOUKI, S.; FERNANDO, A. L.; RODRIGUES, C.; BEN-LAOUANE, R.; AIT-EL-MOKHTAR, M.; OUTZOURHIT, A.; MEDDICH, A. Effects of humic substances and mycorrhizal fungi on drought-stressed cactus: focus on growth, physiology, and biochemistry. **Plants**, v. 12, p. 4156, 2023.

LEE, S.; TRỊNH, C. S.; LEE, W. J.; JEONG, C. Y.; TRUONG, H. A.; CHUNG, N.; KANG, C.; LEE, H. *Bacillus subtilis* strain L1 promotes nitrate reductase activity in *Arabidopsis* and elicits enhanced growth performance in *Arabidopsis*, lettuce, and wheat. **Journal of Plant Research**, v. 133, p. 231-244, 2020.

LEITE, W. S.; MIRANDA, R. S.; ROCHA, M. M.; DUTRA, A. F.; SANTOS, A. S.; SILVA, A. C.; BRITO, F. M.; SOUSA, R. S.; ARAÚJO, S. F.; NASCIMENTO, C. W. A.; ZUFO, A. M.; ALCANTARA NETO, F. Silicon alleviates drought damage by increasing antioxidant and photosynthetic performance in cowpea. **Journal of Agronomy and Crop Science**, v. 00, p. 1-16, 2023.

LIGUORI, G.; INGLESE, G.; PERNICE, F.; SORTINO, G.; INGLESE, P. CO_2 uptake of *Opuntia ficus-indica* (L.) Mill. whole trees and single cladodes, in relation to plant water status and cladode age. **Italian Journal of Agronomy**, v. 8, p. e3, 2013.

LIU, R. N.; JIAO, T. Q.; LI, J.; WANG, A. Y.; LI, Y. X.; WU, S. J.; DU, L. Q.; DIJKWEL, P.; ZHU, J. B. Drought-induced increase in catalase activity improves cotton yield when grown under water-limiting field conditions. **Journal of Agronomy and Crop Science**, v. 00, p. 1-15, 2021.

LIU, X.; LI, Q.; LI, Y.; GUAN, G.; CHEN, S. *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. **PeerJ**, v. 7, p. e7445, 2019.

LUCENA, D. B.; MEDEIROS, R. M.; SABOYA, L. M. F.; NASCIMENTO, P. L. Aptidão e zoneamento agroclimático da palma forrageira para o estado do Piauí. **Revista Brasileira de Agricultura Irrigada**, v. 10, p. 809-819, 2016.

MARQUES, O. F. C.; GOMES, L. P. S.; MOURTHÉ, M. H. F.; BRAZ, T. G. S.; NETO, O. S. P. Palma forrageira: cultivo e utilização na alimentação de bovinos. **Caderno de Ciências Agrárias**, v. 9, p. 75-93, 2017.

MENDONÇA, J. J.; JUNIOR, M. A. L.; CARVALHO, E. X.; FRACETTO, G. G. M.; FRACETTO, F. J. C.; ALVES, M. J. G.; OLIVEIRA, J. P. Diversidade, mecanismos de atuação e potencial agrícola de bactérias promotoras de crescimento de plantas, usando milho como cultura exemplo. **Pesquisa Agropecuária Pernambucana**, v. 25, p. e2282252020, 2020.

MIRANDA, J. R.; FURTADO, D. A.; SILVA, V. C.; NETO, J. D; SOUZA, J. T. A.; ARAÚJO, J. S. Physiology of the forage cactus cultivar *Opuntia stricta* (Haw.) Haw under different irrigation frequencies in the semiarid. **Revista Ceres**, v. 70, p. 24-31, 2023b.

MIRANDA, R. S.; FONSECA, B. S. F.; PINHO, D. S.; BATISTA, J. Y. N.; BRITO, R. R.; SILVA, E. M.; FERREIRA, W. S.; COSTA, J. H.; LOPES, M. S.; SOUSA, R. H. B.; NEVES, L. F.; PENHA, J. A. F.; SANTOS, A. S.; LIMA, J. J. P.; PAULA-MARINHO, S. O.; ALCÂNTARA NETO, F.; AGUIAR, E. S.; SANTOS, C. P.; GOMES-FILHO, E. Selection of soybean and cowpea cultivars with superior performance under drought using growth and biochemical aspects. **Plants**, v. 12, p. 3134, 2023a.

NASCIMENTO, D. B.; MAGALHÃES, A. L. R.; ARAÚJO, G. G. L.; ROCHA, A. T.; OLIVEIRA, G. F.; MACEDO, A.; ARAÚJO, C. A.; NAGAHAMA, H. J.; SILVA, T. G. F.; TURCO, S. H. N.; SOUZA, R. T. A.; GOIS, G. C.; CAMPOS, F. S. Morphological and nutritional indicators of cactus pear in a semiarid region. **Agronomy**, v. 14, p. 2366, 2024.

NASEEM, H.; BANO, A. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. **Journal of Plant Interactions**, v. 9, p. 689-701, 2014.

NEFZAOUI, A. *Opuntia ficus-indica* productivity and ecosystem services in arid areas. **Italus Hortus**, v. 25, p. 29-39, 2018.

NEMESKÉRI, E.; HELYES, L. Physiological responses of selected vegetable crop species to water stress. **Agronomy**, v. 9, p. 447, 2019.

NOBEL, P. S. **Ecophysiology of *opuntia ficus-indica***. In: MONDRAGÓN-JACOBO, C.; PÉREZ-GONZÁLEZ, S. Cactus *(Opuntia* spp.*)* as forage. FAO, 2001. p. 13-20.

OJEDA-PÉREZ, Z. Z.; JIMÉNEZ-BREMONT, J. F.; DELGADO-SÁNCHEZ, P. Continuous high and low temperature induced a decrease of photosynthetic activity and changes in the diurnal fluctuations of organic acids in *Opuntia streptacantha*. **PLoS One**, v. 12, p. e0186540, 2017.

OLIVEIRA, F. T.; SOUTO, J. S.; SILVA, R. P.; ANDRADE FILHO, F. C.; JÚNIOR, E. B. P. Palma forrageira: adaptação e importância para os ecossistemas áridos e semiáridos. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 5, p. 27-37, 2010.

PATACZEK, L.; ARMAS, J. C. B.; PETSCH, T.; HILGER, T.; AHMAD, M.; SCHAFLEITNER, R.; ZAHIR, Z. A.; CADISCH, G. Single-strain inoculation of *Bacillus subtilis* and *Rhizobium phaseoli* affects nitrogen acquisition of an improved mungbean cultivar. **Journal of Soil Science and Plant Nutrition**, v. 24, p. 6746-6759, 2024.

PATANI, A.; PATEL, M.; ISLAM, S.; YADAV, V. K.; PRAJAPATI, D.; YADAV, A. N.; SAHOO, D. K.; PATEL, A. Recent advances in *Bacillus*-mediated plant growth enhancement: a paradigm shift in redefining crop resilience. **World Journal of Microbiology and Biotechnology**, v. 40, p. 77, 2024.

PATEL, M.; ISLAM, S.; HUSAIN, F. M.; YADAV, V. K.; PARK, H. K.; YADAV, K. K.; BAGATHARIA, S.; JOSHI, M.; JEON, B.; PATEL, A. *Bacillus subtilis* ER-08, a multifunctional plant growth-promoting rhizobacterium, promotes the growth of fenugreek (*Trigonella foenum-graecum* L.) plants under salt and drought stress. **Frontiers in Microbiology**, v. 14, p. 1208743, 2023.

PEREIRA, J. S.; CAVALCANTE, A. B.; SIMÕES, W. L.; VOLTOLINI, T. V. Forage yield, structural responses and chemical composition of spineless cactus 'Orelha de Elefante Mexicana' in different water depths and irrigation frequencies. **Ciência Rural**, v. 51, p. e20200324, 2021.

PEREIRA, P. C.; SILVA, T. G. F.; ZOLNIER, S.; MORAIS, J. E. F.; SANTOS, D. C. Morfogênese da palma forrageira irrigada por gotejamento. **Revista Caatinga**, v. 28, p. 184-195, 2015.

PIMIENTA-BARRIOS, E.; CASTILLO-CRUZ, I.; ZAÑUDO-HERNÁNDEZ, J.; MÉNDEZ-MORÁN, L.; NOBEL, P. S. Effects of shade, drought and daughter cladodes on the CO_2

uptake by cladodes of *Opuntia ficus-indica*. **Annals of Applied Biology**, v. 151, p. 137-144, 2007.

PIMIENTA-BARRIOS, E.; ZAÑUDO-HERNÁNDEZ, J.; ROSAS-ESPINOZA, V. C.; VALENZUELA-TAPIA, N.; NOBEL, P. S. Young daughter cladodes affect CO_2 uptake by mother cladodes of *Opuntia ficus-indica*. **Annals of Botany**, v. 95, p. 363-369, 2005.

PISHCHIK, V. N.; CHIZHEVSKAYA, E. P.; CHEBOTAR, V. K.; MIRSKAYA, G. V.; KHOMYAKOV, Y. V.; VERTEBNY, V. E.; KONONCHUK, P. Y.; KUDRYAVTCEV, D. V.; BORTSOVA, O. A.; TIKHONOVICH, I. A. PGPB isolated from drought-tolerant plants help wheat plants to overcome osmotic stress. **Plants**, v. 13, p. 3381, 2024.

QUEIROZ, M. G.; SILVA, T. G. F.; ZOLNIER, S.; JARDIM, A. M. R. F.; SOUZA, C. A. A.; ARAÚJO JÚNIOR, G. N.; MORAIS, J. E. F.; SOUZA, L. S. B. Spatial and temporal dynamics of soil moisture for surfaces with a change in land use in the semi-arid region of Brazil. **Catena**, v. 188, p. 104457, 2020.

QUIROZ, M.; VARNERO, M. T.; CUEVAS, J. G.; SIERRA, H. Cactus pear (*Opuntia ficus-indica*) in areas with limited rainfall for the production of biogas and biofertilizer. **Journal of Cleaner Production**, v. 289, p. 125839, 2021.

RAMOS, J. P. F.; MACÊDO, A. J. S.; SANTOS, E. M.; EDVAN, R. L.; SOUSA, W. H.; PERAZZO, A. F.; SILVA, A. S.; CARTAXO, F. Q. Forage yield and morphological traits of cactus pear genotypes. **Acta Scientiarum. Agronomy**, v. 43, p. 51214, 2021.

REN, J.; SUN, L. N.; ZHANG, Q. Y.; SONG, X. S. Drought tolerance is correlated with the activity of antioxidant enzymes in *Cerasus humilis* seedlings. **BioMed Research International**, v. 2016, p. 9851095, 2016.

ROCHA, J.E.S. **Palma forrageira no Nordeste do Brasil: o estado da arte**. Sobral: Embrapa Caprinos e Ovinos, 2012. 40 p. (Documentos, 106).

SACHDEV, S.; ANSARI, S. A.; ANSARI, M. I.; FUJITA, M.; HASANUZZAMAN, M. Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. **Antioxidants**, v. 10, p. 277, 2021.

SAH, S. K.; REDDY, K. R.; LI, J. Abscisic acid and abiotic stress tolerance in crop plants. **Frontiers in Plant Science**, v. 7, p. 571, 2016.

SAHARAN, B. S.; NEHRA, V. Plant growth promoting rhizobacteria: a critical review. **Life Sciences and Medicine Research**, v. 21, p. 30, 2011.

SALEM, M. A.; ISMAIL, M. A.; RADWAN, K. H.; ABD-ELHALIM, H. M. Unlocking the potential of plant growth-promoting rhizobacteria to enhance drought tolerance in Egyptian wheat (*Triticum aestivum*). **Sustainability**, v. 16, p. 4605, 2024.

SAMPAIO, E. V. S. B.; SALES, A. T.; DUTRA, E.; MENEZES, R. S. C. **Morfologia e fisiologia das palmas forrageiras**. In: SANTOS, M. V. F.; CARVALHO, F. F. R.; FERREIRA, M. A. Palma forrageira: potencial e perspectivas. Editora Universitária da UFPE, 2020. p. 63-88.

SANDHYA, V. S. K. Z.; ALI, S. Z.; GROVER, M.; REDDY, G.; VENKATESWARLU, B. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. **Plant Growth Regulation**, v. 62, p. 21-30, 2010.

SANTOS, D. C.; FARIAS, I.; LIRA, M. A.; SANTOS, M. V. F.; ARRUDA, G. P.; COELHO, R. S. B.; DIAS, F. M.; MELO, J. N. **Manejo e utilização da palma forrageira (*Opuntia* e *Nopalea*) em Pernambuco**. Recife: IPA, 2006. 38 p. (Documentos 30).

SANTOS, W. M.; SOUZA, R. M. S.; SOUZA, E. S.; ALMEIDA, A. Q.; ANTONINO, A. C. D. Variabilidade espacial da sazonalidade da chuva no semiárido brasileiro. **Journal of Environmental Analysis and Progress**, v. 2, p. 368-376, 2017.

SCALISI, A.; MORANDI, B.; INGLESE, P.; BIANCO, R. L. Cladode growth dynamics in *Opuntia ficus-indica* under drought. **Environmental and Experimental Botany**, v. 122, p. 158-167, 2016.

SELEIMAN, M. F.; AL-SUHAIBANI, N.; ALI, N.; AKMAL, M.; ALOTAIBI, M.; REFAY, Y.; DINDAROGLU, T.; ABDUL-WAJID, H. H.; BATTAGLIA, M. L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. **Plants**, v. 10, p. 259, 2021.

SERRAJ, R.; SINCLAIR, T. R. Osmolyte accumulation: can it really help increase crop yield under drought conditions? **Plant, Cell and Environment**, v. 25, p. 333-341, 2002.

SHARMA, P.; JHA, A. B.; DUBEY, R. S.; PESSARAKLI, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. **Journal of Botany**, v. 2012, p. 217037, 2012.

SIDDIQUI, M. H.; AL-KHAISHANY, M. Y.; AL-QUTAMI, M. A.; AL-WHAIBI, M. H.; GROVER, A.; ALI, H. M.; ALI, M. H.; AL-WAHIBI, S. M.; BUKHARI, N. A. Response of different genotypes of faba bean plant to drought stress. **International Journal of Molecular Sciences**, v. 16, p. 10214-10227, 2015.

SILVA, C. C. F.; SANTOS, L. C. Palma forrageira (*Opuntia ficus-indica* Mill) como alternativa na alimentação de ruminantes. **Revista Electrónica de Veterinaria**, v. 7, p. 1-13, 2006.

SILVA, J. A.; DONATO, S. L. R.; DONATO, P. E. R.; SOUZA, E. S.; PADILHA JÚNIOR, M. C.; SILVA JUNIOR, A. A. Yield and vegetative growth of cactus pear at different spacings and under chemical fertilizations. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 20, p. 564 -569, 2016.

SINGH, A.; MEHTA, S.; YADAV, S.; NAGAR, G.; GHOSH, R.; ROY, A.; CHAKRABORTY, A.; SINGH, I. K. How to cope with the challenges of environmental stresses in the era of global climate change: an update on ROS stave off in plants. **International Journal of Molecular Sciences**, v. 23, p. 1995, 2022.

SINGH, D.; THAPA, S.; SINGH, J. P.; MAHAWAR, H.; SAXENA, A. K.; SINGH, S. K.; MAHLA, H. R.; CHOUDHARY, M.; PARIHA, M.; CHOUDHARY, K. B.; CHAKDA, H.

Prospecting the potential of plant growth-promoting microorganisms for mitigating drought stress in crop plants. **Current Microbiology**, v. 81, p. 81-84, 2024.

SOARES, J. C.; SANTOS, C. S.; CARVALHO, S. M. P.; PINTADO, M. M.; VASCONCELOS, M. W. Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. **Plant and Soil**, v. 443, p. 1-26, 2019.

SOARES, M. S. Palma forrageira: aspecto do cultivo e desempenho animal. **Nutritime Revista Eletrônica**, v. 14, p. 6041- 6055, 2017.

SOUSA, A. B.; LEITE, M. R. L.; DAUALA, G. A.; MORAIS, P. G. C.; PAULA-MARINHO, S. O.; MONTEIRO, G. N.; DUARTE, M. H. F.; OLIVEIRA, R. I.; SILVA, R. F.; AVIZ, R. O.; MIRANDA, R. S.; BOECHAT, C. L.; ARAÚJO, A. S. F.; EDVAN, R. L.; COSTA, E. M. *Bacillus* and *Paenibacillus* strains enhance nitrogen metabolism and increase biomass production of *Nopalea cochenillifera* grown in semi-arid soil. **Journal of Soil Science and Plant Nutrition**, v. 25, p. 10782-10798, 2025.

SOUSA, I. S.; BEZERRA, R. M.; SILVA FILHO, E. C.; BEZERRA, L. R.; EDVAN, R. L.; LIMA, S. B.; LOPES, E. C.; CARVALHO, F. L. A.; MOURA, F. A. S.; SOUZA, G. I.; DOURADO, L. R. B. Use of cactus pear meal in the feeding of laying hens in semi-intensive system. **Animals**, v. 14, p. 625, 2024.

SOUZA, L. S. B.; MOURA, M. S. B.; SILVA, T. G. F.; SOARES, J. M.; CARMO, J. F. A.; BRANDÃO, E. O. **Indicadores climáticos para o zoneamento agrícola da palma forrageira (*Opuntia* sp.)**. Petrolina: Embrapa Semiárido, 2008. 6 p. (Documentos, 210).

STINCONE, A.; PRIGIONE, A.; CRAMER, T.; WAMELINK, M. M.; CAMPBELL, K.; CHEUNG, E.; OLIN-SANDOVAL, V.; GRUNING, N. M.; UGER, A. K.; ALAM, M. T.; KELLER, M. A.; BREITENBACH, K.; BRINDE, K. M.; RABINOWITZ, J. D.; RALSER, M. The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. **Biological Reviews**, v. 90, p. 927-963, 2015.

TAIZ, L.; ZEIGER, E.; MOLLER, I. M.; MURPHY, A. **Fisiologia Vegetal**. Porto Alegre: Artmed, 2017. 858 p.

TARIQ, H.; SUBRAMANIAN, S.; GEITMANN, A.; SMITH, D. L. *Bacillus* and *Paenibacillus* as plant growth-promoting bacteria in soybean and cannabis. **Frontiers in Plant Science**, v. 16, p. 1529859, 2025.

VESSEY, J. K. Plant growth promoting rhizobacteria as biofertilizers. **Plant and Soil**, v. 255, p. 571-586, 2003.

VÍLCHEZ, J. I.; GARCÍA-FONTANA, C.; ROMÁN-NARANJO, D.; GONZÁLEZ-LÓPEZ, J.; MANZANERA, M. Plant drought tolerance enhancement by trehalose production of desiccation-tolerant microorganisms. **Frontiers in Microbiology**, v. 7, p. 1577, 2016.

VURUKONDA, S. S. K. P.; VARDHARAJULA, S.; SHRIVASTAVA, M.; SKZ, A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. **Microbiological Research**, v. 184, p. 13-24, 2016.

WANG, P.; LIU, W. C.; HAN, C.; WANG, S.; BAI, M. Y.; SONG, C. P. Reactive oxygen species: multidimensional regulators of plant adaptation to abiotic stress and development. **Journal of Integrative Plant Biology**, v. 66, p. 330-367, 2024.

WEN, W.; LI, K.; ALSEEKH, S.; OMRANIAN, N.; ZHAO, L.; ZHOU, Y.; FERNIE, A. Genetic determinants of the network of primary metabolism and their relationships to plant performance in a maize recombinant inbred line population. **The Plant Cell**, v. 27, p. 1839-1856, 2015.

WU, S.; HU, C.; TAN, Q.; NIE, Z.; SUN, X. Effects of molybdenum on water utilization, antioxidative defense system and osmotic-adjustment ability in winter wheat (*Triticum aestivum*) under drought stress. **Plant Physiology and Biochemistry**, v. 83, p. 365-374, 2014.

YANG, J.; KLOEPPER, J. W.; RYU, C. M. Rhizosphere bacteria help plants tolerate abiotic stress. **Trends in Plant Science**, v. 14, p. 1-4, 2009.

YASMIN, H.; RASHID, U.; HASSAN, M. N.; NOSHEEN, A.; NAZ, R.; ILLYAS, N.; SAJJAD, M.; AZMAT, A.; ALYEMENI, M. N. Volatile organic compounds produced by *Pseudomonas pseudoalcaligenes* alleviated drought stress by modulating defense system in maize (*Zea mays* L.). **Physiologia Plantarum**, v. 172, p. 896-911, 2021.

ZAREI, T.; MORADI, A.; KAZEMEINI, S. A.; AKHGAR, A.; RAHI, A. A. The role of ACC deaminase producing bacteria in improving sweet corn (*Zea mays* L. var. *saccharata*) productivity under limited availability of irrigation water. **Scientific Reports**, v. 10, p. 20361, 2020.

ZHANG, Y. J.; GAO, H.; LI, Y. H.; WANG, L.; KONG, D. S.; GUO, Y. Y.; YAN, F.; WANG, Y. W.; LU, K.; TIAN, J. W.; LU, Y. L. Effect of water stress on photosynthesis, chlorophyll fluorescence parameters and water use efficiency of common reed in the Hexi Corridor. **Russian Journal of Plant Physiology**, v. 66, p. 556-563, 2019.

4 CAPÍTULO 2

PLANT GROWTH-PROMOTING BACTERIA MODULATE METABOLISM AND NITROGEN ACCUMULATION TO COUNTERACT DROUGHT DAMAGE IN CACTUS PEAR PLANTS

(Paper published in *Scientific Reports* - https://doi.org/10.1038/s41598-025-20509-5 - Appendix 1)

ABSTRACT

Searching sustainable management techniques to enhance plant resistance to drought is crucial for agriculture in stressful scenarios. This study aimed to investigate the role of Plant Growth-Promoting Bacteria (PGPB) strains on forage cactus pear performance under drought. Trials were conducted in greenhouse using forage cactus plants in randomized block experimental design, 8 × 2 factorial scheme, consisting of eight inoculation treatments [inoculation with *Paenibacillus* sp. IPACC38 and IPACC55, and *Bacillus subtilis* IPACC29 strains combined with and without nitrogen (N) fertilization] and two water regimes [control at 50% field capacity (FC) and drought at 25% FC]. Drought significantly decreased plant growth across all treatments, regardless of N/inoculation treatment. The best performance under drought was observed in plants grown without N and inoculated with IPACC29 and IPACC38 strains. Improved performance under drought was closely related to PGPB's capacity to provide N accumulation for strategic photosynthetic pigments content, resulting in better root growth and plant development. Our findings reveal that IPACC29 and IPACC38 are the most promising PGPB strains for inoculating forage cactus pear, activating important defense responses against drought. This system offers alternative for cultivating cactus under water restriction and reduced N availability to increase green forage biomass production in dry regions.

Keywords: Nitrogen availability, *Nopalea cochenillifera* Salm-Dyck, Semiarid, Stress tolerance, PGPB inoculation, Water deficit.

4.1 Introduction

Drought is a global phenomenon that affects crops growth and productivity in arid and semi-arid ecosystems worldwide, due to low rainfall and high temperatures throughout a year (Fadiji *et al*., 2022; Singh *et al.,* 2024). Water limitation negatively influences biochemical and physiological processes in plants, depending on intensity and duration of stress, well as plant developmental stage, genotypes and species (Soares *et al.,* 2019; Ali; Khan, 2021; IIyas *et al.,* 2021).

Long periods of drought may impose severe limitations on both native and cultivated species, such as grasses and legumes, causing the decrease in biomass and pasture for animals (Edvan *et al.,* 2020). This environmental constraint represents one of the main challenges faced by small- and medium-scale producers in providing adequate animal feed (Ramos *et al.,* 2021). In this scenario, cultivating cactus pear (*Nopalea cochenillifera* Salm-Dick) emerges as an alternative under water scarcity conditions, as it can serve as a source of food, water and energy for ruminants and other animals.

Cactus pear is a live forage reserve associated with good palatability and satisfactory biomass production, exhibiting high water-use efficiency due to the Crassulacean Acid Metabolism (CAM) photosynthetic pathway (Edvan *et al.,* 2020; Dubeux Jr *et al.,* 2021). However, despite cactus pear plants exhibit good performance in dry regions, prolonged drought periods can negatively impact on the crop, resulting in decrease of nocturnal stomatal conductance, malic acid accumulation, relative water content, chlorophyll content and cladode development (Scalisi *et al.,* 2016; Lahbouki *et al.,* 2023). Water scarcity immediately after cladode planting may lead to plant death, resulting in planting failures and productivity losses under rainfed condition (Edvan *et al.,* 2023).

Therefore, searching for sustainable and low-cost technologies to mitigate drought-related damage in cactus pear cultivation is crucial, particularly for small- and medium-scale farmers in semi-arid and arid regions. Thus, plant growth-promoting bacteria (PGPB) have emerged as a promising strategy for enhancing crop resilience and production of green forage biomass under drought stress, as well as promoting sustainable agriculture by reducing dependence on agricultural fertilizers (Gupta *et al.,* 2022; Salem *et al.,* 2024).

PGPB colonize the endo-rhizosphere or rhizosphere and enhance drought tolerance through direct and indirect mechanisms (Hungria *et al.,* 2010; Ali; Khan, 2021). Some studies have pointed out that plant-PGPB interactions can provide nitrogen for plants as well as promote important alterations in primary and secondary metabolism, while also regulating plant

stress-responsive pathways (Yang; Kloepper; Ryu, 2009; Kavamura *et al.,* 2013; Vurukonda *et al.,* 2016; Ali; Khan, 2021; Danish *et al.,* 2021; Gupta *et al.,* 2022; Salem *et al.,* 2024). In kentucky bluegrass (*Poa pratensis*), inoculation with *Bacillus subtilis* strains promote greater growth and development compared with uninoculated turfgrass under drought stress (Zhang; Rue, 2024). Also, drought-stressed bean plants (*Phaseolus vulgaris*) displayed an important increase in growth, nitrogen content, and nodulation under inoculation with *Paenibacillus s*p. Strains (Riseh *et al.,* 2021; Gupta *et al.,* 2022).

Although numerous reports have evidenced the beneficial role of PGPB in enhancing drought tolerance in various crops, their impact on cactus pear plants under drought conditions remain underexplored. Our investigative study aimed to evaluate the role of plant growth-promoting bacterial strains to enhance the drought tolerance of cactus pear (Miúda cultivar, *O. cochenillifera*), by evaluating plant growth, physiological parameters, and biochemical indicators.

4.2 Material and methods

4.2.1 Study site

The experiments were carried out under greenhouse conditions, between March and September 2023, at the Campus Professora Cinobelina Elvas, Federal University of Piauí (UFPI) (9°04′45.6″ S, 44°19′37.9″ W and 277 m altitude), in Bom Jesus city, Piauí state, Brazil.

4.2.2 Growing conditions, experimental design and treatments

The trials were conducted in a randomized block experimental design, in an 8 × 2 factorial scheme, with four repetitions, totaling 64 experimental units. The first factor consisted of eight inoculation treatments [three PGPB strains [*Paenibacillus* sp. IPACC38 (IC38) and IPACC55 (IC55) and *Bacillus subtilis* IPACC29 (IC29)] associated with and without N, and two uninoculated (UI) controls (with and without N)], while the second factor consisted of two water levels [well irrigated - 50% of soil field capacity (FC), and drought - 25% FC].

The Miúda cultivar from *Nopalea cochenillifera* Salm-Dyck, a cultivated species not protected by intellectual property rights, was selected alongside the inoculation treatments based on findings from preliminary trials (Sousa, 2023**)**. The PGPB strains were obtained from sugarcane tissues (surface disinfected) and identified by Antunes *et al*. (2017). These strains

were selected based on ability to act in different processes (indole-3-acetic acid production and biological N_2 fixation) and promote the growth of plant species such as corn and sorghum (Aquino *et al.,* 2022) and cactus pear (Sousa, 2023).

Soil samples of a dystrophic Yellow Latosol (Oxisol) from the 0-20 cm layer were collected at the Alvorada do Gurguéia experimental farm of UFPI for chemical and physical analyses (Appendix 2). The fertilization was corrected as necessary using P_2O_5 (as simple superphosphate, 18%), K_2O (as potassium chloride, 60%), Cu (as copper sulfate, 26.23%), Zn (as zinc sulfate, 22.73%), and N (as urea, 45%), following recommendations for cactus pear crop (Sousa; Lobato, 2004; Edvan; Carneiro, 2019**)**. The nitrogen was applied as 50 mg dm^{-3} (equivalent to 100 kg ha^{-1}), corresponding to 50% of a dose recommended (100 mg dm^{-3} corresponding to 200 kg ha^{-1}) for cactus pear fertilization (Edvan; Carneiro, 2019). All fertilizers were incorporated to the pots containing 9 dm^3 of soil prior to planting.

Uniform matrix cladodes of Miúda cultivar were collected from the UFPI' Agrostological Experimental Field. The cladodes were selected from 2-year-old plants free from pests and diseases, preferably selecting younger (tertiary) cladodes with similar age, weight, and morphological characteristics such as size, diameter, and thickness. Before planting, PGPB strains were reactivated and checked for purity on TSA (Tryptone Soy Agar) medium at 25%, pH 6.8. For inoculum preparation, the PGPB strains were cultivated in Erlenmeyer flasks containing 50 mL of liquid medium TSB (Tryptone Soya Broth) at 25%, pH 6.8, and incubated on a SL-180/DT shaker table (120 rpm) at 28 ºC for 72 h. Then, the cladodes were inoculated with 1.0 mL of TSB medium containing bacteria strains (at a concentration of approximately 1×10^8 UFC mL^{-1}), applied at the base of cladode.

The plant material was planted by sowing one-third of matrix length in the soil, one per pot. For uninoculated treatment, 1.0 mL of TSB uninoculated medium was applied for plants. The nitrogen fertilization at a rate of 50 mg dm^{-3} was split into two applications: the first dose of 25 mg dm^{-3} was applied 30 days after planting, and the second dose of 25 mg dm^{-3} at 60 days after planting, using urea (45% N) as N source. The environmental conditions inside the greenhouse throughout the experiment are presented in figure 1.

4.2.3 Irrigation management

To ensure the stabilization of the matrix cladodes, soil humidity after planting was reached to 50% FC in all treatments, consisting a well-irrigated condition. The drought treatments were imposed 43 days after planting, by reducing the water level to 25% FC. The

relative soil water content (RSWC) was daily monitored through gravimetric method using the following equation (Xu; Zhou; Shimizu, 2009):

$$RSWC = \frac{(Wsoil - Wpot - DWsoil)}{(WFC - Wpot)}$$

where W_{soil} is the current soil weight, W_{pot} corresponds to weight of empty pot, DW_{soil} is the weight of dried soil, and W_{FC} correspond to the soil weight at field capacity (100%).

Weighing and watering were executed until the pots reached the RSWC targets for control (50% FC) and drought (25% FC) treatments. The plant material was harvested 193 days after planting, corresponding 150 days of drought imposition, and used for all analyzes.

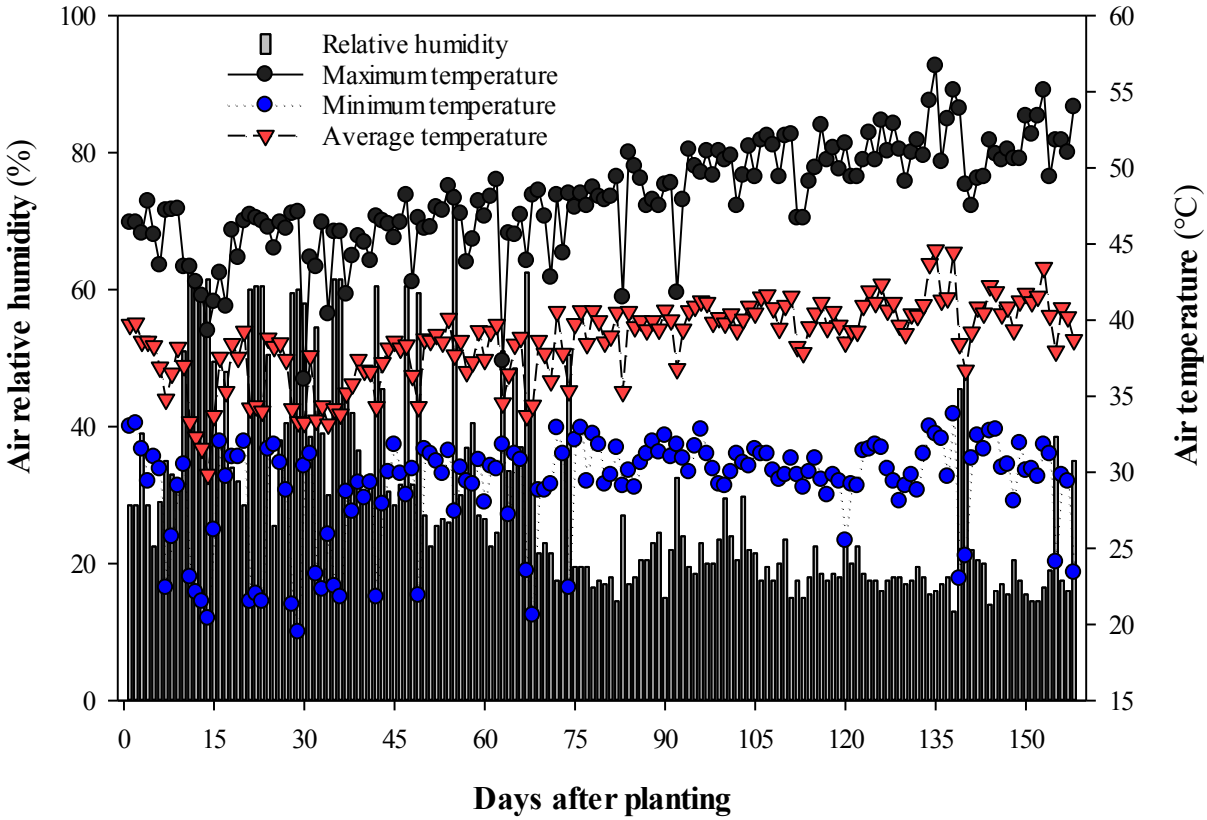


Figure 1 - Relative humidity and maximum, minimum and average air temperatures recorded in the greenhouse during the cultivation of cactus pear plants under different treatments.

4.2.4 Growth parameters and relative tolerance to drought

The harvest time-point was conducted 150 days after drought imposition, when plants exhibited visible symptoms and growth reduction due to water limitation. Non-destructive biometric parameters, including the number of cladodes per plant, plant height, and length and width of cladodes, were estimated. The thickness of primary cladodes was determined at the

midpoint of the third cladode using a precision digital caliper. One representative plant per treatment was also selected for image recording.

The plants were separated into cladodes (matrix, primary and secondary) and roots to determine fresh mass and root length. Cladode area was determined using a digital area meter (LI-3100C, LI-COR), and images of representative roots were acquired using a root scanner (FCLSD-0503, Delta-T Devices). Then, the material was immediately frozen and lyophilized to obtain the dry mass. The relative tolerance to drought was calculated as ratio between total dry mass of drought-stressed plants and total dry mass of control plants (Miranda *et al.,* 2023).

4.2.5 Cladode succulence and temperature

Cladode succulence (CS) was determined based on the following formula (Mantovani, 1999)

$$\mathrm{CS}\ (\mathrm{g\ H_2O\ cm^{-2}}) = \frac{\mathrm{CFM-CDM}}{\mathrm{CA}},$$

where CFM is the cladode fresh mass; CDM is the cladode dry mass; and CA is cladode area.

The temperature of cladodes (°C) was measured one day before harvest using an imaging IR thermometer FLIR (model TG165) starting from 01:00 pm.

4.2.6 Photosynthetic pigments

Photosynthetic pigments (chlorophylls *a*, *b*, total, and carotenoids) were extracted after incubating two 1.0 cm^2 discs of young cladodes in a dimethyl sulfoxide (DMSO) solution saturated with $CaCO_3$. The contents chlorophyll *a*, *b*, total and carotenoids were estimated in supernatant through spectrophotometry readings at 480, 649 and 665 nm (Wellburn, 1994). The discs were then dried in a forced ventilation oven at 65 °C for three days until reaching a constant dry mass, and the pigments content were expressed as μg g^{-1} DM.

4.2.7 Nitrogen, soluble carbohydrates and free amino acids

The nitrogen (N) content (g kg^{-1}) in root and cladode tissues was determined using the Kjeldahl method (Baethgen; Alley, 1989). Then, the N accumulation (g per plant) in plant tissues was calculated using the data from N content and dry mass (DM) (Fageria; Baligar, 2005).

Soluble carbohydrates and free amino acids were extracted by homogenizing powder lyophilized tissues in ethanolic solution (80% v/v) at 75 °C, for 1 h, with agitations each 15 min. Then, the homogenate was centrifuged at 3,000 x g for 10 min at 4 °C, and the resulting supernatant was collected, and the extraction process was repeated twice on the remaining precipitate. The soluble carbohydrates were estimated by absorbance readings at 490 nm, using standard curve of anhydrous D-glucose ranging from 0 to 250 μg mL^{-1} (Dubois *et al.,* 1956). The free amino acids were measured by readings at 570 nm, using standard curve of L-glycine solutions ranging from 0 to 50 μg mL^{-1} (Yemm; Cocking, 1955). The content of these organic compounds was expressed as μmol g^{-1} DM.

4.2.8 Statistical analysis

The data were initially submitted to normality analysis using the Shapiro-Wilk test at a 5% significance level; followed by an analysis of variance (ANOVA) using the F test at 5%. Then, significant interactions ($p \leq 0.05$) were submitted to the Scott-Knott mean test through Sisvar software version 5.6 (Ferreira, 2019**)**. The hierarchical clustering was designed using Excel software, while the graphs were plotted through Sigma Plot 14.0 software. Finally, principal component analysis (PCA) and correlation network were conducted on the datasets analyzed through software R (Development Core Team, 2016)

4.3 Results

4.3.1 Plant growth, phenotypic appearance and root morphology

Under well-irrigated conditions, 50% N-treated plants exhibited plant height higher than those of 0% N treatments. The drought significantly decreased the height of plants from all treatments, irrespective of N/inoculation; however, the highest plant height was reported in IPACC38+0% N stressed plants as compared to other N/inoculation treatments (Figure 2; Appendix 3). Alterations in cladode numbers due to N/inoculation treatments were observed only in well-irrigated plants, where the treatments without N inoculated with IPACC29 or without inoculation (UI) displayed low values as compared to other treatments. Drought stress promoted significant decrease in cladode number only in plants treated with 50% N, under inoculation with IPACC29 and IPACC38 and without inoculation, as compared to well-irrigated plants (Figure 2).

Variables	Irrigation levels	UI + 50% N	IC29 + 50% N	IC38 + 50% N	IC55 + 50% N	NI + 0% N	IC29 + 0% N	IC38 + 0% N	IC55 + 0% N
Plant height	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Bb	Bb	Bb	Bb	Ab	Bb
Cladodes number	Control	Aa	Aa	Aa	Aa	Ba	Ba	Aa	Aa
	Drought	Ab	Ab	Ab	Aa	Aa	Aa	Aa	Aa
Cladode total area	Control	Ba	Ca	Ba	Aa	Ca	Da	Ca	Da
	Drought	Ab	Bb	Ab	Ab	Ab	Ab	Ab	Ab
Root lenght	Control	Aa	Aa	Ba	Aa	Ba	Aa	Ba	Ba
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Primary cladode thickness	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Aa	Aa	Aa	Aa	Ab	Aa	Aa	Aa
Primary cladode length	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Primary cladode width	Control	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Aa
Cladode fresh mass	Control	Aa	Ba	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Ab	Bb	Ab	Ab	Ab	Ab	Bb
Root fresh mass	Control	Aa	Ca	Ca	Aa	Da	Aa	Ca	Da
	Drought	Bb	Bb	Bb	Bb	Aa	Ab	Ab	Aa
Cladode dry mass	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Ab	Ab	Ab	Ab	Ab	Bb
Root dry mass	Control	Aa	Ba	Aa	Aa	Ba	Aa	Ba	Ca
	Drought	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Total dry mass	Control	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Ba
	Drought	Bb	Bb	Ab	Ab	Ab	Ab	Ab	Ab

Figure 2 - Clustering analysis of growth and biomass parameters of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29), *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition. In all cases, blue color specifies an increase, and red denotes a decrease in the analyzed indexes, taking the data from uninoculated (UI) plants treated with 50% nitrogen as reference. White color represents no change. The varying intensities of red and blue express the extent of the change according to fold increase. For each assay, within the same row, different capital letters represent significant differences due to N/inoculation treatments; while, in the same column, different lowercase letters represent significant alterations due to drought stress, according to Scott–Knott's test ($p \leq 0.05$).

The highest cladodes total area was found in well-irrigated plants from IPACC55 + 50% N strain. Drought stress severely decreased cladode total area and root length, and little or no alteration was observed due to N/inoculation treatments. In general, the thickness, length and width of primary cladodes were not altered by N/inoculation treatments, but water limitation significantly decrease the length and width of primary cladodes in plants from all treatments. In addition, the fresh, dry and total mass of cladode and roots were found to be lower in plants growing without N, irrespective of inoculation. For all cases, drought promoted significant decrease in biomass of plant tissues, but the effects were less intense in inoculated plants without N (Figure 2; Appendix 3).

The phenotypic aspects of cactus pear at 150 days after drought imposition reveals more developed plants under well-irrigated conditions combined with 50% N, irrespective of inoculation treatment (Figure 3). Interestingly, the drought-stressed plants from inoculated treatments without N exhibited visual characteristics closer to well-irrigated plants treated with 50% N.

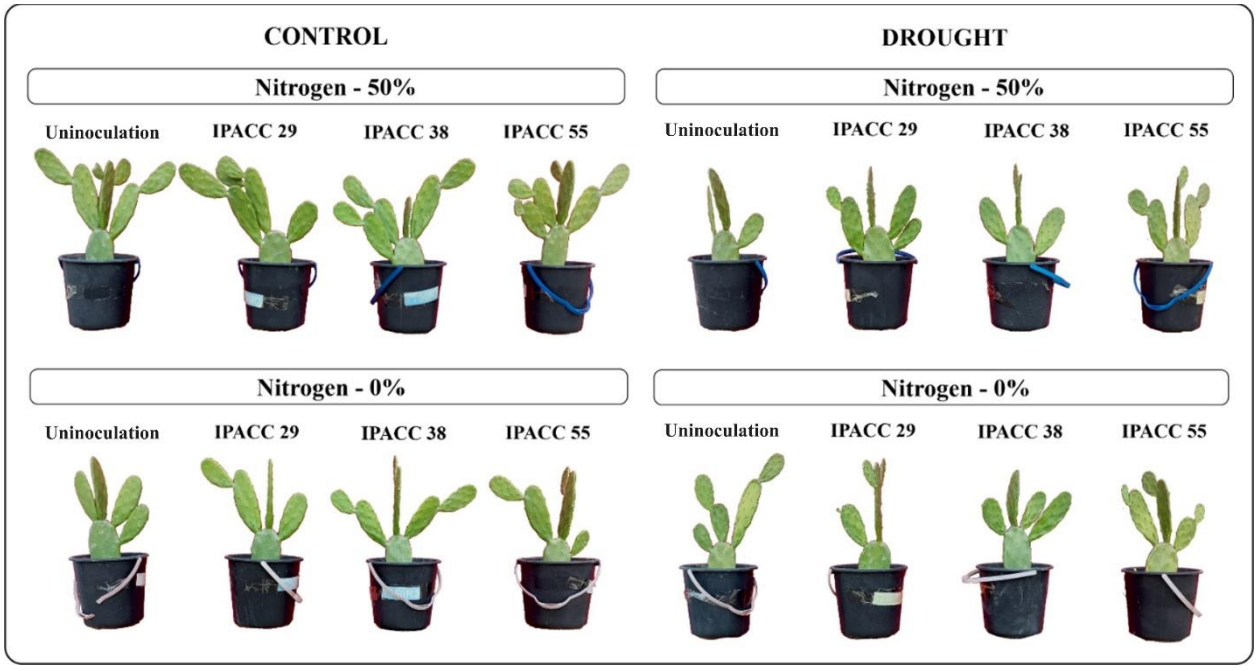


Figure 3 - Phenotypic appearance of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition.

Drought drastically impaired the development of cactus pear roots in plants from all N/inoculation treatments as compared to respective well-irrigated controls (Figure 4). However, the inoculation with IPACC29 strain without N appear to alleviate the drought damage as

compared to treatment without inoculation. Additionally, under drought, all treatments without N appeared in longer roots than those with 50% N (Figure 4).

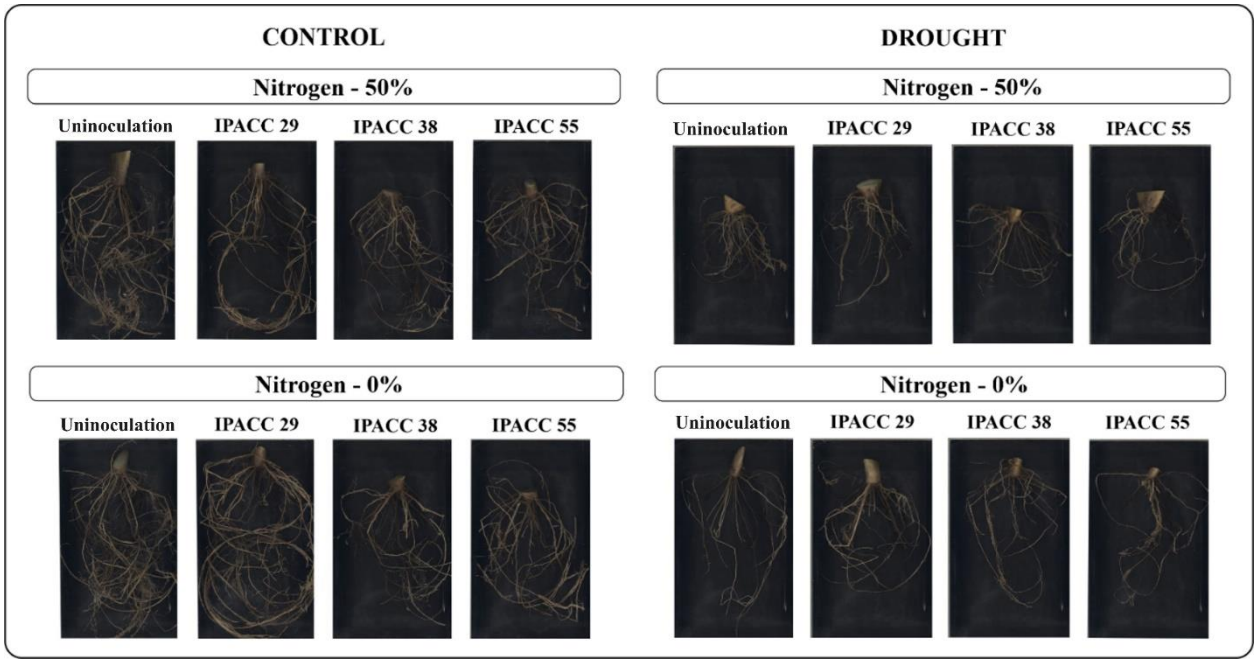


Figure 4 - Root morphology of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition.

4.3.2 Water status and drought tolerance

Under control conditions, the cladode succulence was higher in plants from treatments without N as compared to those from 50% N treatments (Figure 5A). Drought stress similarly decreased cladode succulence as compared to well-irrigated treatment, and no alteration due to N/inoculation was observed in water-stressed plants. The cladode temperature was increased by drought imposition as compared to well-irrigated control, with no significant alteration among the different N/inoculation treatments (Figure 5B).

The negative impacts of drought in dry mass accumulation were reflected in decrease in relative drought tolerance, that was reduced in plants from all N/inoculation treatments. However, cactus pear plants exhibited higher drought tolerance without N (UI) and inoculated with IPACC29-IC29 and IPACC38-IC38 strains, as compared to other treatments (Figure 5C).

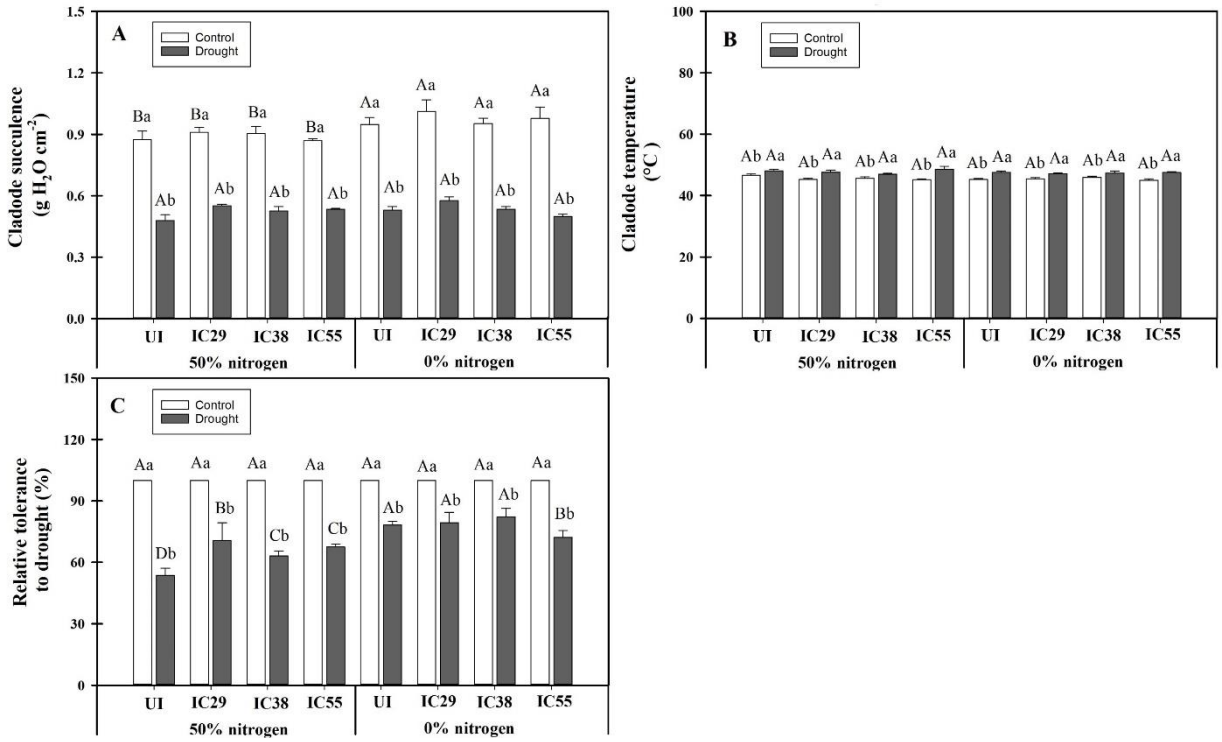


Figure 5 - Cladode succulence (A), relative tolerance to drought (B), and cladode temperature (C) of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p \leq 0.05$).

4.3.3 Nitrogen accumulation

In cladodes, under well-irrigated conditions, the nitrogen content and accumulation were higher in plants from 50% N treatments as compared to those without N (Figures 6A, C). In 0%N treatment, the inoculation with IPACC29-IC29, IPACC38-IC38 and IPACC55-IC55 strains promoted significant increase in nitrogen content and accumulation compared to uninoculated plants (Figures 6A, C). Drought promoted significant increase in nitrogen content of plants from all N/inoculations treatments, but a drought-induced increase in nitrogen accumulation was observed only in plants from 50% N uninoculated and inoculated with IPACC55-IC55, and in plants from 0% N uninoculated and inoculated with IPACC29-IC29 (Figures 6A, C).

In roots, IPACC29-IC29-inoculated plants displayed elevated nitrogen content and accumulation under well-irrigated conditions, as compared to other N/inoculation treatments

(Figures 6B, D). Except for IPACC29-IC29-inoculated plants, drought stress promoted significant increase in nitrogen content in the roots, but in fact resulted in a reduced nitrogen accumulation in subterraneous tissues (Figures 6B, D).

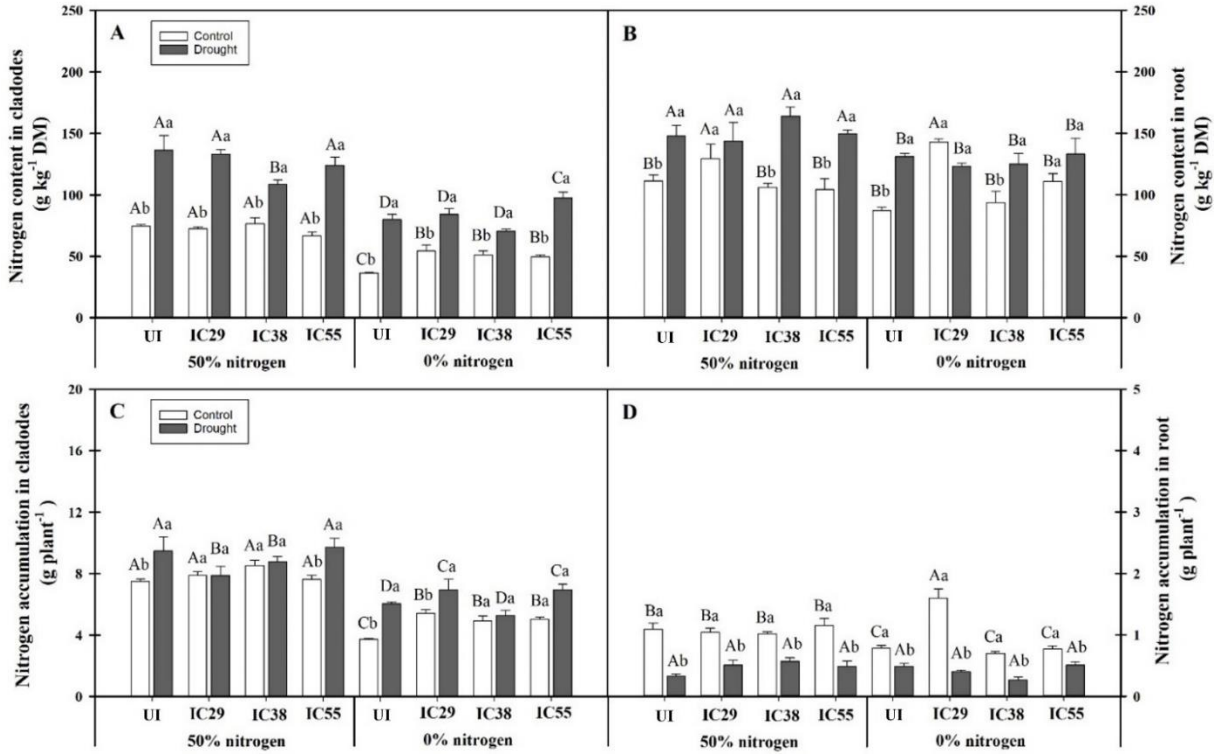


Figure 6 - Content (A, B) and accumulation (C, D) of nitrogen in cladodes and roots of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p \leq 0.05$).

4.3.4 Photosynthetic pigments and organic compounds

The contents of photosynthetic pigments were similar in well-irrigated plants, irrespective of N/inoculation treatment (Figure 7). Plants exposed to drought from all treatments showed significant increase in contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids as compared to well-irrigated control, with rare exceptions. Notably, the UI and IPACC38-IC38 treatments with 50% N, and the UI and IPACC55-IC55 treatments without N, showed the highest levels of chlorophyll *a* and total chlorophyll under deficit conditions (Figures 7A, C). Yet, the highest chlorophyll *b* contents were found in uninoculated plants,

regardless of N nutrition (Figure 7B). Under drought, the highest carotenoids contents were observed in 50% N plants from UI and IPACC38-IC38 treatments (Figure 7D).

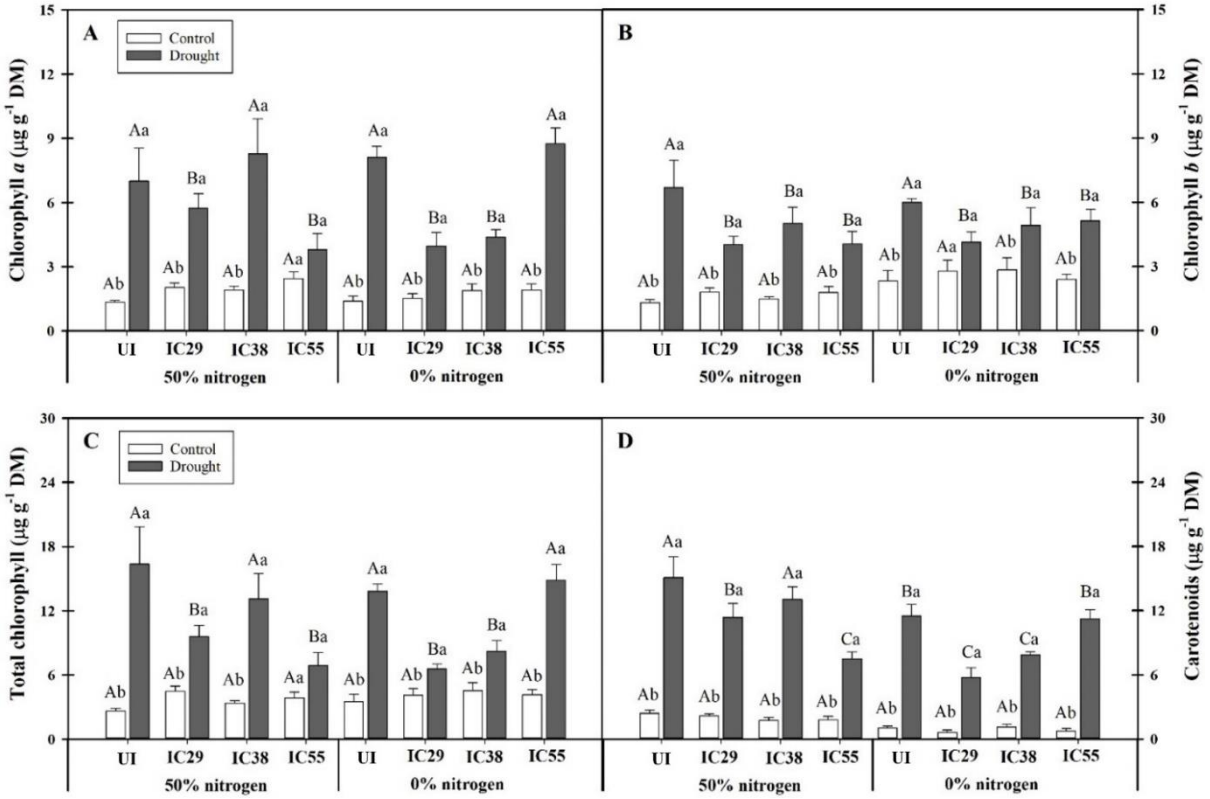


Figure 7 - Content of chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll total (C) and carotenoids (D) of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p \leq 0.05$).

The soluble carbohydrates and free aminoacids were differentially regulated as affected by both N/inoculation treatment and drought imposition (Figure 8). The soluble carbohydrates content in cladodes was decreased by drought stress as compared to well-irrigated control, irrespective of N/inoculation treatment (Figure 8). Conversely, in roots, the soluble carbohydrates were maintained unaltered or increased by drought, particularly in IPACC38-inoculated plants without or with 50%N, as well in IPACC55 + 50% N plants, as compared to control. In general, the highest content of soluble carbohydrate was registered in plants from inoculation treatments.

Drought-stressed plants growing without N displayed increase in cladode free aminoacids contents as compared to well-irrigated ones, whereas, in root, a similar response was registered

in plants growing with 50% N (Figure 8). Under drought, the highest root free aminoacids accumulation was observed in 50% N plants from uninoculated, IPACC38 and IPACC55 treatments compared to other N/inoculation treatments (Figure 8).

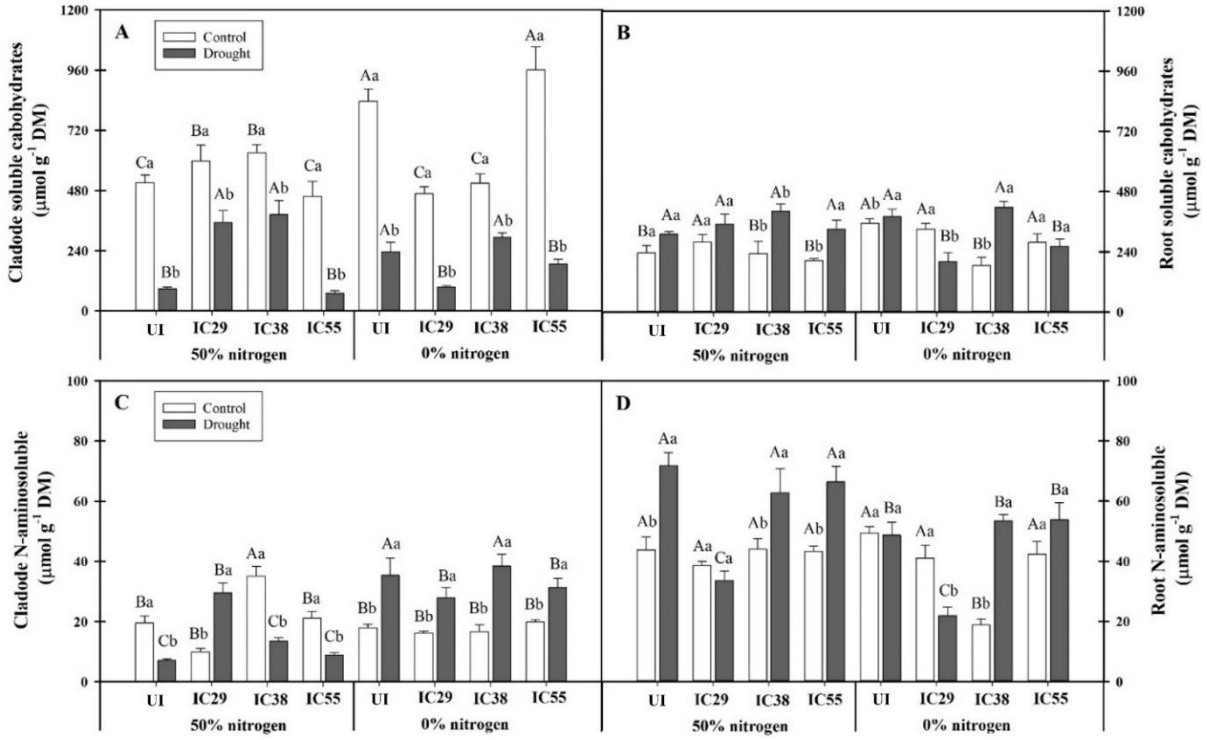


Figure 8 - Content of soluble carbohydrates (A, B) and free aminoacids (C, D) in cladode and roots of cactus pear plants exposed to treatments without (0% N) and with nitrogen (50% N), uninoculated (UI) or combined with inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to drought stress (Control × Drought), using Scott-Knott test ($p \leq 0.05$).

4.3.5 Principal Component Analysis

To explore the main patterns of variation, a preliminary Principal Component Analysis (PCA) with all treatments was performed, identifying PC1 and PC2 as the most informative components with a cumulative variance of 82.07% and 90.90% for treatment without and with 50% N, respectively. For clarity, figure 9 presents a refined PCA to highlight a clear hierarchical clustering trend among the bacterial strains and drought stress.

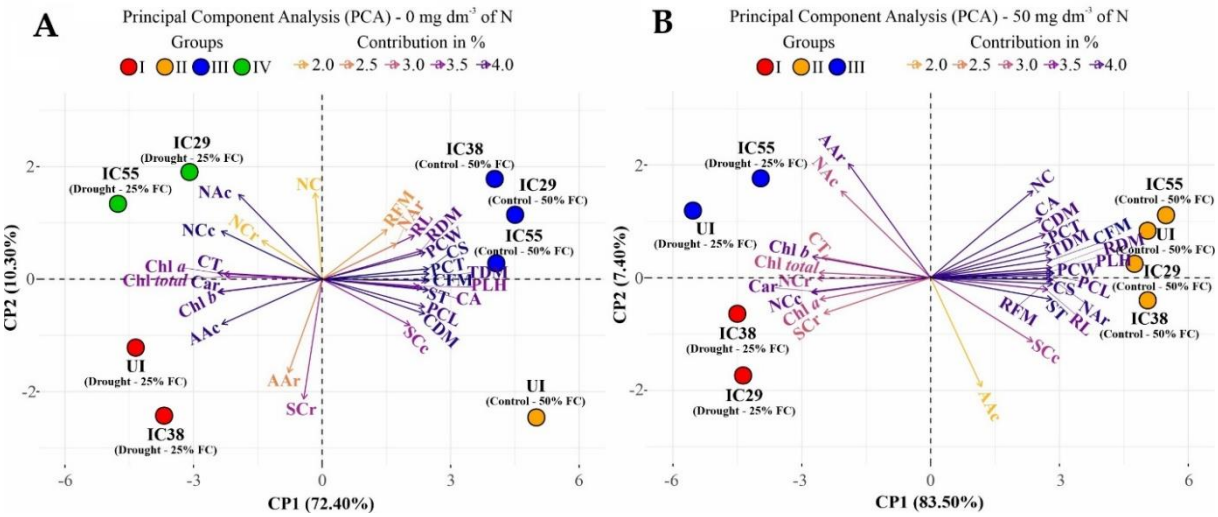


Figure 9 - Principal Component Analysis (PCA) for water regimes (control and drought) and treatments uninoculated (UI) or inoculation with *Bacillus subtilis* (IPACC29-IC29) and *Paenibacillus* sp. (IPACC38-IC38 and IPACC55-IC55) strains, without N (A) and with 50% N (B) in cactus pear plants. Variables: plant height (PLH), number of cladode (NC), cladode area (CA), root length (RL), primary cladode thickness (PCT), primary cladode length (PCL), primary cladode width (PCW), cladode fresh mass (CFM), clad dry mass (CDM), root fresh mass (RFM), root dry mass (RDM), total dry mass (TDM), cladode succulence (CS), stress tolerance (ST), cladode temperature (CT), chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll (Chl total), carotenoids (Car), N content in root (NCr), N accumulation in root (NAr), N content in cladode (NCc), N accumulation in cladode (NAc), root soluble carbohydrates (SCr), cladode soluble carbohydrates (SCc), root aminoacids (AAr), and cladode aminoacids (AAc).

Without N (Figure 9A), the strains clustered distinctly in response to water regimes, forming four groups. Under well-irrigated conditions, the bacterial strains clustered (Group III IPACC29-IC29, IPACC38-IC38 and IPACC55-IC55) distinctly from the uninoculated treatment (Group II). Conversely, under drought, the IPACC29-IC29 and IPACC55-IC55 strains (Group IV) were prominent, promoting higher nitrogen content in cactus pear. In contrast, the IPACC38-IC38 strain clustered with the uninoculated treatment (UI) (Group I), showing higher accumulation of free aminoacids, root soluble carbohydrates, chlorophyll *b*, and carotenoids.

Under 50% N (Figure 9B), three groups were formed, but there was no separation between the strains (IPACC29-IC29 and IPACC55-IC55) and uninoculated (UI) (Group II) treatment in well-irrigated plants, which was strongly related to most biometric parameters, biomass, and water status. Conversely, under drought, a clustering occurred between the IPACC29-IC29 and IPACC38-IC38 strains (Group I), which was more correlated with nitrogen levels in the roots and cladodes, chlorophyll *a*, carotenoids, and root soluble carbohydrates.

Group III was formed by the IPACC55-IC55 strain and UI treatment, which was correlated with chlorophyll *b*, total chlorophyll, cladode temperature, cladode nitrogen accumulation, and root free aminoacids.

The correlation network for each N and inoculation treatment reveals a strong positive correlation between biometric parameters, biomass and water status of the cactus pear plants, regardless of studied treatment (Figure 10). However, the UI and the IPACC38-IC38 and IPACC55-IC55 strains treatments showed a significant negative correlation between carotenoids content with biometric and biomass parameters. Also, UI and IPACC55-IC55 strain showed a positive correlation between soluble carbohydrate and cladode succulence. Finally, the IPACC29-IC29 strain contributed to a higher nitrogen content in roots, which positively correlated with biomass and water status.

4.4 Discussion

4.4.1 Cactus pear resistance against drought can be improved by plant growth-promoting bacteria

In arid and semiarid regions, the soil fertility, high temperatures and limited water availability represent major constraints to agriculture. These limitations are particularly severe for fast-growing species, such as plants with C_3 and C_4 metabolism. In contrast, plants exhibiting CAM metabolism are promising alternatives for agricultural production, and developing sustainable and low-cost technologies plays a crucial role in promoting more resilient and productive agriculture (Dubeux Jr *et al.,* 2021; Hossain; Hossain; Akter, 2023; Lahbouki *et al.,* 2023). In the current study, we investigate the role of selected plant growth-promoting bacteria strains, in association with nitrogen fertilization, on cactus pear performance under drought stress.

The best growth performance of cactus pear plants was registered under well-irrigated regime (50% FC) associated with 50% N in the growth medium (Figures 2, 3 and 4). The data revealed that drought imposed severe limitations on growth of forage cactus, with constraints on plant height, number of cladodes and biomass accumulation in both cladodes and roots (Figure 2). These effects were more evident in plant morphology, where drought-stressed plants seemed to be less vigorous than well-irrigated ones, particularly in the development of the root system (Figures 3, 4).

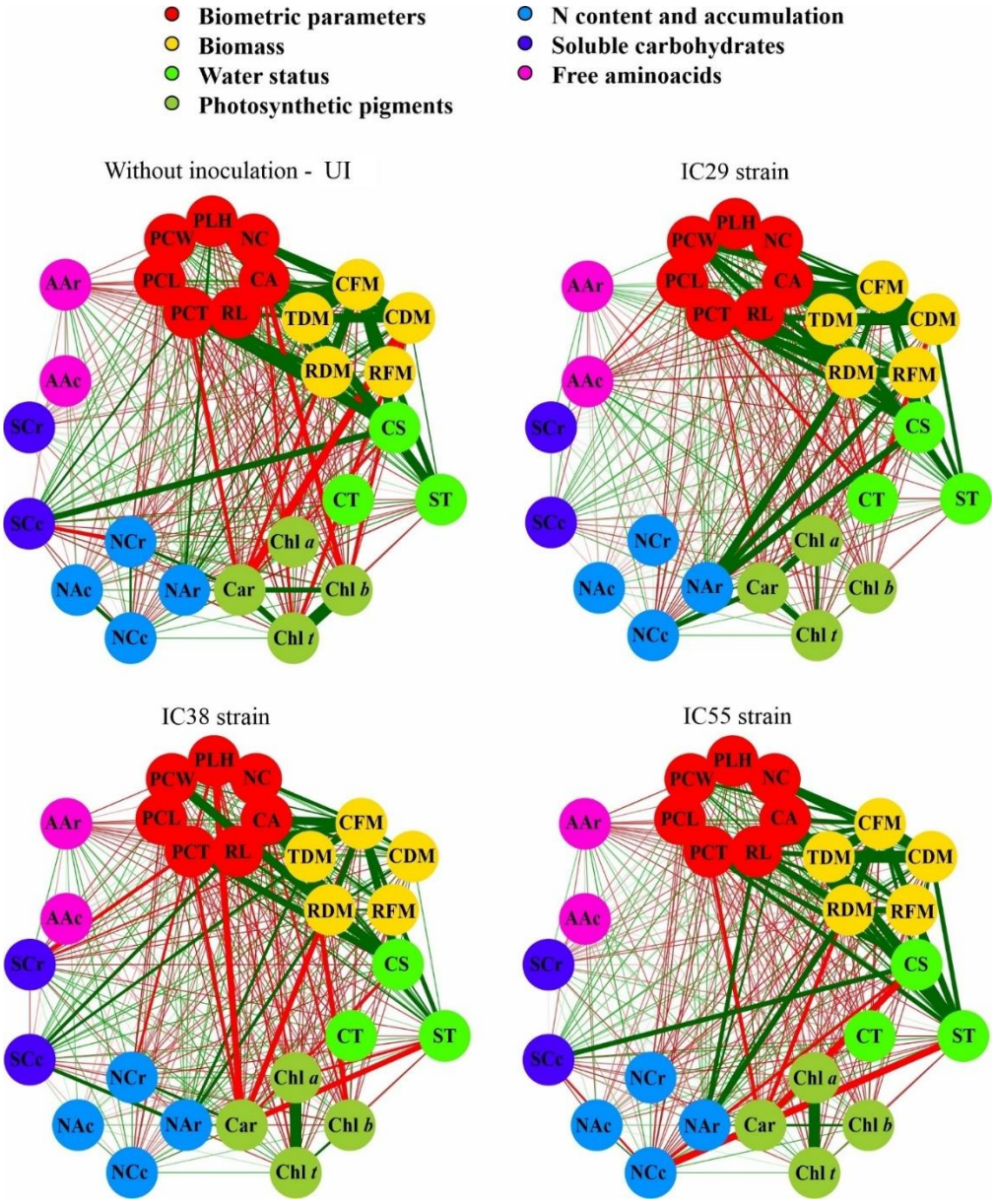


Figure 10 - Correlation network for bacterial strains and uninoculation (UI) treatments in cactus pear plants. The nodes represent individual variables grouped arbitrarily by similarity in nature, while the edges represent the relationships between variables (correlations). The edge thickness and color intensity correspond to magnitude and direction [positive (green) or negative (red)] of the correlation. Variables: plant height (PLH), number of cladode (NC), cladode area (CA), root length (RL), primary cladode thickness (PCT), primary cladode length (PCL), primary cladode width (PCW), cladode fresh mass (CFM), cladode dry mass (CDM), root fresh mass (RFM), root dry mass (RDM), total dry mass (TDM), cladode succulence (CS), stress tolerance (ST), cladode temperature (CT), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *t*), carotenoids (Car), N content in root (NCr), N accumulation in root (NAr), N content in cladode (NCc), N accumulation in cladode (NAc), root soluble carbohydrates (SCr), cladode soluble carbohydrates (SCc), root aminoacids (AAr), and cladode aminoacids (AAc).

The performance of forage cactus was differentially modulated by PGPB inoculation and N fertilization. The beneficial role of PGPB was more evident in plants under drought conditions, with the greatest benefits observed in stressed plants and in the absence of N. The data suggest a mutually beneficial interaction for plant growth, where IPACC29-IC29, IPACC38-IC38 and IPACC55-IC55 strains alleviate drought damage by providing fixed N under water deprivation to better root growth and drought tolerance (Figures 2, 4; 5C). In concordance, the PGPB strains were reported to induce mechanisms capable of enhancing plant growth under adverse conditions through biological nitrogen fixation or phytohormone production (Kavamura *et al.,* 2013; Chippered *et al.,* 2019; Khan; Bano, 2019; Aquino *et al.,* 2021; Danish *et al.,* 2021).

4.4.2 Superior performance under drought induced by plant growth-promoting bacteria is associated with metabolic adjustments

The water content into plant cells has been pointed as critical in drought-stressed plants to increase nitrogen uptake and its efficient use, promoting greater leaf expansion and, consequently, a larger light interception area (Scalisi *et al.,* 2016; Wang *et al.,* 2021). This behavior was not found in our study, and the increase in cladode area (Figure 2) most likely arises from other mechanisms, as drought imposes a similar decrease in cladode succulence and an increase in cladode temperature, without the influence of N/inoculation in these treatments (Figures 5A, B). Another interesting result was that cactus pear plants activated intrinsic defense mechanisms against drought, including positive feedback for nitrogen accumulation in cladodes, which resulted in high contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids (Figures 6, 7). This reprogramming appears to be a constitutive response of cactus pear plants, as there was no influence from the N/inoculation treatments. The accumulation of photosynthetic pigment under drought may reflect a response to maximize photosynthetic efficiency and protect tissues against oxidative damage (Abdallah *et al.,* 2017). Specifically, carotenoids play a protective role as antioxidants, helping to mitigate oxidative stress caused by prolonged exposure to water stress (Dhami; Cazzonelli, 2020).

The data also evidence that superior drought tolerance of IPACC29-IC29-inoculated plants without N (Figure 5C) was associated with root N accumulation (Figures 6, 10) and a fine tuning of photosynthetic pigments contents (Figures 7, 10), a different behavior of plants from other N/inoculation treatments (Figure 9). The lower content of photosynthetic pigments in IPACC29-IC29-inoculated drought-stressed plants (Figure 7) indicates a nitrogen economy

under restricted conditions that can be utilized for other plant processes, including protein synthesis and plant growth. The results show that IPACC29-IC29 strain has an intrinsic ability to promote root growth, regardless of N exogenous. Previous studies have showed that IPACC29-IC29 strain may facilitate nutrient uptake or stimulating the production of phytohormones like auxins (Chiappero *et al.,* 2017), as well act in acetylene reduction (a process catalyzed by nitrogenase from strain) (Aquino *et al.,* 2021), in order to promote the root elongation (Andrade *et al.,* 2023).

4.4.3 Multivariate assays may explain how PGPB modulate cactus pear performance under water restriction

Numerous reports highlight that PGPBs secrete extracellular polysaccharides (EPSs) in response to water stress (Ghosh; Gupta; Mohapatra, 2019; Chieb; Gachomo, 2023). These compounds may modify the root architecture and act as emulsifiers, mitigating the effects of reactive oxygen species (ROS). Moreover, plants inoculated with EPS-producing bacteria show a significant increase in accumulation of proline, carbohydrates and free amino acids, as well as in biomass growth, leaf area expansion, and protein content (Khan; Bano, 2019; Chieb; Gachomo, 2023).

The accumulation of soluble carbohydrates is an important response under drought conditions, often associated with osmotic adjustment, as it helps maintain cell turgor and stabilize cellular structures under water-limited conditions. In this study, PCA and correlation network data indicate that the regulation of carbohydrate accumulation may vary depending on the source of nitrogen and PGPB inoculation (Figures 8, 9, 10). In the absence of inoculation or with strain IPACC55-IC55 inoculation, carbohydrate accumulation was directly related to cladode succulence, indicating its role in osmotic adjustment processes (Figure 10). On the other hand, under IPACC29-IC29 and IPACC38-IC38 inoculation, carbohydrate synthesis was directly related to nitrogen accumulation, which in turn affects succulence and growth parameters (Figure 10). These findings underscore the pivotal role of PGPB inoculation in modulating carbohydrate metabolism and promoting the accumulation of carbon-based energy reserves essential for stress resilience and development (Mohammadi *et al.,* 2023).

The results of free aminoacids in reinforce the role of PGPB in osmoprotection and osmotic adjustment in cladode of drought-stressed plants without N (Figure 8). The aminoacids synthesis pathway was closely related with uninoculated and IPACC38-IC38-inoculated stressed plants (Figures 9, 10), but the correlation network evidence that IPACC29-IC29-

inoculated stressed plants exhibited a close relationship between aminoacids and photosynthetic pigments, suggesting a coordinated response involving nitrogen metabolism and photosynthetic efficiency. Taken together, these results indicate that strain IPACC29-IC29 exhibits the most promising performance among the tested PGPB for inoculation in forage cactus under drought conditions. Future investigations must be performed to explain the specific role on PGPB in nitrogen metabolism and drought tolerance mechanisms in CAM plants such as cactus pear.

4.5 Conclusion

The data reveal that PGPB alleviates the drought deleterious effects in cactus pear plants. The interaction between plant defense mechanisms and PGPB is particularly evident under N absence. The IPACC29 strain was the most promising for inoculation with forage cactus pear, activating important defense responses primarily related to nitrogen and organic compound accumulation, resulting in more developed roots and growth.

The strategic use of bacterial strains can contribute to crop resilience in adverse environments, reducing the need for fertilizers and increasing agricultural sustainability. The data indicate that the IPACC29, IPACC38 and IPACC55 strains can all be effective tools for improving the productivity of *Nopalea cochenillifera* Salm-Dick, Miúda cultivar, in semiarid regions. This biotechnological management offers an alternative for cultivating cactus pear under water restriction and reduced N availability, providing a promising approach to enhance resistance and increasing green forage biomass production

REFERENCES

ABDALLAH, M. A.; METHENNI, K.; NOUAIRI, I.; ZARROUK, M.; YOUSSEF, N. B. Drought priming improves subsequent more severe drought in a drought-sensitive cultivar of olive cv. Chétoui. **Scientia Horticulturae**, v. 221, p. 43-52, 2017.

ALI, S.; KHAN, N. Delineation of mechanistic approaches employed by plant growth promoting microorganisms for improving drought stress tolerance in plants. **Microbiological Research**, v. 249, p. 126771, 2021.

ANDRADE, L. A.; SANTOS, C. H. B.; FREZARIN, E. T.; SALES, L. R.; RIGOBELO, E. C. Plant growth-promoting rhizobacteria for sustainable agricultural production. **Microorganisms**, v. 11, n. 4, p. 1088, 2023.

ANTUNES, J. E. L.; LYRA, M. C. C. P.; OLLERO, F. J.; FREITAS, A. S.; OLIVEIRA, L. M. S.; ARAÚJO, A. S. F.; FIGUEIREDO, M. V. B. Diversity of plant growth promoting

bacteria associated with sugarcane. **Genetics and Molecular Research**, v. 16, p. 16029662, 2017.

AQUINO, J. P. A.; ANTUNES, J. E. L.; BONIFÁCIO, A.; ROCHA, S. M. B.; AMORIM, M. R.; ALCÂNTARA NETO, F.; ARAÚJO, A. S. F. Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. **Theoretical and Experimental Plant Physiology**, v. 33, p. 249-260, 2021.

AQUINO, J. P. A.; NETO, V. P. C.; ANDRADE, M. R. S.; OLIVEIRA, L. M. S.; ANTUNES, J. E. L.; FREITAS, A. S.; NETO, F. A.; ARAÚJO, A. S. F. Plant growth-promoting bacteria increase the yield of green maize and sweet sorghum. **Journal of Plant Nutrition**, v. 46, p. 58-68, 2022.

BAETHGEN, W. E.; ALLEY, M. M. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. **Communications in Soil Science and Plant Analysis**, v. 20, p. 961-969, 1989.

CHIAPPERO, J.; CAPPELLARI, L. R.; ALDERETE, L. G.; PALERMO, T. B.; BANCHIO, E. Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. **Industrial Crops and Products**, v. 139, p. 111553, 2019.

CHIEB, M.; GACHOMO, E. W. The role of plant growth promoting rhizobacteria in plant drought stress responses. **BMC Plant Biology**, v. 23, p. 407, 2023.

DANISH, S.; ZAFAR-UL-HY, M.; MOHSIN, F.; HUSSAIN, M. ACC-deaminase producing plant growth promoting rhizobacteria and biochar mitigate adverse effects of drought stress on maize growth. **PLoS One**, v. 15, p. e0230615, 2020.

DHAMI, N.; CAZZONELLI, C. I. Environmental impacts on carotenoid metabolism in leaves. **Plant Growth Regulation**, v. 92, p. 455-477, 2020.

DUBEUX JR, J. C. B.; SANTOS, M. V. F.; CUNHA, M. V.; SANTOS, D. C.; SOUZA, R. T. A.; MELLO, A. C. L.; SOUZA, T. C. Cactus (*Opuntia* and *Nopalea*) nutritive value: a review. **Animal Feed Science and Technology**, v. 275, p. 114890, 2021.

DUBOIS, M.; GILLES, K. A.; HAMILTON, J. K.; REBERS, P. T.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v. 28, p. 350-356, 1956.

EDVAN, R. L.; CARNEIRO, M. S. S. **Palma forrageira: cultivo e uso na alimentação animal**. Appris: Curitiba, Brazil, 2019. 93 p.

EDVAN, R. L.; BARROS, D. M.; NASCIMENTO, R. R.; ARAÚJO, M. J.; SOUSA, H. R.; NERIS, L. M. L.; BEZERRA, L. R.; SILVA-FILHO, E. C. Potential use of hydrogel from natural fiber in the initial growth of Nopalea Cochenillifera under water stress: water source of slow release. **Communications in Soil Science and Plant Analysis**, v. 54, p. 3063-3075, 2023.

EDVAN, R. L.; MOTA, R. R. M.; DIAS, S. T. P.; NASCIMENTO, R. R.; SOUSA, S. V.; SILVA, A. L.; ARAÚJO, J. S. Resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. **Scientific Reports**, v. 10, p. 10040, 2020.

FADIJI, A. E.; SANTOYO, G.; YADAV, A. N.; BABALOLA, O. O. Efforts towards overcoming drought stress in crops: revisiting the mechanisms employed by plant growth-promoting bacteria. **Frontiers in Microbiology**, v. 13, p. 962-427, 2022.

FAGERIA, N. K.; BALIGAR, V. C. Enhancing nitrogen use efficiency in crop plants. **Advances in Agronomy**, v. 88, p. 97-185, 2005.

FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Brazilian Journal of Biometrics**, v. 37, p. 529-535, 2019.

GHOSH, D.; GUPTA, A.; MOHAPATRA, S. A comparative analysis of exopolysaccharide and phytohormone secretions by four drought-tolerant rhizobacterial strains and their impact on osmotic-stress mitigation in Arabidopsis thaliana. **World Journal of Microbiology and Biotechnology**, v. 35, p. 90, 2019.

GUPTA, A.; MISHRA, R.; RAI, S.; BANO, A.; PATHAK, N.; FUJITA, M.; HASANUZZAMAN, M. Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. **International Journal of Molecular Sciences**, v. 23, p. 3741, 2022.

HOSSAIN, M. A.; HOSSAIN, M. S.; AKTER, M. Challenges faced by plant growth-promoting bacteria in field-level applications and suggestions to overcome the barriers. **Physiological and Molecular Plant Pathology**, v. 126, p. 102029, 2023.

HUNGRIA, M.; CAMPO, R. J.; SOUZA, E. M.; PEDROSA, F. O. Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. **Plant and Soil**, v. 331, p. 413-425, 2010.

IIYAS, M.; NISAR, M.; KHAN, N.; HAZRAT, A.; KHAN, A. H.; HAYAT, K.; FAHAD, S.; KHAN, A.; ULLAH, A. Drought tolerance strategies in plants: a mechanistic approach. **Journal of Plant Growth Regulation**, v. 40, n. 3, p. 926-944, 2021.

KAVAMURA, V. N.; SANTOS, S. N.; SILVA, J. L.; PARMA, M. M.; ÁVILA, L. A.; VISCONTI, A.; ZUCCHI, T.; TAKETANI, R. G.; ANDREOTE, F.; MELO, I. S. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. **Microbiological Research**, v. 168, p. 183-191, 2013.

KHAN, N.; BANO, A. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. **PLoS One**, v. 14, p. e0222302, 2019.

LAHBOUKI, S.; BEN-LAOUANE, R.; ANLI, M.; BOUTASKNIT, A.; AIT-RAHOU, Y.; AIT-EL-MOKHTAR, M.; EL GABARDI, S.; DOUIRA, A.; WAHBI, S.; OUTZOURHIT, A. Arbuscular mycorrhizal fungi and/or organic amendment enhance the tolerance of prickly pear (*Opuntia ficus-indica*) under drought stress. **Journal of Arid Environments**, v. 199, p. 104703, 2022.

LAHBOUKI, S.; FERNANDO, A. L.; RODRIGUES, C.; BEN-LAOUANE, R.; AIT-EL-MOKHTAR, M.; OUTZOURHIT, A.; MEDDICH, A. Effects of humic substances and mycorrhizal fungi on drought-stressed cactus: focus on growth, physiology, and biochemistry. **Plants**, v. 12, p. 4156, 2023.

MANTOVANI, A. A method to improve leaf succulence quantification. **Brazilian Archives of Biology and Technology**, v. 42, p. 9-14, 1999.

MIRANDA, R. S.; FONSECA, B. S. F.; PINHO, D. S.; BATISTA, J. Y. N.; BRITO, R. R.; SILVA, E. M.; FERREIRA, W. S.; COSTA, J. H.; LOPES, M. S.; SOUSA, R. H. B.; NEVES, L. F.; PENHA, J. A. F.; SANTOS, A. S.; LIMA, J. J. P.; PAULA-MARINHO, S. O.; ALCÂNTARA NETO, F.; AGUIAR, E. S.; SANTOS, C. P.; GOMES-FILHO, E. Selection of soybean and cowpea cultivars with superior performance under drought using growth and biochemical aspects. **Plants**, v. 12, p. 3134, 2023.

MOHAMMADI, A. S.; ZAHRA, N.; HAJIAGHAEI, K. M.; ASGARI, L. B.; NOBAHARAN, K.; ASTATKIE, T.; SIDDIQUE, K. H. M.; FAROOQ, M. Role of root hydraulics in plant drought tolerance. **Journal of Plant Growth Regulation**, v. 42, p. 6228-6243, 2023.

R DEVELOPMENT CORE TEAM. R: **A Language and Environment for Statistical Computin***g*; R Foundation for Statistical Computing: Vienna, Austria (2016). Available online: http://www.R-project.org (accessed June 2024).

RAMOS, J. P. F.; MACÊDO, A. J. S.; SANTOS, E. M.; EDVAN, R. L.; SOUSA, W. H..; PERAZZO, A. F.; SILVA, A. S.; CARTAXO, F. Q. Forage yield and morphological traits of cactus pear genotypes. **Acta Scientiarum. Agronomy**, v. 43, p. 51214, 2021.

RISEH, R. S.; EBRAHIMI-ZARANDI, M.; VAZVANI, M. G.; SKORIK, Y. A. Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. **International Journal of Molecular Sciences**, v. 22, p. 12979, 2021.

SALEM, M. A.; ISMAIL, M. A.; RADWAN, K. H.; ABD-ELHALIM, H. M. Unlocking the potential of plant growth-promoting rhizobacteria to enhance drought tolerance in Egyptian wheat (*Triticum aestivum*). **Sustainability**, v. 16, p. 4605, 2024.

SCALISI, A.; MORANDI, B.; INGLESE, P.; BIANCO, R. L. Cladode growth dynamics in *Opuntia ficus-indica* under drought. **Environmental and Experimental Botany**, v. 122, p. 158-167, 2016.

SINGH, D.; THAPA, S.; SINGH, J. P.; MAHAWAR, H.; SAXENA, A. K.; SINGH, S. K.; MAHLA, H. R.; CHOUDHARY, M.; PARIHA, M.; CHOUDHARY, K. B.; CHAKDA, H. Prospecting the potential of plant growth-promoting microorganisms for mitigating drought stress in crop plants. **Current Microbiology**, v. 81, p. 81-84, 2024.

SOARES, J. C.; SANTOS, C. S.; CARVALHO, S. M. P.; PINTADO, M. M.; VASCONCELOS, M. W. Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. **Plant and Soil**, v. 443, p. 1-26, 2019.

SOUSA, A. B. **Bactérias promotoras do crescimento de plantas e adubação nitrogenada no desenvolvimento de cultivares de palma forrageira em região semiárida**. 2023. 76 f. Dissertação (Mestrado em Agronomia) – Universidade Federal do Piauí, Teresina, 2023.

SOUSA, D. M. G.; LOBATO. E. **Cerrado: Correção do Solo e Adubação**. Brasília: Embrapa Informação Tecnológica, 2004. 416 p.

VURUKONDA, S. S. K. P.; VARDHARAJULA, S.; SHRIVASTAVA, M. S.; SKZ, A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. **Microbiological Research**, v. 184, p. 13-24, 2016.

WANG, H.; WU, L.; WANG, X.; ZHANG, S.; CHENG, M.; FENG, H.; FAN, J.; ZHANG, F.; XIANG, Y. Optimization of water and fertilizer management improves yield, water, nitrogen, phosphorus and potassium uptake and use efficiency of cotton under drip fertigation. **Agricultural Water Management**, v. 245, p. 106662, 2021.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of Plant Physiology**, v. 144, p. 307-313, 1994.

XU, Z.; ZHOU, G.; SHIMIZU, H. Are plant growth and photosynthesis limited by pre-drought following rewatering in grass? **Journal of Experimental Botany**, v. 60, p. 3737-3749, 2009.

YANG, J.; KLOEPPER, J. W.; RYU, C. M. Rhizosphere bacteria help plants tolerate abiotic stress. **Trends in Plant Science**, v. 14, p. 1-4, 2009.

YEMM, E. W.; COCKING, E. C. The determination of amino acids with ninhydrin. **Analyst**, v. 80, p. 209-213, 1955.

ZHANG, Q.; RUE, K. Effects of plant growth-promoting microorganisms on the early growth of kentucky bluegrass under drought and salinity. **HortTechnology**, v. 34, p. 405-411, 2024.

5 CAPÍTULO 3

BIOCHEMICAL AND METABOLIC ADJUSTMENTS UNDERLYING *Bacillus subtilis* -INDUCED DROUGHT TOLERANCE IN CACTUS PEAR PLANTS

ABSTRACT

Cactus pear is a strategic forage species adapted to semiarid regions, supplying both energy and water to livestock. Despite this adaptation, alternative strategies to increase plant tolerance to drought are still required, and the inoculation of plant growth-promoting bacteria (PGPB) represents a promising biological approach. Our study aimed to provide biochemical and metabolic evidence that inoculation with *Bacillus subtilis* stimulates drought tolerance and promotes growth of *Nopalea cochenillifera* (variety Doce Miúda). Experiments were conducted in a greenhouse using a randomized block design, 2 × 2 factorial scheme, consisting of two inoculation treatments [uninoculated (UI) and inoculated with *B. subtilis*] and two water regimes [well-watered at 50% field capacity (FC) and drought at 25% FC]. Drought significantly decreased dry biomass accumulation regardless of inoculation. However, under drought conditions, inoculated plants showed superior performance, associated with increased nitrogen content and greater accumulation of proline, soluble carbohydrates, soluble proteins, and organic acids, as well as increased activity of antioxidant enzymes SOD and APX. These metabolic adjustments alleviated oxidative damage in cladodes, as evidenced by decreased H_2O_2 and MDA levels. The data suggest that the regulation of essential metabolites, including citric acid, malic acid, inositol, sucrose, fructose, pyroglutamic acid, glycine, and proline, promoted an integrated metabolic response capable of minimizing the drought damages. In conclusion, *B. subtilis*-induced drought tolerance is associated with biochemical adjustments, reduced oxidative damage, and metabolic regulation in cactus pear plants.

Keywords: Drought, Metabolic profile, *Nopalea cochenillifera* Salm-Dyck, PGPB inoculation, Plant defense, Semiarid.

5.1 Introduction

Drought is a limiting environmental factor for crop performance and productivity, especially in arid and semiarid regions (Fadiji *et al.,* 2022; Singh *et al.,* 2024). This condition

of low water availability increases the content of malondialdehyde (MDA), and synthesis of reactive oxygen species (ROS) in plants, which can severely damage cellular components, such as nucleic acids, proteins, and membrane lipids, which consequently promote cell death (Wang *et al.,* 2024). Although plants can partially mitigate these deleterious effects through enzymatic and non-enzymatic antioxidant systems, including catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), and pathways for osmotic adjustment, these defense mechanisms often impose metabolic constraints, resulting in decreased plant biomass (Edvan *et al.,* 2020; IIyas *et al.,* 2021; Wang *et al.,* 2024).

Numerous plant species present adaptation to dry environments which represent a viable and sustainable strategy for agriculture in semiarid regions. In Brazilian semiarid, cactus pear such as *Nopalea* spp. plays a crucial role as a forage resource for livestock due to its high digestibility, elevated soluble carbohydrates, and high capacity to store water in cladodes (Dubeux Jr. *et al.,* 2021; Edvan *et al.,* 2020, 2023). Furthermore, cactus pear shows high water-use efficiency due to crassulacean acid metabolism (CAM), contributing to the reduction of seasonal variability in forage production throughout the year (Araújo Júnior *et al.,* 2021; Dubeux Jr. *et al.,* 2021). Despite its well-known adaptation to drought, cactus pear may present strong decrease in growth and changes in physiological and biochemical responses under prolonged drought (Scalisi *et al.,* 2016; Araújo Júnior *et al.,* 2021; Lahbouki *et al.,* 2022), highlighting the need of alternative and sustainable strategies for increasing drought tolerance.

In recent years, inoculation with plant growth-promoting bacteria (PGPB) has been shown as a promising and low-cost technology for mitigating drought-induced damage. PGPB can induce drought tolerance through multiple mechanisms, including osmolyte synthesis, metabolic adjustment, and production of phytohormones such as indole-3-acetic acid (IAA), which plays a key role in promoting root and shoot elongation (Vurukonda *et al.,* 2016; Ali; Khan, 2021). In addition, the PGPB produce exopolysaccharides (EPS) and form biofilms that protect the root system against desiccation, stimulate antioxidant enzymes, and improve the nutrient availability to plants through phosphorus and potassium solubilization, biological nitrogen fixation, and siderophore production (Chieb; Gachomo, 2023; Singh *et al.,* 2024; Khan *et al.,* 2026).

In cactus pear, the *Bacillus subtilis* inoculation increased plant growth under drought, a response associated with increased nitrogen accumulation, which favored the formation of photosynthetic pigments and promoted root and plant growth (Dauala *et al.,* 2025). However, evidence into *B. subtilis*-induced antioxidant defense (CAT, SOD, and APX) to scavenging

oxidative damage (MDA and hydrogen peroxide) and reprograming metabolic profile of cactus pear remains unexplored.

Our working hypothesis was that inoculation with the *B. subtilis* IPACC29 induce specific alterations in primary metabolites associated with cellular water status regulation and antioxidant responses, thereby increasing drought tolerance in cactus pear plants. To address this hypothesis, we assessed growth, organic and inorganic compounds, oxidative and antioxidant indicators, and metabolic profile of cactus pear under greenhouse conditions.

5.2 Material and methods

5.2.1 Study site and environmental conditions

The experiments were carried out in a greenhouse at the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas Campus (CPCE), located in Bom Jesus, Piauí, Brazil (9°04′45.6″ S, 44°19′37.9″ W; 277 m altitude). The regional climate is classified as Aw (tropical semi-arid) according to Koppen classification (1931), characterized by dry winter and rainy summer (Alvares *et al.,* 2014). During the experimental period, the environmental conditions inside the greenhouse were as follows: maximum and minimum temperatures of 48.0 ± 3.5 ºC and 29.0 ± 2.9 ºC, respectively; relative air humidity of approximately 30.0 ± 5.2%.

5.2.2 Growing conditions, experimental design and treatments

The trials were conducted using a randomized block experimental design in a 2 × 2 factorial scheme, corresponding to two inoculation treatments [uninoculated (UI) and inoculated with *B. subtilis* strain IPACC29] and two water levels [well-watered (WW - control) - 50% of soil field capacity (FC), and water deficit (WD - drought) - 25% FC], with four replicates, except for metabolomic profile, which used three biological replicates.

The inoculation treatments and Doce Miúda cactus pear (*Nopalea cochenillifera* Salm-Dyck) variety were selected alongside based on previous studies (Sousa *et al.,* 2025; Dauala *et al.,* 2025 in Chapter 2). The IPACC29 strain, isolated from sugarcane tissues and identified by Antunes *et al*. (2017), was selected due to its ability to enhance nitrogen and organic compound accumulation and to improve drought tolerance in cactus pear when combined with 50% of the recommended nitrogen dose (Dauala *et al.,* 2025). This strain is also known to produce

phytohormones, such as indole-3-acetic acid (IAA), promoting growth in maize and sorghum under both greenhouse and field conditions (Aquino *et al.,* 2021, 2022).

Soil samples of a typical dystrophic Yellow Latosol (Oxisol) were collected from the 0-20 cm layer at the UFPI Experimental Farm in Alvorada do Gurguéia for physical and chemical analyses (Appendix 2). Fertilization was adjusted using simple superphosphate (18% P_2O_5), potassium chloride (60% K_2O), copper sulfate (26.23% Cu), zinc sulfate (Zn 22.73%) and urea (45% N), following recommendations for cactus pear cultivation (Sousa; Lobato, 2004; Edvan; Carneiro, 2019). Nitrogen was applied at a total dose of 100 kg ha^{-1} (equivalent to 50 mg dm^{-3}), corresponding to 50% of the dose recommended rate of 200 kg ha^{-1} (100 mg dm^{-3}) for cactus pear fertilization (Edvan; Carneiro, 2019). The fertilizers were incorporated into pots containing 9 dm^3 of soil during and after planting. Nitrogen fertilization (50 mg dm^{-3}) was split into two applications: the first (25 mg dm^{-3}) at 30 days after planting (DAP) and the second (25 mg dm^{-3}) at 60 DAP, using urea (45% N) as N source.

Matrix cladodes of the Doce Miúda variety were collected from the UFPI Agrostological Experimental Field. Cladodes were selected from two-year-old plants showing uniformity and free from pests and diseases, with similar age, weight, length, width, and thickness; young and older cladodes were discarded. After selection, cladodes were placed in a shaded environment for 15 days to allow healing (Edvan; Carneiro, 2019)

Prior to planting, the IPACC29 strain was reactivated and its purity confirmed on TSA (Tryptone Soy Agar, at 25%, pH 6.8) medium. For inoculum preparation, the strain was cultivated in Erlenmeyer flasks containing 50 mL of TSB (Tryptone Soy Broth, at 25%, pH 6.8) medium and incubated on an SL-180/DT orbital shaker (120 rpm) at 28 ºC for 72 h at the Soil Microbiology Laboratory at UFPI. Matrix cladodes were then inoculated with 1.0 mL of TSB medium containing approximately 1×10^8 UFC mL^{-1} of the bacterial suspension, applied at their base, while UI plants received 1.0 mL of uninoculated TSB medium. Each cladode was planted vertically, with one-third of its length buried in soil, one per pot.

5.2.3 Irrigation management

At the beginning of the experiment, all treatments were maintained at 50% FC to ensure uniform establishment of the matrix cladodes under well-watered conditions. Thew drought was imposed 43 DAP by reducing soil humidity to 25% FC. The relative soil water content (RSWC) was monitored daily using gravimetric method, following the equation described by

Xu, Zhou and Shimizu (2009). Weighing and irrigation were executed until the pots reached the levels for well-watered (50% FC) and drought (25% FC) treatments.

5.2.4 Plant harvest and dry biomass

The plant material was harvested 193 DAP, corresponding to 150 days of drought imposition. During harvest, plants were separated into cladodes and roots. Samples of both organs were immediately frozen for biochemical analyses, while the remaining material was chopped and dried in a forced air oven at 65 °C until a constant weight was reached. Subsequently, the dry mass of cladodes, roots, and total biomass (g per plant) was determined using a precision balance.

5.2.5 Carbohydrates and organic acids

Ethanolic extracts were prepared from lyophilized root and cladode samples. Soluble carbohydrates were determined according to Dubois *et al*. (1956) by absorbance readings at 490 nm using anhydrous D-glucose as standard.

The organic acids (malic, citric, succinic, and lactic acids) in cladodes were quantified by high-performance liquid chromatography (HPLC; Shimadzu LCMS2020, UV detector) following Kung Jr. and Ranjit (2001) with minor modifications. Briefly, crude extract was prepared from 10 g of lyophilized cladodes homogenized in 90 mL of Milli-Q water. The homogenate was filtered through microfilters (PVDF membrane, 0.22 μm) and 10 mL of filtrate were mixed with 1 mL of 20% (m/v) metaphosphoric acid and 50 μL of 50% (v/v) sulfuric acid (H_2SO_4). Then, the solution was centrifuged at 13,000 × g for 15 min, and the supernatant was transferred to 2 mL vials. Analyses were performed on an HPX-87H column using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL min^{-1}, with detection at 210 nm. Organic acid contents were determined from standard curves using reagents purchased from Sigma Aldrich.

5.2.6 Inorganic ions and proline

Aqueous extracts for inorganic ions were prepared from cladode and root lyophilized tissues. Nitrate (NO_3^-) content was determined according to Cataldo *et al*. (1975) by measuring absorbance at 410 nm, using a KNO_3 standard curve, while ammonium (NH_4^+) content was estimated at 625 nm, using $(NH_4)_2SO_4$ as standard (Weatherburn, 1967). Proline content was

determined by absorbance readings at 520 nm using L-proline as standard (Bates; Waldren; Teare, 1973).

5.2.7 Nitrate reductase activity

Nitrate reductase (NR) activity was obtained after incubating 300 mg of fresh roots with buffer [0.1 M potassium phosphate buffer (pH 7.5), 50 mM KNO_3, 1% (v/v) isopropanol, and 15 mg L^{-1} chloramphenicol] at 30 °C for 30 min. NR activity was measured *in vivo* according to Coombs and Hall (1987) and Snell and Snell (1949). Nitrite (NO_2^-) content was determined in a reaction mixture containing buffer, 1% (m/v) sulphanilamide and 0.02% (m/v) NED [N-(1-naphthyl) ethylenediamine dihydrochloride] for 15 min, in the absence of light followed by absorbance readings at 540 nm using a $NaNO_2$ standard curve.

5.2.8 Lipid peroxidation and hydrogen peroxide

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) content as described by Heath and Packer (1968). MDA content was calculated from the absorbance difference at 532 and 600 nm, using a molar extinction coefficient of 155 mM^{-1} cm^{-1}. Hydrogen peroxide (H_2O_2) content was extracted following Cheeseman (2006) and Alexieva *et al.* (2001). H_2O_2 content was measured at 390 nm using a standard curve prepared with H_2O_2 solutions.

5.2.9 Protein content and antioxidant enzymes

Crude extracts were prepared from 1.0 g of fresh cladodes macerated with liquid nitrogen and homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) at 4 °C. Following homogenate was centrifuged at 12,000 × g at 4 °C, for 15 min, and the supernatant was collected and used to determine enzymatic activities and protein contents.

Total soluble protein concentration was measured at 595 nm according to Bradford (1976) using bovine serum albumin (BSA) as standard. Ascorbate peroxidase (APX) activity was determined according to Nakano and Asada (1981) by monitoring the decrease in absorbance at 290 nm, due to ascorbate oxidation, using a molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. Catalase (CAT) activity was measured by monitoring H_2O_2 consumption at 240 nm, as described by Beers and Sizer (1952), employing a molar extinction coefficient of 36 M^{-1} cm^{-}

[1]. Superoxide dismutase (SOD) activity was measured by absorbance reading at 560 nm, as described by Beyer and Fridovich (1987). SOD activity was expressed as the amount of enzyme required to cause 50% inhibition of NBT photoreduction, expressed as enzyme unit (EU mg^{-1} protein).

5.2.10 Metabolic profile

Cladodes of the same age were collected at 10:00 to 11:00 h, immediately frozen in liquid nitrogen, and stored at -80 °C until processing. Extraction of polar metabolites was developed as described by Lisec *et al*. (2006) with minor modifications. Primary metabolites were extracted from lyophilized sample in solution containing methanol, chloroform and ultrapure water (2:1:2, v/v/v). Ribitol at 20 mg mL^{-1} (in ultrapure water) was added as internal standard. Thereafter, aliquot of the upper phase (polar) was dried in a vacuum concentrator at room temperature, and the dried samples were derivatized with methoxylamine hydrochloride (20 mg mL^{-1} in anhydrous pyridine) at 37 °C for 2 h, followed by the addition of N-methyl-N-(trimethylsilyl)- trifluoro acetamide (MSTFA) at 37 °C for 30 min.

The metabolic profiles were obtained using Gas Chromatography coupled to Mass Spectrometry (GC-MS, QP-PLUS 2010, Shimadzu, Japan). Samples (1.0 µL) were injected in split mode (1:10 ratio) using helium as carrier gas at flow rate of 0.97 mL min^{-1}. Metabolites were separated on a RTX-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with an initial of 80 °C for 5 min, followed by a ramp of 10 °C per min to 300 °C and held for 10 min. Injection and MS interface temperatures were kept at 230 and 250 °C, respectively, with ion source at 200 °C. The MS operated at 70 eV (EI) with a scan range of 80-700 (*m/z*). Quality control was prepared by pooled of experimental sample to verify the accuracy and stability of the method. Metabolites were identified using Xcalibur™ 2.1 software by comparison with National Institute of Standards and Technology (NIST) library and in-house standards. The data were further analyzed using MetaboAnalyst 6.0 server (http://www.metaboanalyst.ca) (Pang *et al.,* 2024).

5.2.11 Statistical analysis

Dry biomass and biochemical data were initially tested for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests, respectively, at a 5% significance level; followed by an analysis of variance (ANOVA) using the F test at 5%. Then, significant

interactions ($p \leq 0.05$) were submitted to the Tukey mean test through Sisvar software, version 5.6 (Ferreira, 2019). Graphs were plotted through Sigma Plot software, version 14.0, while principal component analysis (PCA) and dendrograms were performed using R software version 4.5.1 (R Development Core Team, 2016).

For metabolic analysis, the data were normalized to the internal standard (ribitol) and sample dry mass, and expressed as relative area concentration. Prior to statistical analysis, the data were log transformed and Pareto-scaled. Chemometric analyses included Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) to compare four treatments: well-watered uninoculated, well-watered inoculated with IPACC29, drought uninoculated, and drought inoculated with IPACC29. Differential metabolites were identified using variable importance in projection (VIP $\geq$ 1.0) (Xia; Wishart, 2011). The metabolites were further analyzed by one-way ANOVA, with means separated by Tukey test ($p \leq 0.01$). A heatmap was also generated to visualize hierarchical clustering among treatments.

5.3 Results

5.3.1 Plant growth responses

Under well-watered conditions, plants inoculated showed higher root dry mass (RDM), cladode dry mass (CDM), and total dry mass (TDM) compared with uninoculated plants (Figure 11). Drought significantly decreased biomass in all plants; however, the damage was less intense in inoculated plants, whose RDM increased by 62% relative to uninoculated (UI) plants (Figure 11A).

5.3.2 Accumulation of organic solutes

Soluble carbohydrates were differentially regulated by inoculation and drought (Figure 12). Under well-watered conditions, no significant difference in carbohydrate content was observed between inoculated and UI plants in either roots or cladodes (Figures 12A, B). In roots, drought increased carbohydrate accumulation, with inoculated plants under drought showing 30% more than UI plants (Figure 12A). In cladodes, drought decreased carbohydrate content in UI plants compared to the well-watered control, whereas inoculated plants maintained unaltered values, which were 371% higher than those of UI plants (Figure 12B). Drought promoted a significant increase in organic acids in the cladodes of inoculated plants,

and no alteration was reported for UI plants (Figures 12C-F). In all cases, inoculation increased malic, citric, succinic, and lactic acid contents, with values in plants under drought found to be 624%, 219%, 309%, and 123% higher than those of UI plants.

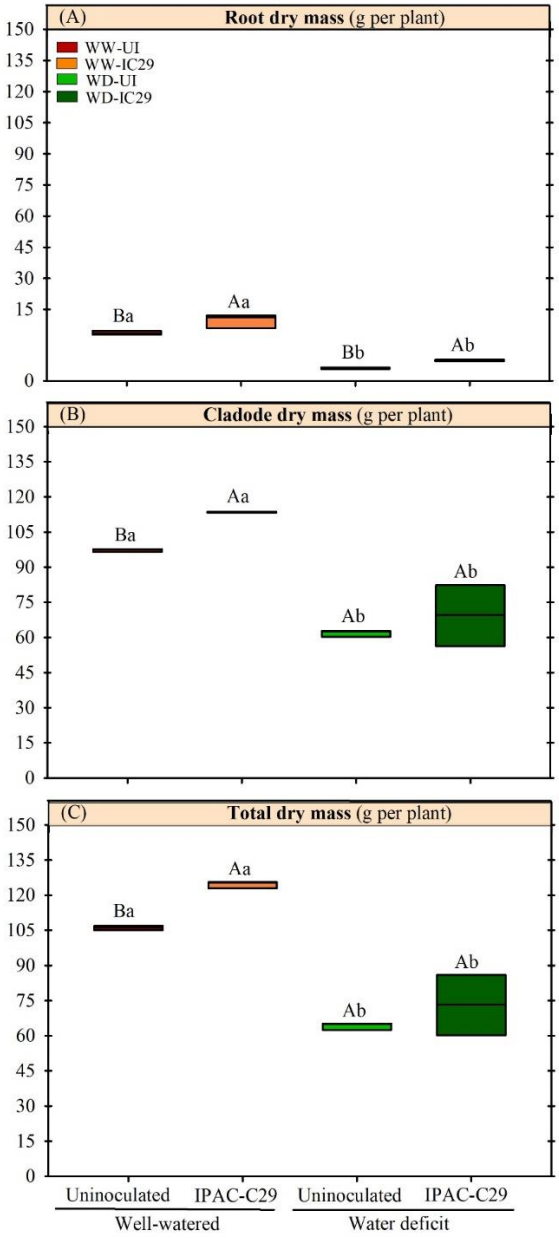


Figure 11 - Dry mass of root (A) and cladode (B), and total dry mass (C) of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29-IC29)] and water levels [well-watered (WW - control) - 50% of soil field capacity (FC) and water deficit (WD - drought) - 25% FC], 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to water levels, using Tukey test ($p \leq 0.05$).

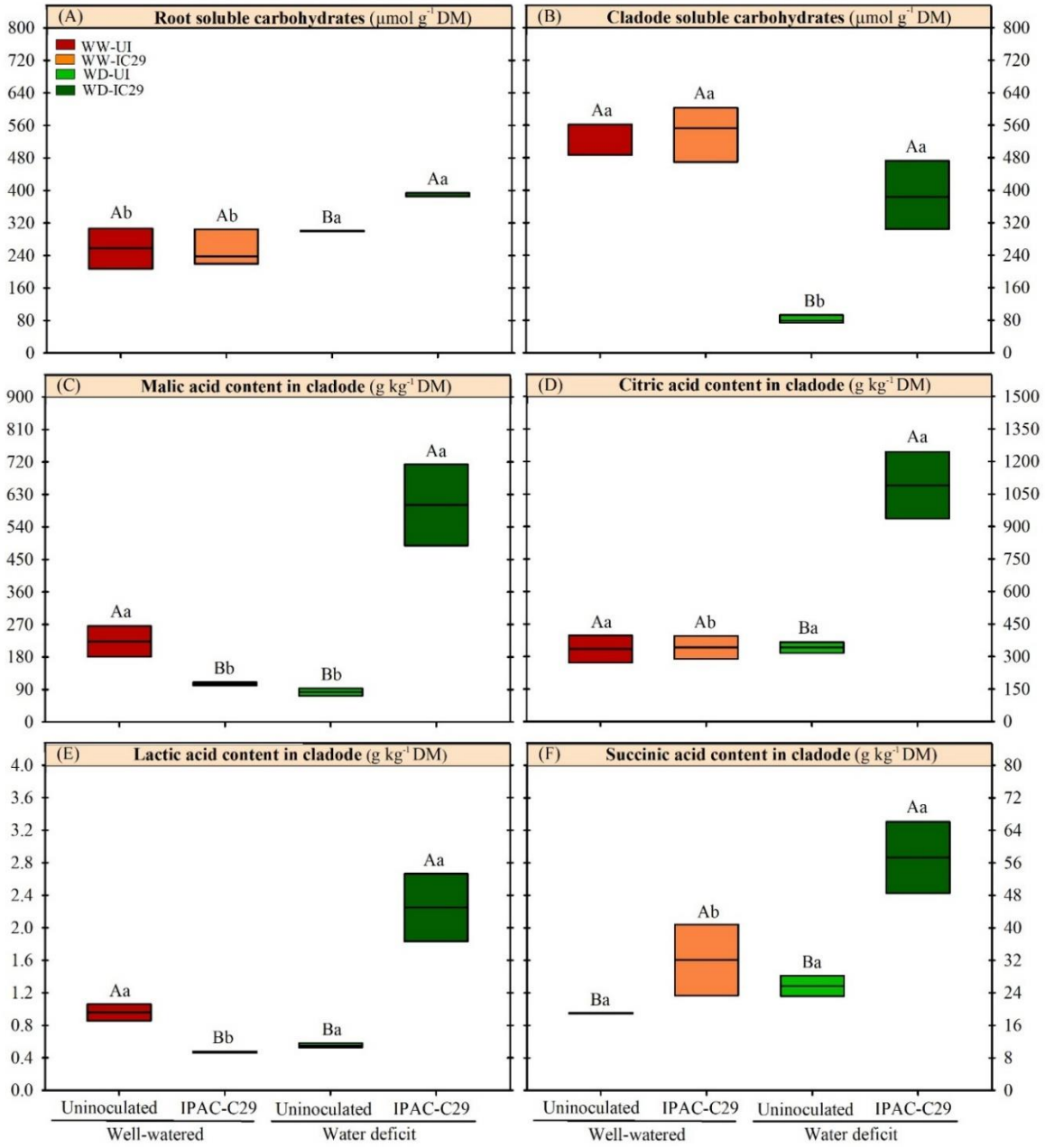


Figure 12 - Content of soluble carbohydrates in roots (A) and cladodes (B), and organic acids malic (C), citric (D), lactic (E) and succinic (F) in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29-IC29)] and water levels [well-watered (WW - control) - 50% of soil field capacity (FC) and water deficit (WD - drought) - 25% FC], 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to water levels, using Tukey test ($p \leq 0.05$)

5.3.3 NO_3^- and NH_4^+ contents and NR activity

Under well-watered conditions, no significant difference in nitrate (NO_3^-) and ammonium (NH_4^+) contents was recorded between inoculation treatments in either roots or

cladodes (Figures 13A-D). In roots, drought increased NO_3^- and NH_4^+ contents only in inoculated plants compared to well-watered plants (Figures 13A, C). In cladodes, drought significantly decreased the NO_3^- contents in UI plants relative to well-watered conditions (Figure 13B). No significant difference in NH_4^+ levels was observed among cladode treatments (Figure 13D). Overall, NO_3^- and NH_4^+ concentrations were highest in roots, with ammonium representing the predominant nitrogen form (Figures 13A, C). Under well-watered conditions, the highest NR activity occurred in UI plants as compared to inoculated plants. The drought decreased NR activity only in UI plants as compared to well-watered control, but no significant alteration was reported in inoculated plants, which showed NR activity in roots significantly higher than UI plants (Figure 13E).

5.3.4 Proline, hydrogen peroxide and lipid peroxidation

The drought enhanced proline accumulation in both roots and cladodes as compared to well-watered treatments, in both UI and inoculated plants (Figures 14A, B), but the relative increase was higher in roots (67%) and cladodes (55%) of inoculated plants than UI plants (Figures 14A, B). In addition, under drought, the H_2O_2 content and lipid peroxidation (MDA content) in cladodes were higher in UI plants as compared to inoculated ones (Figures 14C, D).

5.3.5 Protein content and antioxidant enzymatic system

Drought promoted a 121% increase in soluble protein content of cladodes from inoculated plants, compared to well-watered plants, whereas UI plants showed a 32% reduction. Under drought, soluble protein accumulation was 190% higher in inoculated plants than in UI plants (Figure 15A). Drought increased SOD activity in cladodes of both inoculation treatments, whereas APX activity increased only in UI plants compared with well-watered conditions (Figures 15B, C). On the contrary, water deficit decreased CAT activity relative to well-watered control, although inoculated plants maintained higher activity than UI plants (Figure 15D). Under drought, no significant difference in APX and SOD activity was observed between inoculation treatments (Figures 15B, C).

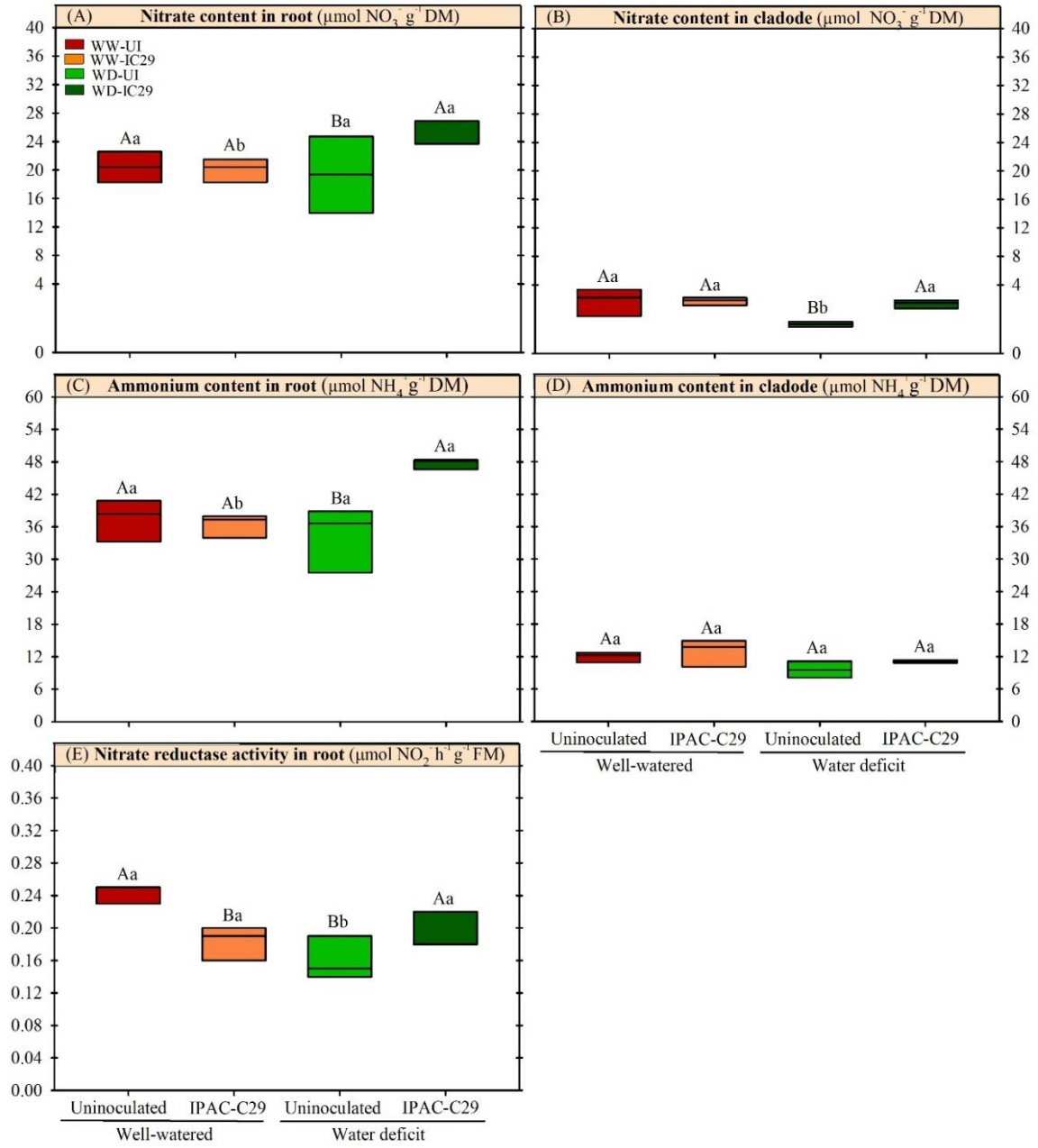


Figure 13 - Content of nitrate (NO_3^-) (A, B), ammonium (NH_4^+) (C, D) in roots and cladodes, and activity of nitrate reductase (E) in roots of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29-IC29)] and water levels [well-watered (WW - control) - 50% of soil field capacity (FC) and water deficit (WD - drought) - 25% FC], 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to water levels, using Tukey test ($p \leq 0.05$).

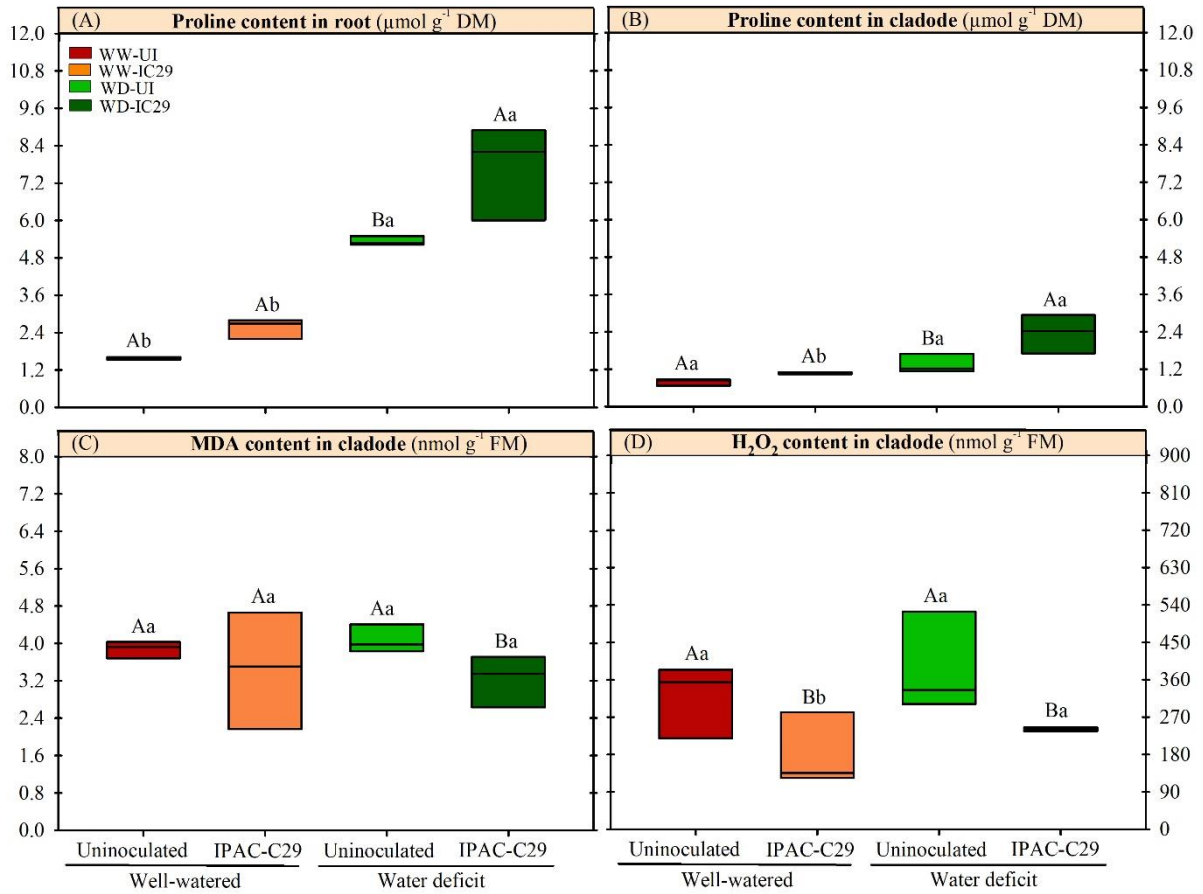


Figure 14 - Contents of proline in roots (A) and cladodes (B), and malondialdehyde (MDA, C) and hydrogen peroxide (H_2O_2, D) in cladodes of cactus pear (var. Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29-IC29)] and water levels [well-watered (WW - control) - 50% of soil field capacity (FC) and water deficit (WD - drought) - 25% FC], 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to water levels, using Tukey test ($p \leq 0.05$).

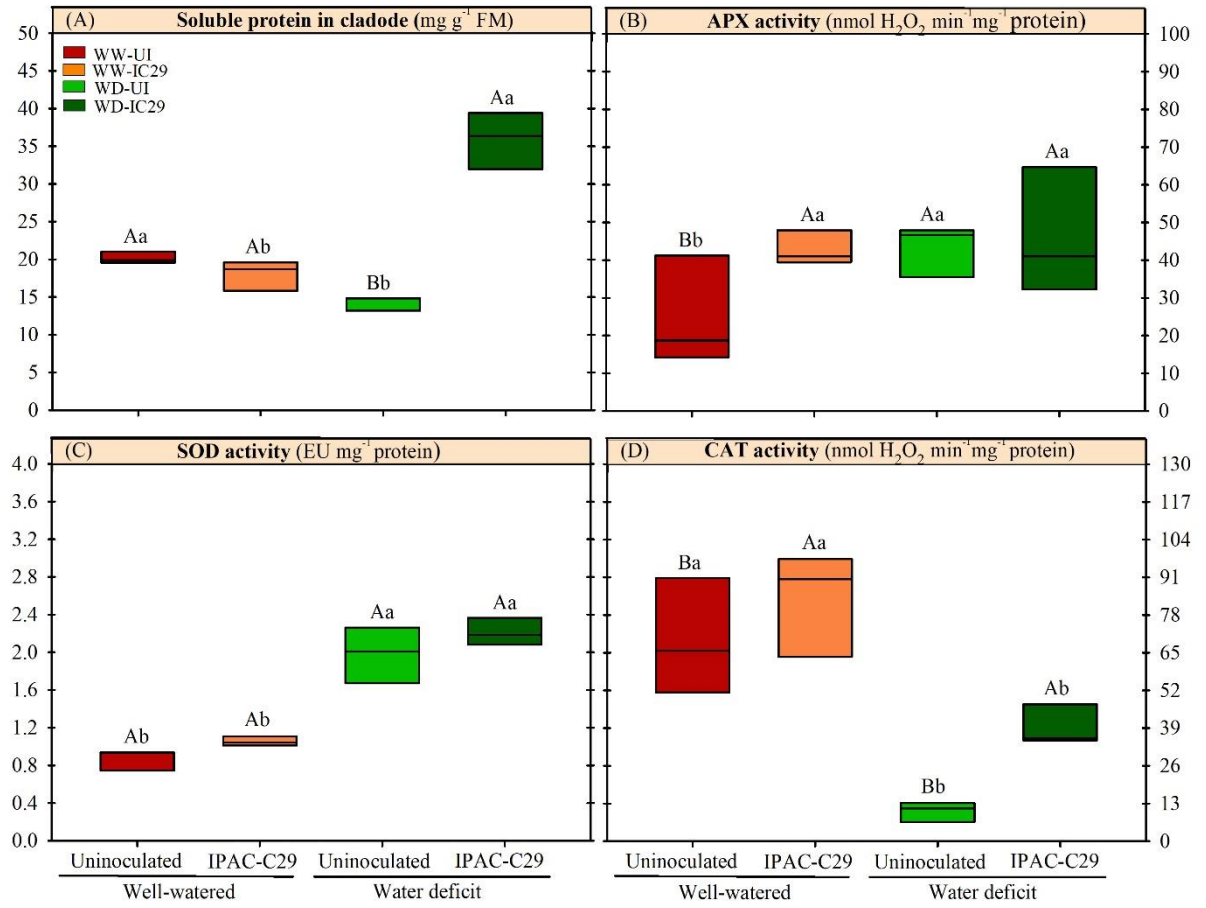


Figure 15 - Contents of soluble protein (A) and activity of ascorbate peroxidase (APX, B), superoxide dismutase (SOD, C) and catalase (CAT, D) in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29-IC29)] and water levels [well-watered (WW - control) - 50% of soil field capacity (FC) and water deficit (WD - drought)- 25% FC], 150 days after drought imposition. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to water levels, using Tukey test ($p \leq 0.05$).

5.3.6 PCA of growth and biochemical parameters

Cluster analysis and principal component analysis (PCA) were performed to explore the relationship between inoculated and UI cactus pear plants under well-watered and drought conditions, revealing three distinct groups (Figure 16). PC1 explained 54.1% of the total variance and PC2 36.2%, totaling 90.3% (Figure 16A). Under well-watered conditions, inoculated and UI plants clustered together in Group III, associated with dry biomass, inorganic nitrogen (NO_3^- and NH_4^+), soluble carbohydrates, and nitrate reductase activity in roots or cladodes (Figures 16A, B). Under drought, inoculated plants formed a distinct cluster (Group I), clearly separated from UI plants (Group II). Inoculated plants showed higher accumulation

of organic solutes (organic acids, soluble proteins, proline, and soluble carbohydrates), inorganic nitrogen, and antioxidant enzymes (APX and SOD). In contrast, UI plants (Group II) were characterized by higher levels of oxidative stress markers (MDA and H_2O_2) in cladodes (Figure 16A). These results are supported by negative correlation between oxidative damage indicators and antioxidant enzyme activities in inoculated plants under drought stress.

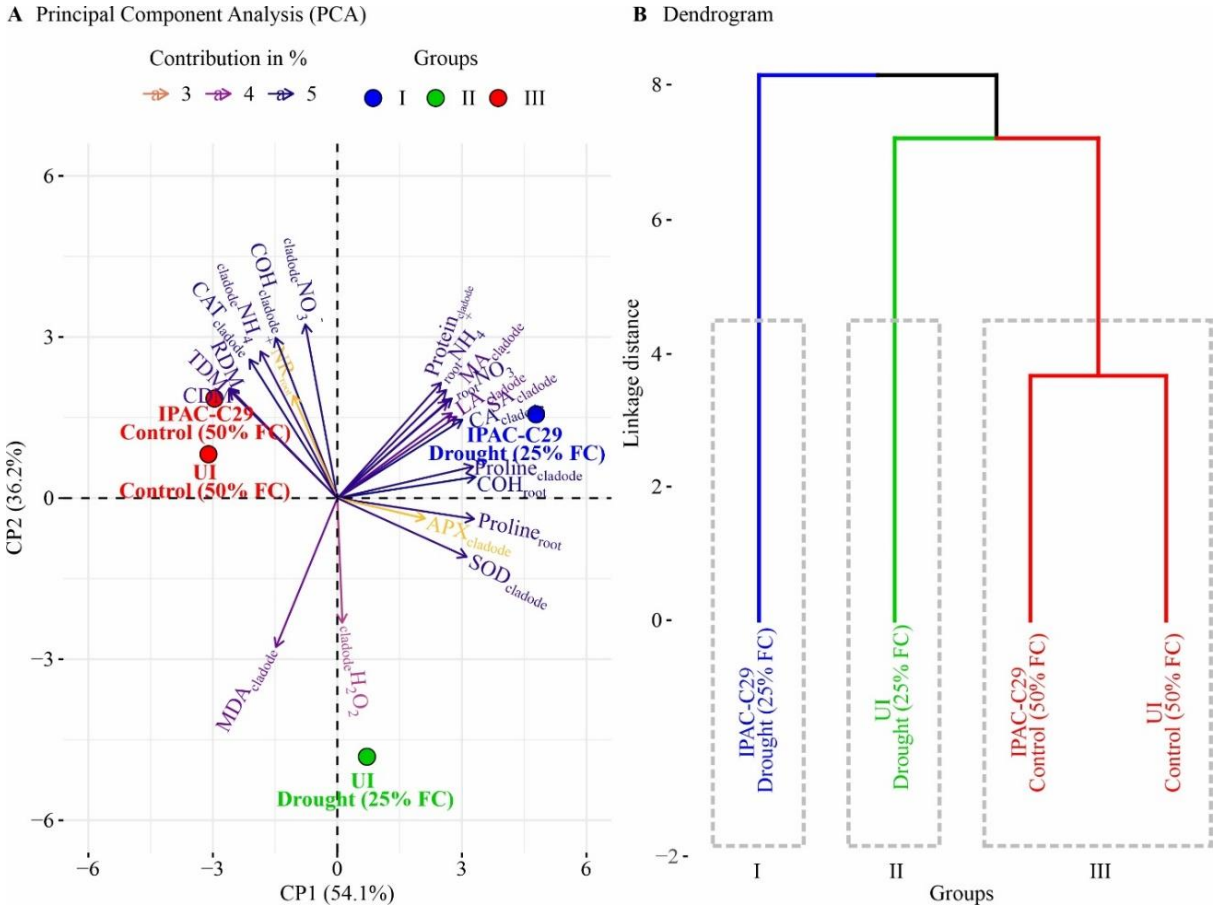


Figure 16 - Principal Component Analysis (PCA) (A) and dendrogram (B) of growth and biochemical parameters in cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC], 150 days after drought imposition. Parameters: content of malic acid ($MA_{cladode}$), citric acid ($CA_{cladode}$), lactic acid ($LA_{cladode}$) and succinic acid ($SA_{cladode}$) in cladode; carbohydrates content in root (CHO_{root}) and cladode ($CHO_{cladode}$); dry mass in root (RDM) and cladode (CDM); total dry mass (TDM); nitrate content in root ($_{root}NO_3^-$) and cladode ($_{cladode}NO_3^-$); ammonium content in root ($_{root}NH_4^+$) and cladode ($_{cladode}NH_4^+$); proline content in root ($Proline_{root}$) and cladode ($Proline_{cladode}$); malondialdehyde in cladode ($MDA_{cladode}$); hydrogen peroxide in cladode ($_{cladode}H_2O_2$); soluble protein in cladode ($Protein_{cladode}$); activity of nitrate reductase in root (NR_{root}); and activity of ascorbate peroxidase ($APX_{cladode}$), superoxide dismutase ($SOD_{cladode}$) and catalase ($CAT_{cladode}$) in cladode.

5.3.7 Metabolomic profiling

GC-MS analysis identified 52 primary metabolites, including ribitol, in cladodes of cactus pear subjected to different inoculation treatments and water levels (Figure 17). The metabolites were classified according to retention time, KEGG ID, and relative concentration (Appendixes 4, 5). Organic acids and carbohydrates were the most abundant groups (18 metabolites each), followed by amino acids (9), sugar alcohols (4), and other compounds (3).

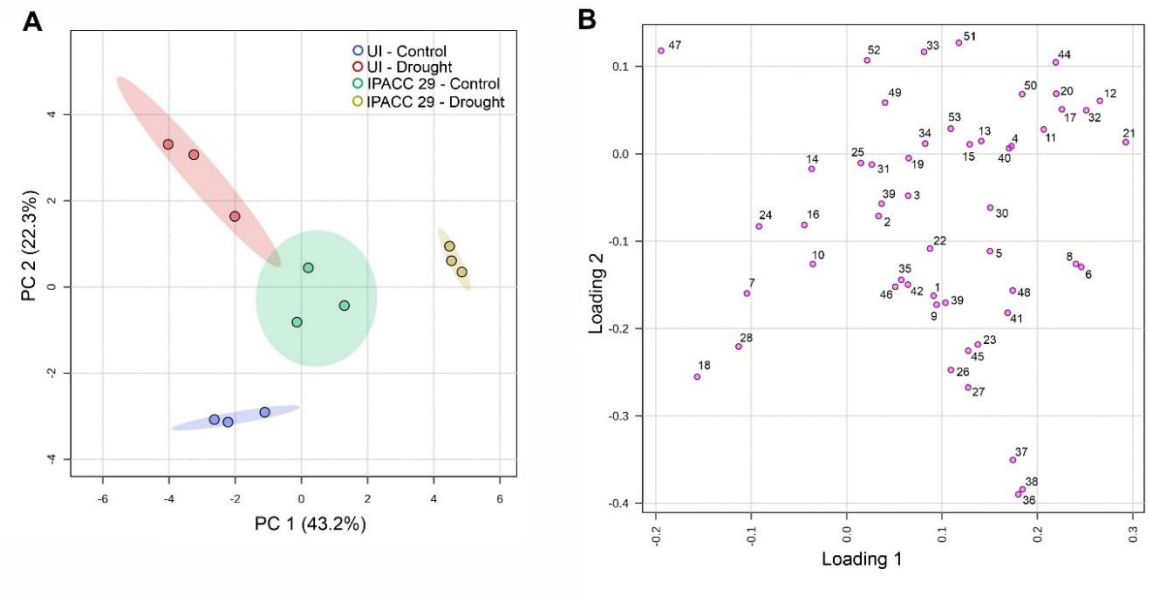


Figure 17 - Partial least squares discriminant analysis - PLS-DA (A) of the metabolic profiles and loading plot of metabolites (B) in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC], 150 days after drought imposition. The loading plot shows the positive and negative contributions of each metabolite to PLS-DA. Metabolite enumeration is presented in Appendix 4.

To assess the influence of inoculation treatments under different water conditions on the metabolic profile, an unsupervised principal component analysis (PCA) was performed, explaining 71.4% of the total variation (Appendix 6). A partial least squares discriminant analysis (PLS-DA) further confirmed the clear separation among treatment groups, with slight overlap between UI-stressed plants and well-watered inoculated plants (Figure 17A). The PLS-DA model showed strong predictive capacity (R^2 = 0.93 and Q^2 = 0.76) and a significant permutation test (p = 0.006 in 2000 permutations). The first two principal components explained 65.5% of the total variance, with PC1 accounting for 43.2% and PC2 for 22.3% (Figure 17A). Component 1 was positively influenced by 44 metabolites and negatively by 8,

whereas component 2 was positively influenced by 19 metabolites and negatively by 33 (Figure 17B). Key metabolites contributing to group separation included 4-amino butyric acid or GABA (21), proline (12), citric acid (32), valine (6), glycine (8), and malic acid (17) for PC1, and trehalose (47), dehydroascorbic acid (33), melezitose (52), sucrose (44) an unidentified metabolite (51) for PC2 (Figure 17B; Appendix 4).

The VIP score analysis identified 20 metabolites (VIP ≥ 1.0) as major contributors to treatment differentiation (Figure 18). Under well-watered conditions, UI plants showed higher levels of erythritol, serine, and ribose; whereas inoculated plants showed greater accumulation of maltotriose and isomaltose. Under drought, UI plants showed increased trehalose levels, while inoculated plants accumulated higher contents of organic acids (citric, malic, maleic and GABA), amino acids (proline, pyroglutamic acid, glycine, valine and alanine), carbohydrates (sucrose, galactonic acid and palatinose), phosphoric acid, and an unidentified compound.

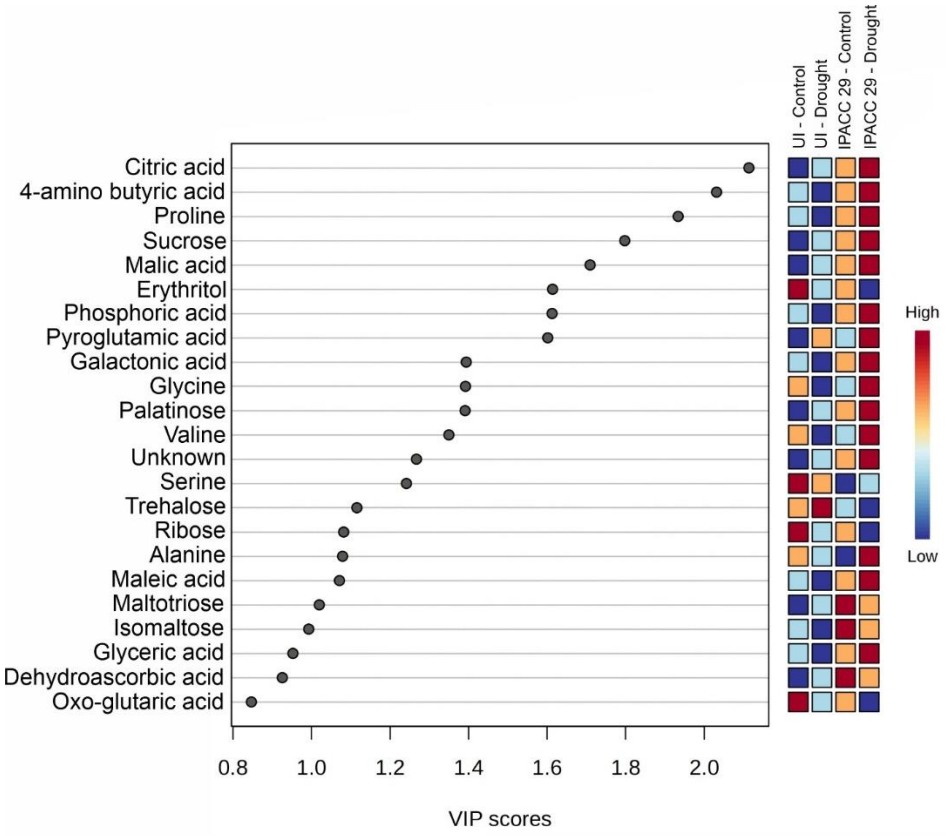


Figure 18 - VIP scores plot of metabolites in cladodes of cactus pear (variety Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC], 150 days after drought imposition. Red or blue squares on the right indicate high and low abundance of the corresponding metabolite in each treatment, respectively.

Among the 52 identified metabolites, 27 showed significant variation ($p \leq 0.01$) according to Tukey test (Appendix 5). Heatmap analysis further revealed that inoculation with IPAC-C29 under drought increased the relative abundance of several 9 organic acids (citric, malic,GABA, saccharic, lactic, maleic, glycolic, erythronic, and quinic), 5 amino acids (pyroglutamic acid, glycine, proline, valine, and alanine), 11 carbohydrates and derivates (palatinose, sucrose, galactonic acid, maltotriose, cellobiose, glucose, fructose, galactose, arabinose, xylose, and isomaltose) and 2 other compounds (phosphoric acid and unidentified compound) compared with UI plants (Figure 19). In contrast, UI plants showed higher levels of only erythritol and trehalose. Under well-watered conditions, inoculated plants accumulated higher levels of certain carbohydrates and organic acids, whereas UI plants showed greater abundance of succinic acid and lactic acid dimer.

5.4 Discussion

5.4.1 The PGPB inoculation boosts organic compounds accumulation and intensify antioxidant defense in cactus pear plants under drought

Water restriction significantly decreased the dry biomass accumulation of cactus pear (Figure 11), corroborating previous studies demonstrating the negative impacts of prolonged drought on growth (Scalisi *et al.,* 2016; Araújo Júnior *et al.,* 2021; Lahbouki *et al.,* 2022). However, inoculation with IPACC29 strain significantly mitigated the damage of drought, resulting in increased root dry mass (RDM) of Doce Miúda variety (Figure 11A). These findings reinforce the potential of PGPB as an efficient strategy to enhance plant acclimatation to drought (Vurukonda *et al.,* 2016; Singh *et al.,* 2024).

The improved performance of inoculated plants was associated with higher accumulation of organic solutes, such as proline in roots and cladodes, soluble carbohydrates in roots, soluble proteins and organic acids (citric, malic, succinic and lactic) in cladodes. Concurrently, increased contents in inorganic N (NO_3^- and NH_4^+) in roots and upregulation of antioxidant SOD and APX enzymes were observed (Figure 16A). The increase in NO_3^- and NH_4^+ may be related to enhanced N uptake and assimilation and/or to the contribution of biological nitrogen fixation promoted by IPACC29 (Aquino *et al.,* 2021). In contrast, UI plants showed markedly different biochemical responses (Figure 16A), highlighting the efficiency of PGPB in inducing drought tolerance in cactus pear, as previously reported by Dauala *et al*. (2025).

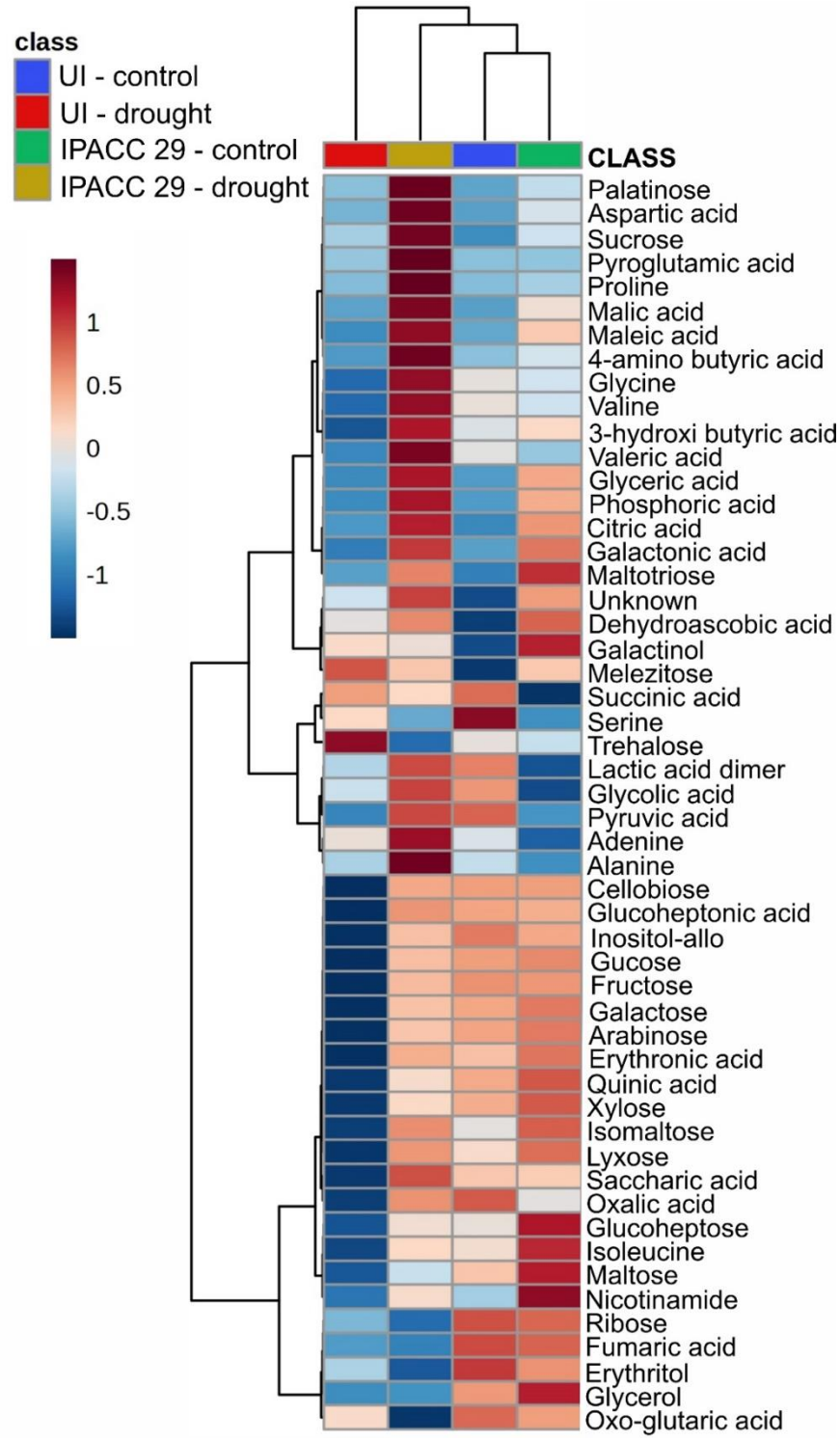


Figure 19 - Heatmap representation of the relative abundance of 52 detected metabolites in cladodes of cactus pear (var. Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC] 150 days after drought imposition.

The accumulation of compatible solutes, particularly proline (Figures 14A, B; 16A) and soluble carbohydrates (Figures 12A; 16A), likely contributed to maintaining cellular membrane integrity, preventing dehydration and protecting against oxidative damage (Chieb; Gachomo, 2023; Buqori *et al.,* 2025). These osmotic adjustments were reflected in increased RDM (Figure 11A), possibly supported by higher N content (Figures 13A, B, C; 16A), enhanced synthesis of soluble proteins (Figures 15A; 16A), and accumulation of Krebs cycle intermediate organic acids (Figures 12C-F; 6A), which are essential for energy generation and photosynthetic performance under drought (Batool *et al.,* 2020). Moreover, the increase in cladode protein content represents a relevant nutritional gain for forage use, given the typically low crude protein content of cactus pear (Dubeux Jr. *et al.,* 2021).

Another relevant aspect was the maintenance of soluble carbohydrate content in cladodes of inoculated plants under drought stress (Figure 12B), suggesting preservation of photosynthetic activity. These findings suggest that PGPB favored the maintenance of PEP-case activity in inoculated-stressed plants, as supported by increased malic acid content (Figures 12C; 16A). The PEP-case is a key enzyme of CAM metabolism responsible for CO_2 assimilation, resulting in higher photosystem II (PSII) efficiency and an intensified Krebs cycle (Liguori *et al.,* 2013; Ojeda-Pérez *et al.,* 2017). Additionally, the ability of IPACC29 strain to produce indole-3-acetic acid (IAA) (Aquino *et al.,* 2021) may have stimulated root growth, promoting greater root elongation and increasing water and nutrient uptake (Kavamura *et al.,* 2013; Andrade *et al.,* 2023). The role of exopolysaccharides and bacterial biofilm formation should also be considered, an additional mechanism to promote water retention in rhizosphere under drought conditions (Ali; Khan, 2021; Carezzano *et al.,* 2023).

In parallel, inoculated plants showed a significant decrease in H_2O_2 and MDA levels (Figures 14C, D; 16A), indicating higher efficiency of antioxidant systems and, consequently, lower lipid peroxidation (Wang *et al.,* 2024). The coordinated action of SOD, APX, proline and soluble proteins (Figure 16A) likely contributed to ROS scavenging, thereby preserving cellular integrity (Wang *et al.,* 2024). In contrast, UI plants showed higher H_2O_2 and MDA levels (Figures 14C, D; 6A), reflecting lower antioxidant efficiency and higher oxidative damage. These findings reinforce that, although cactus pear displays intrinsic tolerance to semi-arid conditions, the biochemical responses can still be severely compromised under prolonged drought conditions (Scalisi *et al.,* 2016; Lahbouki *et al.,* 2022).

5.4.2 Metabolic profiling reveals key primary metabolites induced by IPACC29 strain to counteract drought damage in cactus pear cladodes

The metabolomic approach revealed the metabolic dynamics among treatments, with 27 metabolites showing significant differences according to Tukey test (Appendix 5), with those exhibiting higher relative concentrations considered essential metabolites associated with drought tolerance. The metabolic separation between treatments was clearly evidenced by multivariate analyses, including PLS-DA, VIP scores, and heatmap clustering (Figures 17A, 18, 19; Appendix 5).

The UI plants showed a marked decrease in carbohydrates and derivatives, amino acids, and organic acids, particularly intermediates of the Krebs cycle and glycolysis pathways (Appendix 5; Figure 19), indicating a limited capacity for metabolic adjustment. This pattern was reinforced by decreased levels of N (Figure 13B), soluble carbohydrates (Figure 12B), proline (Figures 14B; 6A), soluble proteins (Figures 15A; 16A), and organic acids (citric, malic, succinic and lactic) (Figures 12C-F; 16A), associated with a significant increase in H_2O_2 and MDA levels (Figures 14C, D; 16A). Additionally, the higher accumulation of erythritol and trehalose in UI plants suggests rapid utilization or depletion of carbon reserves under drought conditions (Kang *et al.,* 2019; Khan; Bano; Babar, 2019), which may have contributed to the lower accumulation of RDM and organic acids (Figures 11A; 12C-F; Appendix 5). Collectively, the data indicate low photosynthetic performance, and increased oxidative damage due to low efficiency of antioxidant systems in UI plants (Wang *et al.,* 2024).

In contrast, plants inoculated with the IPACC29 strain showed overaccumulation of organic acids, particularly citric, malic and 4-amino butyric acids, as well as carbohydrates and derivatives such as sucrose, fructose and inositol, and amino acids including pyroglutamic acid, glycine and proline (Appendix 5). The findings reflect a more efficient metabolic adjustment and highlight the role of IPACC29 strain in modulating the metabolic profile of cactus pear plants.

The higher accumulation of organic acids, particularly malic acid, suggests the maintenance of photosynthetic activity and redirection of carbon toward the synthesis of compatible solutes, energy production, and physiological support (Panchal; Miller; Giri, 2021), as reflected by increased carbohydrate content and RDM (Figures 11A; 12A, B; Appendix 5). In parallel, the accumulation of sucrose, fructose, and inositol reinforces the role of carbohydrates and derivatives as energy sources, osmotic regulators and signaling molecules in stress responses (Lastdrager; Hanson; Smeekens, 2014; Khan; Bano; Babar, 2019). Furthermore,

the increased accumulation of amino acids, including proline, glycine and pyroglutamic acid, likely contributed to osmoregulation, ROS detoxification, membrane stabilization and maintenance of cellular water status, which was also associated with increased soluble protein content (Figure 15A; Appendix 5).

5.5 Conclusion

The findings revealed key mechanisms underlying drought responses of cactus pear plants inoculated with IPACC29 strain. The data suggest that essential metabolites associated with drought tolerance in cladodes include citric acid, malic acid, inositol, sucrose, fructose, pyroglutamic acid, glycine and proline. The drought tolerance induced by IPACC29 was attributed to: (i) increase nitrogen content, which stimulated the accumulation of compatible solutes such as proline, soluble carbohydrates, soluble proteins and organic acids, contributing to cellular water balance and higher root biomass; (ii) enhanced antioxidant capacity, evidenced by lower H_2O_2 levels and lipid peroxidation, resulting from the coordinated action of enzymatic (SOD and APX) and non-enzymatic (proline, soluble proteins and organic acids) antioxidant; and (iii) regulation of key metabolites involved in osmotic adjustment, ROS scavenging and energy balance, promoting greater metabolic efficiency under drought conditions.

REFERENCES

ALEXIEVA, V.; SERGIEV, I.; MAPELLI, S.; KARANOV, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. **Plant, Cell and Environment**, v. 24, p. 1337-1344, 2001.

ALI, S.; KHAN, N. Delineation of mechanistic approaches employed by plant growth promoting microorganisms for improving drought stress tolerance in plants. **Microbiological Research**, v. 249, p. 126771, 2021.

ALVARES, C. A.; STAPE, J. L.; SENTELHAS, P. C.; GONÇALVES, J. L. M.; SPAROVE, G. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, p. 711-728, 2013.

ANDRADE, L. A.; SANTOS, C. H. B.; FREZARIN, E. T.; SALES, L. R.; RIGOBELO, E. C. Plant growth-promoting rhizobacteria for sustainable agricultural production. **Microorganisms**, v. 11, n. 4, p. 1088, 2023.

ANTUNES, J. E. L.; LYRA, M. C. C. P.; OLLERO, F. J.; FREITAS, A. S.; OLIVEIRA, L. M. S.; ARAÚJO, A. S. F.; FIGUEIREDO, M. V. B. Diversity of plant growth promoting

bacteria associated with sugarcane. **Genetics and Molecular Research**, v. 16, p. 16029662, 2017.

AQUINO, J. P. A.; ANTUNES, J. E. L.; BONIFÁCIO, A.; ROCHA, S. M. B.; AMORIM, M. R.; ALCÂNTARA NETO, F.; ARAÚJO, A. S. F. Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. **Theoretical and Experimental Plant Physiology**, v. 33, p. 249-260, 2021.

AQUINO, J. P. A.; NETO, V. P. C.; ANDRADE, M. R. S.; OLIVEIRA, L. M. S.; ANTUNES, J. E. L.; FREITAS, A. S.; NETO, F. A.; ARAÚJO, A. S. F. Plant growth-promoting bacteria increase the yield of green maize and sweet sorghum. **Journal of Plant Nutrition**, v. 46, p. 58-68, 2022.

ARAÚJO JÚNIOR, G. N.; SILVA, T. G. F.; SOUZA, L. S. B.; ARAÚJO, G. G. L.; MOURA, M. S. B.; ALVES, C. P.; SALVADOR, K. R. SILVA; SOUZA, C. A.; MONTENEGRO, A. A. A.; SILVA, M. J. Phenophases, morphophysiological indices and cutting time in clones of the forage cacti under controlled water regimes in a semiarid environment. **Journal of Arid Environments**, v. 190, p. 104510, 2021.

BATES, L. S.; WALDREN, R. P.; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, v. 39, p. 205-207, 1973.

BATOOL, T.; ALI, S.; SELEIMAN, M. F.; NAVEED, N. H.; ALI, A.; AHMED, K.; ABID, M.; RIZWAN, M.; SHAHID, M. R.; ALOTAIBI, M.; AL ASHKAR, I.; MUBUSHAR, M. Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. **Scientific Reports**, v. 10, p. 16975, 2020.

BEERS JR, R. F.; SIZER, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. **Journal of Biological Chemistry**, v. 195, p. 133-140, 1952.

BEYER, W. F.; FRIDOVICH, I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. **Analytical Biochemistry**, v. 161, p. 559-566, 1987.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, p. 248-254, 1976.

BUQORI, D. M. A. I.; SUGIHARTO, B.; SUHERMAN; SISWOYO, T. A.; HARIYONO, K. Mitigating drought stress by application of drought-tolerant *Bacillus* spp. enhanced root architecture, growth, antioxidant and photosynthetic genes expression in sugarcane. **Scientific Reports**, v. 15, p. 5259, 2025.

CAREZZANO, M. E.; ÁLVAREZ STRAZZI, F. B.; PÉREZ, V.; BOGINO, P.; GIORDANO, W. Exopolysaccharides synthesized by rhizospheric bacteria: a review focused on their roles in protecting plants against stress. **Applied Microbiology**, v. 3, p. 1249-1261, 2023.

CATALDO, D. A.; HAROON, M.; SCHRADER, L. E.; YOUNGS, V. L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis**, v. 6, p. 71-80, 1975.

CHEESEMAN, J. M. Hydrogen peroxide concentrations in leaves under natural conditions. **Journal of Experimental Botany**, v. 57, p. 2435-2444, 2006.

CHIEB, M.; GACHOMO, E. W. The role of plant growth promoting rhizobacteria in plant drought stress responses. **BMC Plant Biology**, v. 23, p. 407, 2023.

COOMBS, J.; HALL, D. O. **Técnicas de bioprodutividade e fotossíntese**. Fortaleza: Edições UFC, Brazil, 1987. 292 p.

DAUALA, G. A.; FONSECA, B. S. F.; SOUSA, A. B.; PAULA-MARINHO, S. O.; MORAIS, P. G. C.; LEITE, M. R. L.; SILVA, R. F.; AVIZ, R. O.; DUARTE, M. H. F.; NASCIMENTO, R. R.; COSTA, E. M.; BOECHAT, C. L.; ARAÚJO, A. S. F.; SOUSA, R. O.; COSTA, J. H.; EDVAN, R. L.; NETO, F. A.; MIRANDA, R. S. Plant growth-promoting bacteria modulate metabolism and nitrogen accumulation to counteract drought damage in cactus pear plants. **Scientific Reports**, v. 15, p. 36432, 2025.

DUBEUX JR, J. C. B.; SANTOS, M. V. F.; CUNHA, M. V.; SANTOS, D. C.; SOUZA, R. T. A.; MELLO, A. C. L.; SOUZA, T. C. Cactus (*Opuntia* and *Nopalea*) nutritive value: a review. **Animal Feed Science and Technology**, v. 275, p. 114890, 2021.

DUBOIS, M.; GILLES, K. A.; HAMILTON, J. K.; REBERS, P. T.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v. 28, p. 350-356, 1956.

EDVAN, R. L.; BARROS, D. M.; NASCIMENTO, R. R.; ARAÚJO, M. J.; SOUSA, H. R.; NERIS, L. M. L.; BEZERRA, L. R.; SILVA-FILHO, E. C. Potential use of hydrogel from natural fiber in the initial growth of *Nopalea cochenillifera* under water stress: water source of slow release. **Communications in Soil Science and Plant Analysis**, v. 54, p. 3063-3075, 2023.

EDVAN, R. L.; CARNEIRO, M. S. S. **Palma forrageira: cultivo e uso na alimentação animal**. Appris: Curitiba, Brazil, 2019. 93 p.

EDVAN, R. L.; MOTA, R. R. M.; DIAS, S. T. P.; NASCIMENTO, R. R.; SOUSA, S. V.; SILVA, A. L.; ARAÚJO, J. S. Resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. **Scientific Reports**, v. 10, p. 10040, 2020.

FADIJI, A. E.; SANTOYO, G.; YADAV, A. N.; BABALOLA, O. O. Efforts towards overcoming drought stress in crops: revisiting the mechanisms employed by plant growth-promoting bacteria. **Frontiers in Microbiology**, v. 13, p. 962427, 2022.

FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Brazilian Journal of Biometrics**, v. 37, p. 529-535, 2019.

HEATH, R. L.; PACKER, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics**, v. 125, p. 189-198, 1968.

IIYAS, M.; NISAR, M.; KHAN, N.; HAZRAT, A.; KHAN, A. H.; HAYAT, K.; FAHAD, S.; KHAN, A.; ULLAH, A. Drought tolerance strategies in plants: a mechanistic approach. **Journal of Plant Growth Regulation**, v. 40, n. 3, p. 926-944, 2021.

KANG, Z.; BABAR, M. A.; KHAN, N.; GUO, J.; KHAN, J.; ISLAM; SHRESTHA, S.; SHAHI, D. Comparative metabolomic profiling in the roots and leaves in contrasting genotypes reveals complex mechanisms involved in post-anthesis drought tolerance in wheat. **PLoS One**, v. 14, p. e0213502, 2019.

KAVAMURA, V. N.; SANTOS, S. N.; SILVA, J. L.; PARMA, M. M.; ÁVILA, L. A.; VISCONTI, A.; ZUCCHI, T.; TAKETANI, R. G.; ANDREOTE, F.; MELO, I. S. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. **Microbiological Research**, v. 168, p. 183-191, 2013.

KHAN, I.; ULLAH, N.; MAQBOOL, H.; HASHMI, A.; KHAN, B.; KHALID, A.; NAZ, I.; PAKER, N. P.; MUNIS, M. F. H.; CHAUDHARY, H. J. Holistic review on *Paenibacillus*: The multifunctional genus in heavy metal detoxification, pesticide degradation, and abiotic stress mitigation for sustainable agriculture. **Plant and Soil**, v. 518, p. 1245-1280, 2026.

KHAN, N.; BANO, A.; BABAR, M. A. Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum L*. **PloS One**, v. 14, p. e0213040, 2019.

KHAN, N.; BANO, A. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. **PLoS One**, v. 14, p. e0222302, 2019.

KUNG JR, L.; RANJIT, N. K. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. **Journal of Dairy Science**, v. 84, p. 1149-1155, 2001.

LAHBOUKI, S.; BEN-LAOUANE, R.; ANLI, M.; BOUTASKNIT, A.; AIT-RAHOU, Y.; AIT-EL-MOKHTAR, M.; EL GABARDI, S.; DOUIRA, A.; WAHBI, S.; OUTZOURHIT, A. Arbuscular mycorrhizal fungi and/or organic amendment enhance the tolerance of prickly pear (*Opuntia ficus-indica*) under drought stress. **Journal of Arid Environments**, v. 199, p. 104703, 2022.

LASTDRAGER, J.; HANSON, J.; SMEEKENS, S. Sugar signals and the control of plant growth and development. **Journal of Experimental Botany**, v. 65, p. 799-807, 2014.

LIGUORI, G.; INGLESE, G.; PERNICE, F.; SORTINO, G.; INGLESE, P. CO_2 uptake of *Opuntia ficus-indica* (L.) Mill. whole trees and single cladodes, in relation to plant water status and cladode age. **Italian Journal of Agronomy**, v. 8, p. e3, 2013.

LISEC, J.; SCHAUER, N.; KOPKA, J.; WILLMITZER, L.; FERNIE, A. R. Gas chromatography mass spectrometry-based metabolite profiling in plants. **Nature Protocols**, v. 1, p. 387-396, 2006.

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. **Plant and Cell Physiology**, v. 22, p. 867-880, 1981.

OJEDA-PÉREZ, Z. Z.; JIMÉNEZ-BREMONT, J. F.; DELGADO-SÁNCHEZ, P. Continuous high and low temperature induced a decrease of photosynthetic activity and changes in the diurnal fluctuations of organic acids in *Opuntia streptacantha*. **PLoS One**, v. 12, p. e0186540, 2017.

PANCHAL, P.; MILLER, A. J.; GIRI, J. Organic acids: versatile stress-response roles in plants. **Journal of Experimental Botany**, v. 72, p. 4038-4052, 2021.

PANG, Z.; LU, Y.; ZHOU, G.; HUI, F.; XU, L.; VIAU, C.; SPIGELMAN, A. F.; MACDONALD, P. E.; WISHART, D. S.; LI, S.; XIA, J. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. **Nucleic Acids Research**, v. 52, p. 398-406, 2024.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2016. Disponível em: http://www.R-project.org. Acesso em: nov. 2025.

SCALISI, A.; MORANDI, B.; INGLESE, P.; BIANCO, R. L. Cladode growth dynamics in *Opuntia ficus-indica* under drought. **Environmental and Experimental Botany**, v. 122, p. 158-167, 2016.

SINGH, D.; THAPA, S.; SINGH, J. P.; MAHAWAR, H.; SAXENA, A. K.; SINGH, S. K.; MAHLA, H. R.; CHOUDHARY, M.; PARIHA, M.; CHOUDHARY, K. B.; CHAKDA, H. Prospecting the potential of plant growth-promoting microorganisms for mitigating drought stress in crop plants. **Current Microbiology**, v. 81, p. 81-84, 2024.

SNELL, F.D., SNELL, C.T. Colorimetric methods of analysis. New York: Van Nostrand, 1949.

SOUSA, A. B.; LEITE, M. R. L.; DAUALA, G. A.; MORAIS, P. G. C.; PAULA-MARINHO, S. O.; MONTEIRO, G. N.; DUARTE, M. H. F.; OLIVEIRA, R. I.; SILVA, R. F.; AVIZ, R. O.; MIRANDA, R. S.; SOUZA; BOECHAT, C. L.; ARAÚJO, A. S. F.; EDVAN, R. L.; COSTA, E. M. *Bacillus* and *Paenibacillus* strains enhance nitrogen metabolism and increase biomass production of *Nopalea cochenillifera* grown in semi-arid soil. **Journal of Soil Science and Plant Nutrition**, v. 25, p. 10782-10798, 2025.

SOUSA, D. M. G.; LOBATO, E. **Cerrado: Correção do Solo e Adubação**. Brasília: Embrapa Informação Tecnológica, 2004. 416 p.

VURUKONDA, S. S. K. P.; VARDHARAJULA, S.; SHRIVASTAVA, M. SKZ.; A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. **Microbiological Research**, v. 184, p. 13-24, 2016.

WANG, P.; LIU, W. C.; HAN, C.; WANG, S.; BAI, M. Y.; SONG, C. P. Reactive oxygen species: multidimensional regulators of plant adaptation to abiotic stress and development. **Journal of Integrative Plant Biology**, v. 66, p. 330-367, 2024.

WEATHERBURN, M. W. Phenol-hypochlorite reaction for determination of ammonia. **Analytical Chemistry**, v. 39, p. 971-974, 1967.

XIA, J.; WISHART, D. S. Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. **Nature Protocols**, v. 6, p. 743-760, 2011.

XU, Z.; ZHOU, G.; SHIMIZU, H. Are plant growth and photosynthesis limited by pre-drought following rewatering in grass? **Journal of Experimental Botany**, v. 60, p. 3737-3749, 2009.

6 CAPÍTULO 4

MICROBIAL INOCULANTS BOOST WATER AND BIOMASS ACCUMULATION IN CACTUS PEAR UNDER CONTRASTING WATER LEVELS IN FIELD-PRODUCTION SYSTEM

ABSTRACT

Drought stress is one of the most severe constraints preventing plants from reaching their full potential, particularly in arid and semi-arid regions. Plant growth-promoting bacteria (PGPB) represent a promising strategy to enhance crops resilience and yield under such conditions. This study aimed to investigate the role of PGPB in the performance of two cactus pear varieties, Doce Miúda and Baiana (*Nopalea cochenillifera* Salm-Dyck) under drought stress. Two parallel and independent field trials were conducted, one for each variety, using a randomized block design. Treatments consisted of three inoculation levels [two PGPB strains (*Bacillus subtilis* IPACC29 and *Paenibacillus* sp. IPACC55) and an uninoculated (UI)] combined with two irrigation intervals with a fixed depth of 8.5 mm (control - irrigation every 7 days, and drought stress - irrigation every 30 days) over 491 days. After 108 days of drought imposition, growth parameters, biomass, water status, photosynthetic pigments and yield were assessed. Drought significantly decreased plant growth in both varieties, regardless of inoculation treatment. Inoculating cactus pear with IPACC29 significatively increased water content, dry mass accumulation and yield, especially under well-watered conditions. The IPACC55 inoculation modulated the photosynthetic pigments, cladode succulence and tissue hydration for both Doce Miúda and Baiana varieties, but no microbial inoculants promote increase in biomass accumulation and drought tolerance under water restriction. The findings reveals that the IPACC29 is promisor for inoculating cactus pear under well-watered conditions, activating important responses to accumulate water and biomass. The PGPB hold potential as sustainable biotechnological tools to cultivating cactus pear in semi-arid regions, contributing to agricultural sustainability.

Keywords: *Napolea cochenillifera* Salm-Dyck, PGPB inoculation, Plant defense, Semiarid, Water stock, Yield.

6.1 Introduction

Arid and semi-arid regions usually present low water availability, an essential resource for plant growth and metabolism that constitutes a significant portion of fresh biomass (Seleiman *et al*., 2021). Drought is considered the primary environmental stressor limiting the growth of native and cultivated species, such as grasses and legumes with high water requirements, causing the decrease in biomass, pasture and forage availability for animal (Edvan *et al*., 2020; Ilyas *et al*., 2021; Seleiman *et al*., 2021). In plants, drought stress impairs physiological and biochemical processes, primarily affecting photosynthesis and antioxidant defense mechanisms, and may also alter the function and interactions of soil microorganisms (Ilyas *et al*., 2021; Wang *et al*., 2022).

Cultivating drought-tolerant fodder species with low water requirements and high nutritional value can help mitigate drought damage and increase feed availability for livestock in dry regions, thereby contributing to food security (Nefzaoui, 2018; Dubeux Jr *et al*., 2021). Among fodder crops, the cactus pear species *Napolea cochenillifera* is particularly important due to its relevance for animal production in arid and semi-arid regions, especially the Doce Miúda and Baiana varieties (Cavalcante *et al*., 2014; Edvan *et al*., 2020, 2023). This crop stores elevated amounts of water in its cladodes and has been widely used as a source of water and energy for ruminants and other animals, due to its high carbohydrate and nutrient content. Furthermore, it exhibits good palatability, satisfactory biomass production, and high water-use efficiency, attributes associated with its Crassulacean Acid Metabolism (CAM) photosynthetic mechanism (Nefzaoui, 2018; Dubeux Jr *et al*., 2021).

Although well adapted to dry environments, several studies have demonstrated that prolonged drought periods negatively affect growth, yield, biomass composition, physiological performance, and biochemical indicators of cactus pear under both greenhouse (Scalisi *et al*., 2016; Lahbouki *et al*., 2022, 2023) and field conditions (Araújo Júnior *et al*., 2021; Miranda *et al*., 2023a). Thus, research focusing on developing management strategies devoted to counteract drought damage are critical for crop yield.

Inoculating plants with symbiotic microorganisms has been widely cited as a promising strategy for sustainable agriculture, as it enhances crop resilience to drought in dry regions. Plant growth-promoting bacteria (PGPB) represent an effective approach to stimulating plant responses and improving green biomass production, thereby contributing to food security and agricultural sustainability (Andrade *et al*., 2023; Giller *et al*., 2025). These bacteria colonize

the rhizosphere and enhance drought tolerance through direct and indirect mechanisms (Bittencourt *et al*., 2023).

Previous studies have reported that plant-PGPB interactions positively influence plant morphology as well as physiological and biochemical processes, enhancing drought resilience and promoting growth (Vurukonda *et al*., 2016; Ali; Khan, 2021; Khan *et al*., 2026). In plants, association with PGPB may increase leaf water content, improve water-use efficiency, enhance nutrient uptake, increase chlorophyll content, stimulate photosynthetic performance and ultimately improve yield in several cultivated species (Vurukonda *et al*., 2016; Gupta *et al*., 2022; Singh *et al*., 2024). However, studies focusing on cactus pear responses under PGPB interactions in field are poorly addressed.

We hypothesized that inoculating the Doce Miúda and Baiana varieties with IPACC29 (*Bacillus subtilis*) and IPACC55 (*Paenibacillus* sp.) strains enhances plant resilience to drought under field condition and represents a sustainable strategy for cactus pear cultivation in semi-arid regions. Our investigative study aimed to evaluate the role of PGPB strains in the performance of Doce Miúda and Baiana cactus pear varieties (*Nopalea cochenillifera*) under drought stress. Growth, physiological and yield indicators were assessed.

6.2 Material and methods

6.2.1 Study site and environmental conditions

The experiments were carried out under field conditions, from May 2023 to September 2024, at the Campus Professora Cinobelina Elvas, Federal University of Piauí (UFPI) (9°04′45.6″ S, 44°19′37.9″ W; approximately 277 m altitude), located in Bom Jesus city, Piauí state, Brazil. The regional climate is classified as Aw (tropical semi-arid), characterized by summer rainfall and dry winter, according to the Koppen classification described by Alvares *et al*. (2014). Environmental data were obtained from an automatic meteorological station located near the experimental site. The air temperature and relative humidity during drought imposition are shown in figure 20. High maximum air temperatures and low relative humidity were also recorded, characterizing a typical semi-arid region.

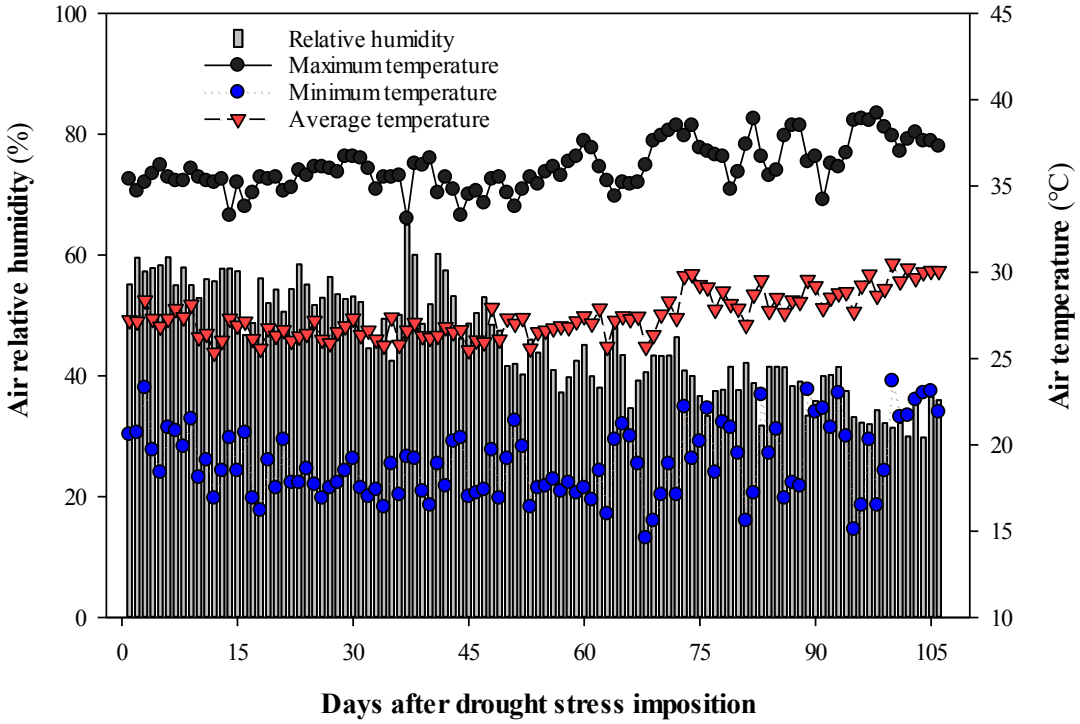


Figure 20 - Dynamics of maximum, minimum, average temperature and air relative humidity, during the drought imposition in the field experiment with cactus pear varieties and inoculation treatments.

6.2.2 Growing conditions, experimental design and treatments

Two parallel and independent trials were carried out using one cactus pear variety each (*Napolea cochenillifera* Salm-Dyck), Baiana or Doce Miúda (Appendix 7). The experiments were conducted in a randomized block design with four repetitions, in a 3 × 2 factorial scheme, corresponding to three inoculation levels [two PGPB strains (*Bacillus subtilis* IPACC29 and *Paenibacillus* sp. IPACC55) and an uninoculated (UI)] and two irrigation intervals with a fixed depth of 8.5 mm (control - irrigation every 7 days, and drought stress - irrigation every 30 days), totaling six treatments and 48 experimental units.

The fixed irrigation depth was defined based on reference evapotranspiration (ETo) and a water level to maintain 50% in an accumulated manner, according to previous study (Castro *et al*., 2021; Miranda *et al*., 2023a; Araújo Júnior *et al*., 2025). ETo was calculated using the Penman-Monteith method (FAO-56), as described by Allen *et al*. (1998). Irrigation intervals were established according to the methodology described by Pereira *et al*. (2021), Neto *et al*. (2020) and Miranda *et al*. (2023a).

The timeline of activities during the trials is illustrated in figure 21. The cactus pear varieties and inoculation levels were defined based on preliminary experiments (Sousa *et al*., 2025; Dauala *et al*., 2025 in Chapter 2). The bacterial strains IPACC29 and IPACC55 were isolated from sugarcane tissues and identified by Antunes *et al*. (2017). Both strains are known for their ability to produce phytohormones, such as indole-3-acetic acid (IAA), and to perform biological nitrogen fixation through acetylene-reducing activity (ARA) (Aquino *et al*., 2021). In addition, these PGPB strains have been shown to promote growth of maize and sorghum under greenhouse (Aquino *et al*., 2021) and field conditions (Aquino *et al*., 2022).

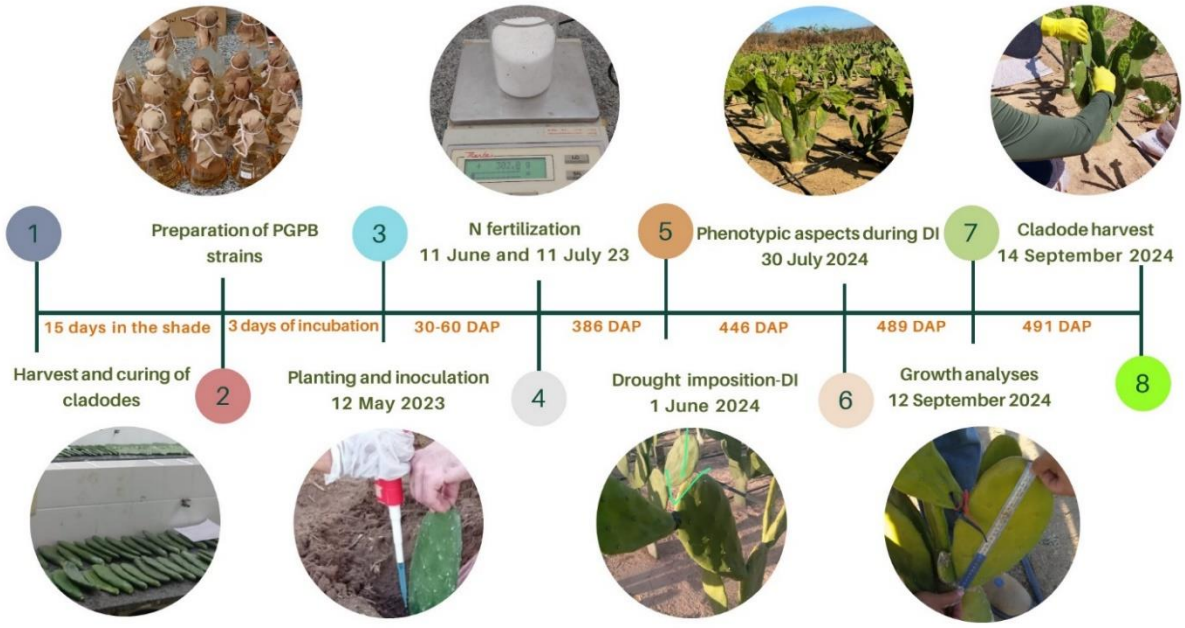


Figure 21 - Timeline of main activities conducted during the field experiment with cactus pear varieties under different irrigation intervals and inoculation treatments.

Before starting the experiment, soil samples of a dystrophic Yellow Latosol (Oxisol) were collected from 0-20 cm layer in the experimental area of UFPI for chemical and physical analyses (Appendix 8). Fertilization was corrected as necessary using P_2O_5 (as simple superphosphate, 18%) and N (as urea, 45%) as source, following recommendations for cactus pear cultivation (Sousa; Lobato, 2004; Edvan; Carneiro, 2019). Nitrogen was applied as 100 kg ha^{-1}, corresponding to 50% of the recommended dose (200 kg ha^{-1}) for cactus pear fertilization (Edvan; Carneiro, 2019). Nitrogen fertilization was applied in two doses, at 30 and 60 days after planting (DAP).

Prior to planting, the matrix cladodes of Baiana and Miúda varieties were collected from the Agrostological Experimental Field at UFPI. Subsequently, the cladodes were placed in a shaded environment for healing for 15 days (Edvan; Carneiro, 2019). The plant material used

was healthy, free from pests and diseases, and presented uniformity. Then, matrix cladodes of Baiana and Miúda varieties were inoculated with 1.0 mL of a medium (containing approximately 1×10^8 UFC mL^{-1} of the bacterial suspension) prepared in 50 mL of liquid medium TSB (Tryptone Soya Broth), after incubation on a SL-180/DT shaker table (120 rpm) for 72 h in the Soil Microbiology Laboratory at UFPI.

The plant material was planted by inserting one-third of cladode length into soil, one per hole, spaced 0.5 m and 1.0 m between rows and plants, respectively, resulting in a planting density of 20.000 plants per hectare (Appendix 7). For UI treatment, 1.0 mL of TSB uninoculated medium was applied for plants.

6.2.3 Irrigation management

Irrigation management was performed using a drip irrigation system, with drip lines spaced 0.50 m and 0.2 m between drippers. To ensure the stabilization of the matrix cladodes, irrigation after planting was maintained every 7 days in all treatments from May to October 2023. From November 2023 to May 2024, the experiment coincided with the rainy season at the study site. Drought treatments were subsequently imposed from June to September 2024 by increasing the irrigation interval to 30 days (Figure 22A).

The irrigation depths applied during the drought imposition are shown in figure 22B. Total irrigation depths during the experiment were 414.5 mm and 321.4 mm for control and drought treatments, respectively, resulting in a difference of 93 mm. The accumulated rainfall between November 2023 and May 2024 was 1012.3 mm, and total water depths (irrigation + rainfall) were 1426.8 mm and 1333.7 mm for control and drought treatments, respectively (Figures 22A, C).

6.2.4 Plant harvest, growth and biomass parameters

The plant material was harvested 491 DAP, corresponding 108 days of drought imposition, with two plants per plot considered as one replicate. Firstly, non-destructive biometric parameters number of cladodes per plant, plant height, and length and width of the cladodes were measured. Cladode area (CA, cm^2) was estimated using the equation CA = length × width × 0.632, as described by Cortázar and Nobel (1991). The total cladode area per plant was calculated by summing the CA from each order. The thickness of primary cladodes was determined at the third middle part using a precision digital caliper. Cladodes were harvested

above the main cladode, preserving the mother plant (matrix) to maintain crop perenniality, as illustrated in figure 21. The harvested material was weighed to obtain total fresh mass and production per plant. Subsequently, cladode samples were immediately frozen for laboratory analyses, while the remaining material was placed in a forced air oven at 65 °C until constant weight. The dry samples were then weighed to determine total dry mass and production per plant. Fresh and dry mass yields (t ha^{-1}) were calculated by multiplying the biomass production per plant (kg) by the planting density (20.000 plants per hectare), then dividing the result by 1.000.

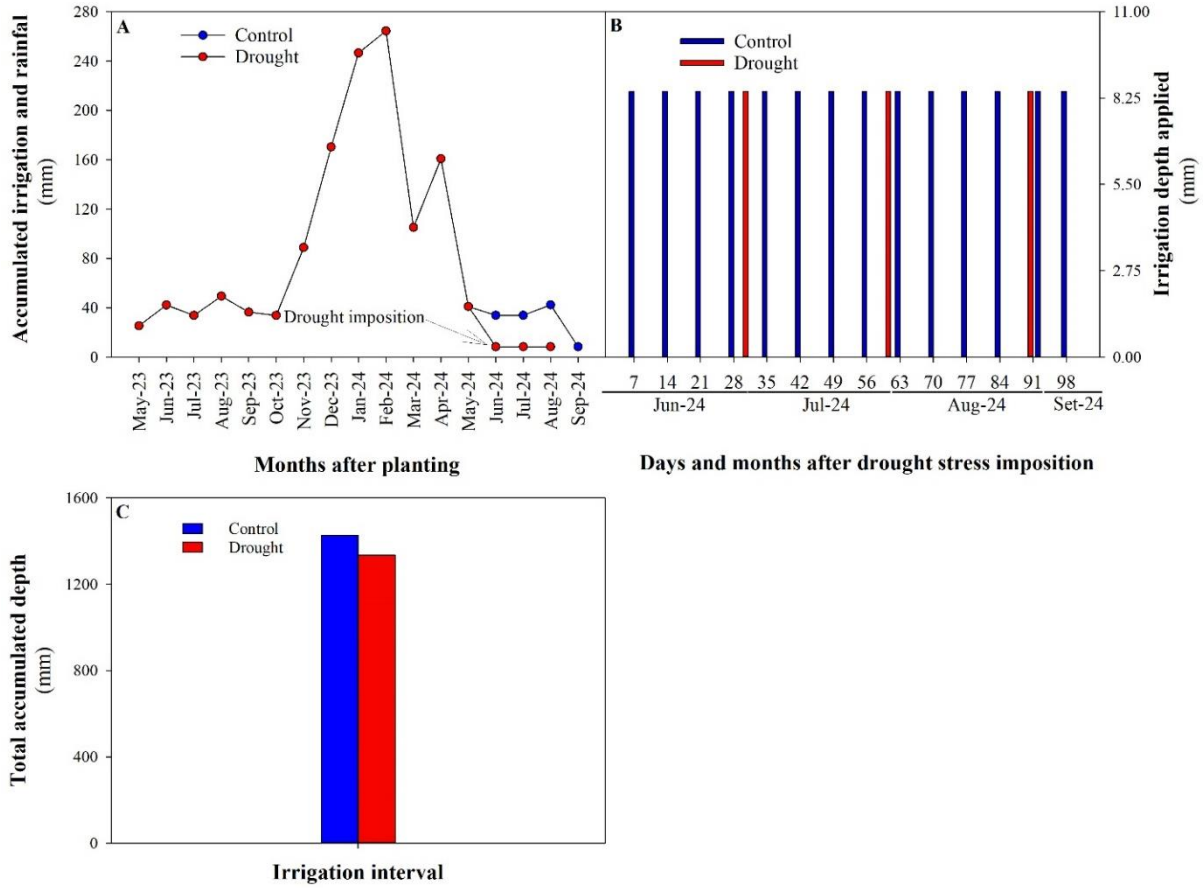


Figure 22 - Monthly accumulated irrigation and rainfall (A), irrigation depth applied during the drought imposition (B), and total accumulated depth (C) in the field experiment with cactus pear varieties subjected to different irrigation intervals and inoculation treatments. No rainfall was recorded during the drought stress imposition.

6.2.5 Relative tolerance to drought, water use efficiency and plant accumulated water

Relative tolerance to drought (RTd) was calculated based on the following formula adapted from Miranda *et al*. (2023b):

$$\text{RTd (\%)} = \frac{\text{TDMstress}}{\text{TDMcontrol}} \times 100$$

where TDM_{stress} is the total dry mass of drought-stressed plants; and $TDM_{control}$ is the total dry mass of control plants cladode.

Water use efficiency (WUE) was determined based on the following formula adapted from Pereira *et al*. (2021):

$$\text{WUE (kg DM mm}^{-1}) = \frac{\text{DMY}}{\text{TID} + \text{RF}} \times 1000$$

where DMY is the dry mass yield (t ha^{-1}); TID is the total irrigation depth for each treatment (mm); and RF is the rainfall recorded during the experiment (mm).

Plant accumulated water (PAW) was estimated based on the following formula adapted from Edvan *et al*. (2020) and Ramos *et al*. (2021)

$$\text{PAW (t ha}^{-1}) = \text{FMY} \times (\frac{100 - \text{DMC}}{100})$$

where FMY is the fresh mass yield (t ha^{-1}); and DMC is the dry matter content (%).

6.2.6 Cladode succulence and temperature

Cladode succulence (CS) was determined based on the following formula proposed by Mantovani (1999):

$$\text{CS (g H}_2\text{O cm}^{-2}) = \frac{\text{CFM} - \text{CDM}}{\text{CA}}$$

where CFM is the cladode fresh mass; CDM is the cladode dry mass; and CA is cladode area.

The temperature of cladodes (°C) was measured one day before harvest, at the center of highest cladode on plant, using an imaging IR thermometer FLIR (model TG165) starting from 01:00 pm.

6.2.7 Photosynthetic pigments

Photosynthetic pigments (chlorophylls *a*, *b*, total, and carotenoids) were extracted after incubating two discs of young cladodes in a dimethyl sulfoxide (DMSO) solution saturated with $CaCO_3$. The contents chlorophyll *a*, *b*, total and carotenoids were estimated through spectrophotometry readings at 480, 649 and 665 nm, according to the methodology proposed by Wellburn (1994). The cladode discs were dried in a forced ventilation oven at 65 °C until reaching a constant dry mass, and the pigments content were expressed as μg g^{-1} DM.

6.2.8 Statistical analysis

The data were first tested for normality using the Shapiro-Wilk test at a 5% significance level. Subsequently, analysis of variance (ANOVA) was performed using the F test at the same significance level. When significant interactions were detected ($p \leq 0.05$), means were grouped using the Scott-Knott test with the aid of Sisvar software (version 5.6) (Ferreira, 2019). The graphs were generated using SigmaPlot 14.0. Finally, principal component analysis (PCA) and dendrogram analysis were conducted using R software (Development Core Team, 2016).

6.3 Results

6.3.1 Growth parameters

In general, drought stress promoted a significant decrease in the growth of Baiana and Doce Miúda varieties, but the damages were less intense in plants from inoculated treatments (Figure 23). Plant height exhibited minimal variation under well-watered (control) and drought stress conditions in both varieties, regardless of inoculation treatment (Figure 23A). In Baiana variety, under well-watered conditions, inoculation with IPACC29 strain resulted in the highest number of cladodes per plant (NCP) and total cladode area (TCA), whereas in Doce Miúda variety, increase by inoculation with IPACC29 and IPACC55 was reported only in total cladode area (Figure 23B, C). Under drought, the inoculation with IPACC55 provided the highest NCP in Doce Miúda (Figure 23B), and highest TCA in both varieties compared to other treatments (Figure 23C). Yet, drought stressed plants showed similar increase in primary cladode thickness under both inoculation treatments as compared to UI plants, irrespective of cactus pear variety (Figure 23D). Cladode length showed minimal variation, with similar values among inoculation treatments in both varieties (Figure 23E). Additionally, IPACC55 promoted greater cladode width in Doce Miúda, whereas in Baiana this parameter was similar across all treatments (Figure 23F).

6.3.2 Biomass parameters and water use efficiency

The fresh and dry mass production and yield of cladodes, as well as water use efficiency (WUE), were significantly influenced by inoculation treatments and irrigation intervals in both varieties (Figure 24). Under well-watered conditions, inoculation with IPACC29 resulted in the

highest cladode production, yield and WUE in both varieties (Figure 24). Drought stress significantly decreased all these parameters in both varieties; however, the effects were less intense in Doce Miúda plants inoculated with IPACC55, which exhibited higher production, yield, and WUE values compared to other treatments (Figure 24).

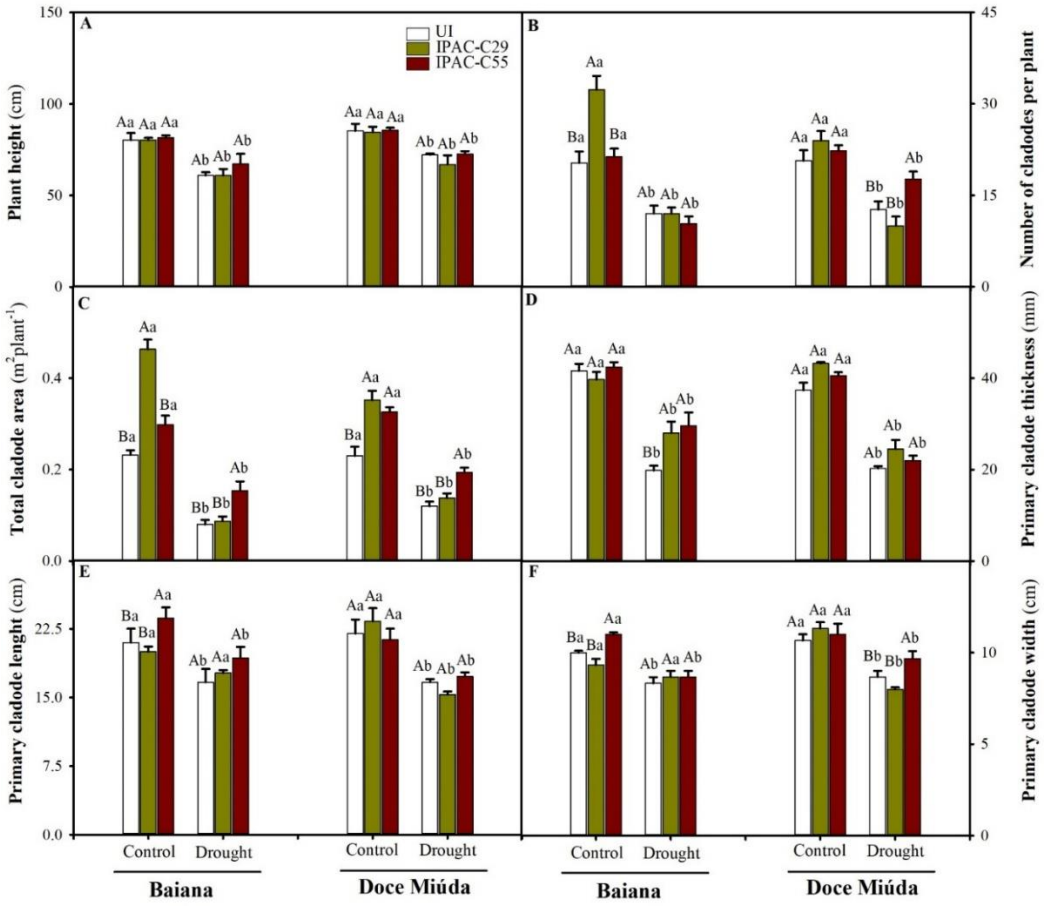


Figure 23 - Plant height (A), number of cladodes per plant (B), total cladode area (C), thickness, length, and width of the primary cladode (D, E, F) of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation treatments [uninoculated (UI), inoculation with *Bacillus subtilis* strain (IPACC29) and *Paenibacillus* sp. (IPACC55)] and irrigation intervals [control (irrigation every 7 days) and drought (irrigation every 30 days)], 108 days after drought imposition under field conditions. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to irrigation intervals (Control and Drought), using Scott-Knott test ($p \leq 0.05$).

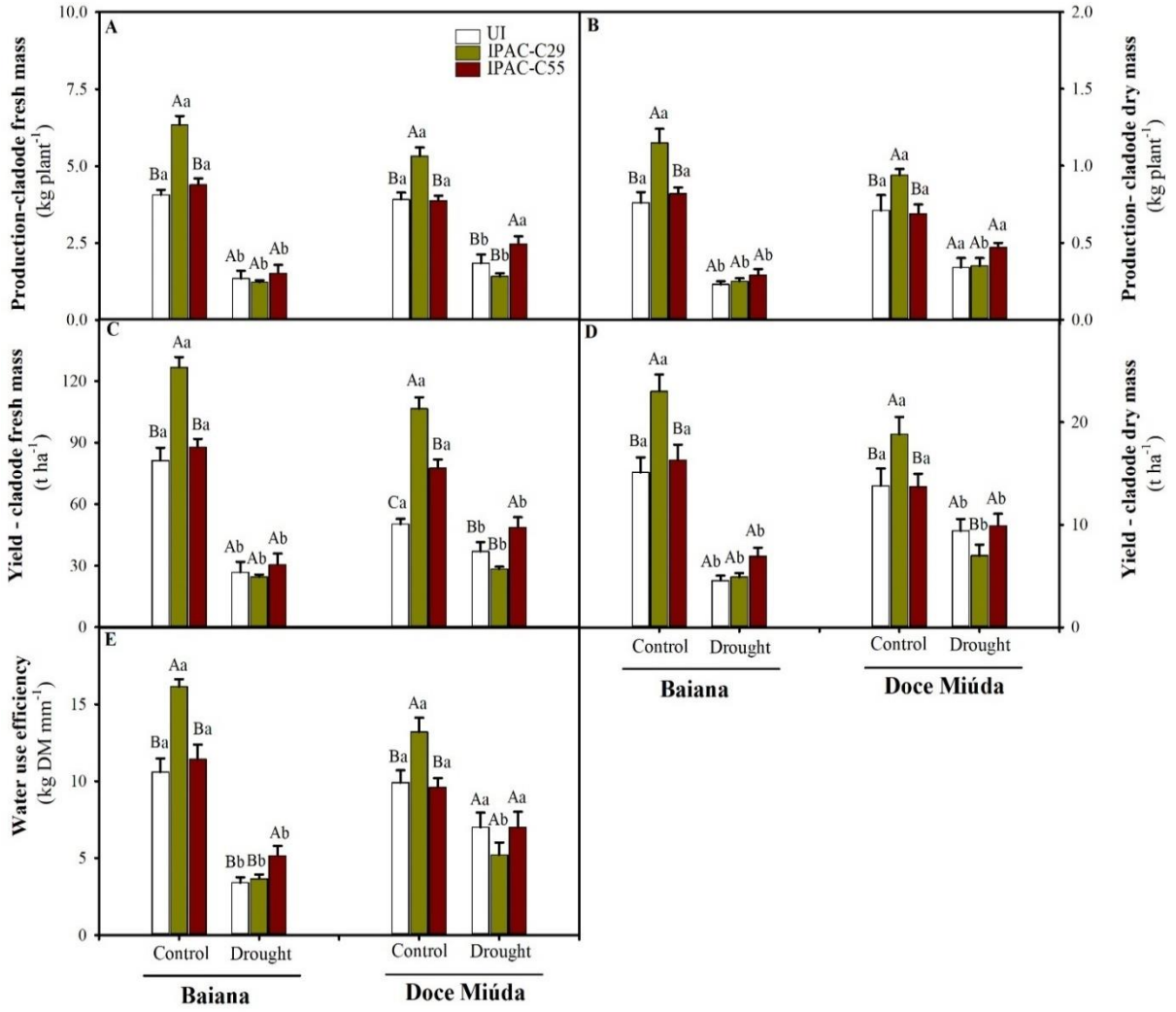


Figure 24 - Production (A, B), yield (C, D) of fresh and dry biomass of cladodes, and water use efficiency (E) of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation treatments [uninoculated (UI), inoculation with *Bacillus subtilis* strain (IPACC29) and *Paenibacillus* sp. (IPACC55)] and irrigation intervals [control (irrigation every 7 days) and drought (irrigation every 30 days)], 108 days after drought imposition under field conditions. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to irrigation intervals (Control and Drought), using Scott-Knott test ($p \leq 0.05$).

6.3.3 Water status and drought tolerance

In Baiana variety, cladode succulence was not significantly altered by inoculation treatments or water regimes (Figure 25A). In contrast, in Doce Miúda under drought, inoculation with IPACC55 resulted in highest succulence compared to other treatments (Figure 25A). The cladode temperature was unaltered by inoculation and drought treatments, except Doce Miúda from UI treatment that showed increased values under drought stress compared to

well-watered control, without significant differences among inoculation treatments (Figure 25B). The negative impacts of drought on cladode dry mass accumulation caused a decrease in relative drought tolerance (RTd) across all inoculation treatments in both varieties, and the damages were more pronounced in IPACC29 inoculated plants (Figure 25C).

Under well-watered conditions, inoculation with IPACC29 provided higher plant accumulated water (PAW) in both varieties (Figure 25D). Drought stress significantly decreased PAW in both varieties; however, the effects were less severe in Doce Miúda plants inoculated with IPACC55, which exhibited the highest value under these conditions (Figure 25D).

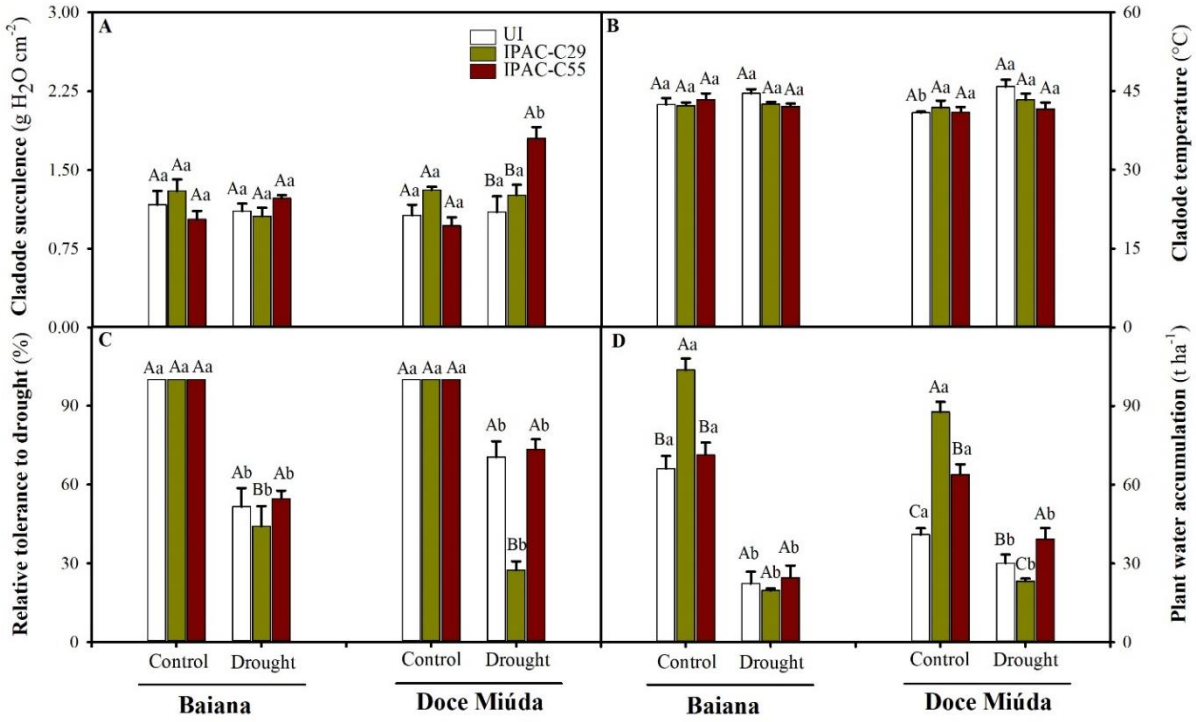


Figure 25 - Cladode succulence (A), cladode temperature (B), relative tolerance to drought (C), and plant accumulation water (D) of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation treatments [uninoculated (UI), inoculation with *Bacillus subtilis* strain (IPACC29) and *Paenibacillus* sp. (IPACC55)] and irrigation intervals [control (irrigation every 7 days) and drought (irrigation every 30 days)], 108 days after drought imposition under field conditions. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to irrigation intervals (Control and Drought), using Scott-Knott test ($p \leq 0.05$).

6.3.4 Photosynthetic pigments

For Baiana variety under well-watered conditions, the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl total) and carotenoids were similar across all

inoculation treatments (Figure 26). In contrast, in Doce Miúda variety, inoculation with IPACC29 increased the contents of Chl *a*, Chl total and carotenoids compared to other treatments (Figures 26A, C, D).

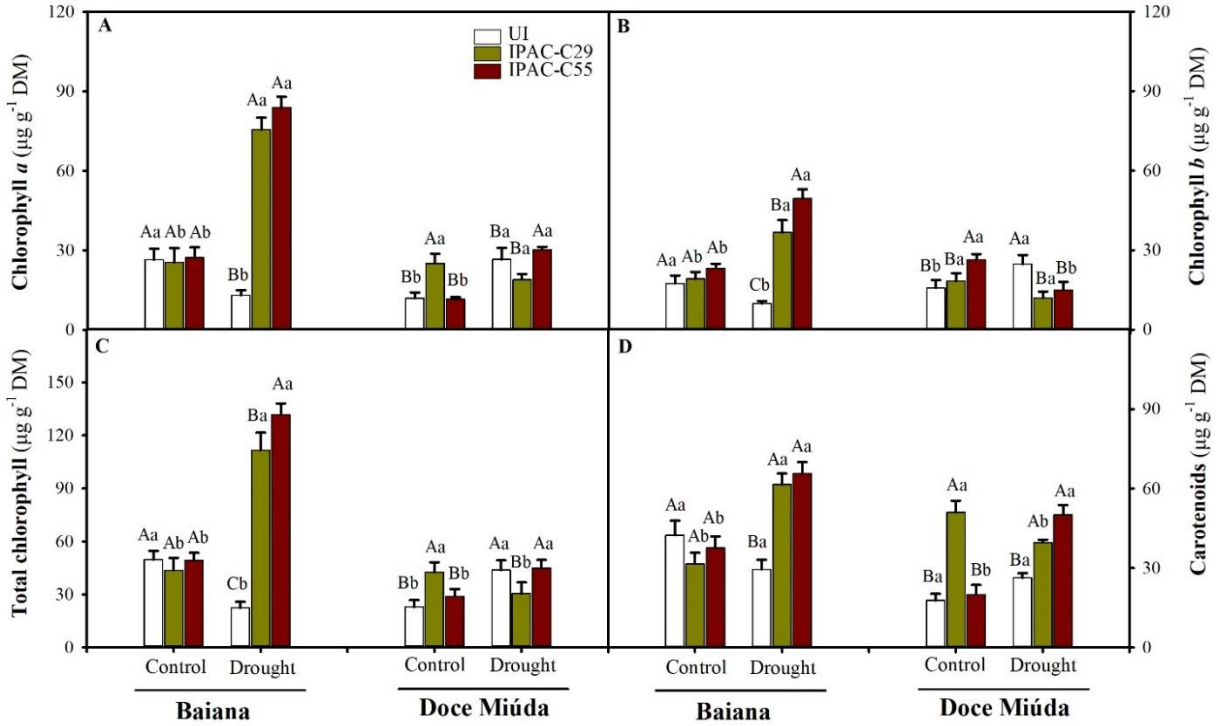


Figure 26 - Content of chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll total (C) and carotenoids (D) of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation treatments [uninoculated (UI), inoculation with *Bacillus subtilis* strain (IPACC29) and *Paenibacillus* sp. (IPACC55)] and irrigation intervals [control (irrigation every 7 days) and drought (irrigation every 30 days)], 108 days after drought imposition under field conditions. Means followed by different capital letters indicate significant differences due to inoculation treatments, whereas different lowercase letters represent significant differences due to irrigation intervals (Control and Drought), using Scott-Knott test ($p \leq 0.05$).

Under drought stress, the Baiana variety inoculated with IPACC29 and IPACC55 showed significant increases in contents of Chl *a*, Chl *b*, Chl total and carotenoids compared to well-watered control (Figure 26). On the other hand, drought-stressed Doce Miúda plants showed significant increase in Chl *a*, Chl total, and carotenoids as compared to well-watered control (Figures 26A, C, D). Overall, drought-stressed plants inoculated with IPACC55 exhibited the highest contents of photosynthetic pigments in both varieties, with few exceptions. Notably, in Baiana, plants inoculated with IPACC55 and IPACC29 showed the highest contents of photosynthetic pigments compared to UI treatment under drought (Figure 26). The highest Chl *b* contents were observed in Baiana plants inoculated with IPACC55 and

UI in Doce Miúda plants (Figure 26B). Under drought, the highest carotenoids contents were recorded in plants inoculated with IPACC55 and IPACC29 in both varieties, particularly in Baiana (Figure 26D).

6.3.5 Principal Component Analysis

Principal Component Analysis (PCA) was designed to investigate the correlations within each variety of cactus pear (Doce Miúda and Baiana) and the parameters that best separate the strains regarding drought tolerance (Figure 27). The data indicated that the evaluated parameters were sufficient to reveal correlations and the multiple effects of treatments combining bacterial inoculation and irrigation intervals on both cactus pear varieties.

In Doce Miúda, PCA explained 84.8% of the total variation, with the first and second components accounting for 66.2 and 18.6%, respectively (Figure 27A). The bacterial strains clustered distinctly according to irrigation intervals, forming four groups (Figures 27A, B). Under well-watered conditions (control), the IPACC29 strain clustered (Group III) separately from the IPACC55 strain and UI treatment (Group IV), showing a strong correlation with most biometric parameters, biomass, and water status. Under drought stress, the IPACC55 strain (Group I) was prominent, increasing the contents of photosynthetic pigments (Chl *a*, total Chl, and carotenoids) and cladode succulence, thereby demonstrating its ability to modulate physiological responses in cactus pear. In contrast, the IPACC29 strain clustered with the UI treatment (Group II), which was associated with higher cladode temperature.

For Baiana variety, PCA explained 87.5% of total variation, with the first and second components contributing 66.6 and 20.9%, respectively (Figure 27C). Three groups were formed (Figures 27C, D). Under well-watered conditions, no separation was observed between the strains (IPACC29 and IPACC55) and UI treatment (Group I). This group was strongly correlated with the most biometric parameters, biomass and water status, particularly in association with the IPACC29 strain. Conversely, under drought, the IPACC29 and IPACC55 strains clustered together (Group III), promoting higher accumulation of photosynthetic pigments (Chl *a*, Chl *b*, total Chl, and carotenoids), with IPACC55 strain showing greater prominence. The findings highlight the ability of studied strains to modulate the physiological responses of cactus pear under water deficit. Group II, formed by UI treatment, was associated with higher cladode temperature.

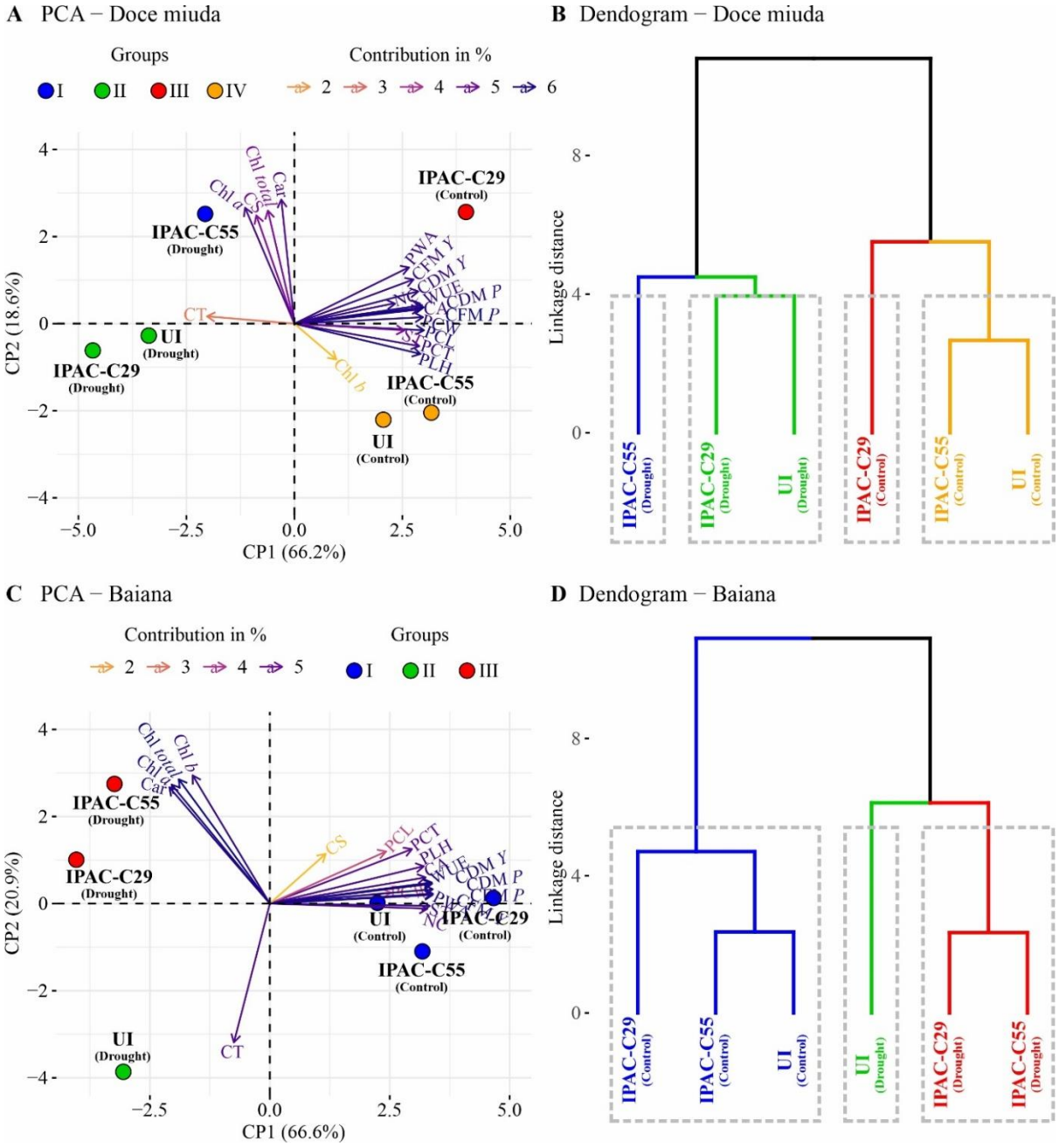


Figure 27 - Principal Component Analysis (PCA) (A, C) and dendrogram (B, D) of cactus pear (Baiana and Doce Miúda varieties) subjected to inoculation treatments [uninoculated (UI), inoculation with *Bacillus subtilis* strain (IPACC29) and *Paenibacillus* sp. (IPACC55)] and irrigation intervals [control (irrigation every 7 days) and drought (irrigation every 30 days)], under field conditions. Variables: plant height (PLH), number of cladode (NC), primary cladode thickness (PCT), primary cladode length (PCL), primary cladode width (PCW), cladode fresh mass production (CFM-P), cladode dry mass production (CDM-P), cladode fresh mass yield (CFM-Y), cladode dry mass yield (CDM-Y), cladode succulence (CS), stress tolerance (ST), cladode temperature (CT), plant accumulation water (PWA), water use efficiency (WUE), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl total) and carotenoids (Car).

Overall, PCA highlighted the ability of IPACC29 and IPACC55 strains to modulate performance of cactus pear varieties under drought stress. The dendrograms (Figures 27B, D) confirmed PCA groupings, highlighting the interaction between bacterial inoculation and irrigation intervals in both varieties.

6.4 Discussion

6.4.1 PGPB inoculation enhances biomass accumulation and water storage in cactus pear under well-watered conditions

In the present study, inoculation with plant growth-promoting bacteria (PGPB), particularly the IPACC29 strain, promoted significant improvements in growth and yield of cactus pear varieties, especially under well-watered conditions (Figures 23, 24, 25). These data indicate that applying 8.5 mm of water every seven days was sufficient to prevent drought damage, representing an optimal management strategy for cactus pear cultivation, as supported by irrigation records and cumulative water depth throughout the experiment (Figure 22A, C). This argument is consistent with previous studies demonstrating that adequate water availability is a key factor for maximizing cactus pear productivity, even in species adapted to dry environments (Scalisi *et al*., 2016; Pereira *et al*., 2021; Miranda *et al*., 2023a).

The positive interaction between bacterial inoculation and irrigation management resulted in increased fresh and dry biomass, higher cladode succulence, and greater water accumulation in plant tissues, which are critical traits for forage production under water-limited environments. This response is particularly relevant considering the importance of cactus pear as a strategic feed resource for livestock in semiarid regions. The ability of PGPB to enhance carbon assimilation, nutrient uptake, and water storage reinforces their potential as biotechnological tools for crops with crassulacean acid metabolism (CAM), such as cactus pear. However, the benefits of inoculation were more pronounced under improved water availability, suggesting that microbial inoculants maximize plant performance primarily when water supply is not severely limiting.

6.4.2 Microbial inoculants activate physiological responses but are insufficient to overcome severe water restriction in cactus pear

Although cactus pear is inherently adapted to semiarid environments through CAM metabolism, severe drought markedly constrained growth, cladode development and biomass accumulation, regardless of inoculation treatments (Figures 23, 24, 27). Under prolonged water limitation, the magnitude of stress likely exceeded the buffering capacity of CAM-related carbon fixation, restricting nocturnal CO_2 assimilation and limiting carbon supply for growth (Lahbouki *et al*., 2022; Miranda *et al*., 2023a). While inoculation with PGPB strains, particularly IPACC55, activated important physiological adjustments, these responses were not sufficient to sustain biomass production under severe drought.

Under drought, inoculating Doce Miúda plants with IPACC55 resulted in highest fresh and dry cladode biomass (Figures 24A, B), that reflected in higher yield (fresh mass) (Figure 24C), greater succulence (Figures 25A, 27A), water-use efficiency (Figure 24E) and plant accumulated water (Figure 25D). The modulation of osmotic balance through increased succulence suggests improved cellular water retention, a key trait in CAM plants. These responses may be linked to bacterial-mediated production of phytohormones such as indole-3-acetic acid (Vurukonda *et al*., 2016; Ali; Khan, 2021; Aquino *et al*., 2021; Chieb; Gachomo, 2023), which stimulates root growth and enhances water uptake, as well as to the synthesis de exopolysaccharides and biofilm formation, improving rhizosphere water retention. The data also suggest that increase in succulence induced by IPACC55 was related to greater water accumulation in plants (Figure 25D), which likely contributed to the maintenance of cladode number, area, thickness, biomass, and temperature even under drought conditions (Figures 23, 24, 25). Notably, plants inoculated with IPACC55 exhibited higher carotenoids contents compared with UI treatment (Figures 26D, 27A), highlighting the role of PGPB in inducing antioxidant defense mechanisms (Vurukonda *et al*., 2016; Batool *et al*., 2020). The inoculation benefits were not observed in Baiana, suggesting a variety-depending reprograming response for cactus pear.

Several studies have also reported that PGPB application can significantly increase photosynthetic pigment content under drought stress (Batool *et al*., 2020; Salem *et al*., 2024). In agreement, our results revealed a significant increase in Chl *a*, Chl *b*, Chl total and carotenoids contents in both evaluated varieties (Figure 26), reinforcing the role of PGPB in activating defense mechanisms that enhance drought tolerance (Vurukonda *et al*., 2016; Batool *et al*., 2020). In Baiana variety, the IPACC55 and IPACC29 strains markedly increased the

accumulation of photosynthetic pigments (Figures 26, 27C), a response clearly distinct from that observed in UI plants. This accumulation likely represents a defense response to maximize photosynthetic efficiency and protect tissues against oxidative damage (Abdallah *et al*., 2017; Singh *et al*., 2024). Among these pigments, carotenoids play a particularly important role due to their antioxidant function, helping to mitigate oxidative stress caused by prolonged drought exposure (Batra; Sharma; Kumari, 2014; Dhami; Cazzonelli, 2020).

In summary, cactus pear plants have shown a persistence of limited biomass accumulation under drought highlighting that metabolic and physiological adjustments were insufficient to fully offset the constraints imposed by severe water deficit. Under extreme conditions, restrictions on carbon assimilation, energy metabolism, and cell expansion prevail over PGPB-induced benefits. Therefore, although microbial inoculants effectively activate stress-responsive pathways in CAM plants, their capacity to enhance drought tolerance is strongly dependent on stress intensity. These findings reinforce that the agronomic benefits of PGPB in cactus pear are maximized under moderate water availability, where improved metabolic efficiency can be translated into increased growth and forage yield.

6.5 Conclusion

Low water availability markedly constrained the performance of Doce Miúda and Baiana cactus pear, resulting in limited growth, biomass accumulation and yield, despite the CAM metabolism. Inoculating with plant growth-promoting bacteria (PGPB) triggered physiological responses under drought such as modulation of photosynthetic pigments, improved tissue hydration, and activation of stress-related pathways, but these adjustments were insufficient to sustain biomass production under severe drought.

The IPACC29 markedly increased plant performance, promoting greater fresh and dry biomass, increased cladode succulence and higher water storage capacity under well-watered condition, highlighting the potential of PGPB as biotechnological tools for CAM species. The IPACC29 and IPACC55 represent promising inoculants for cactus pear cultivation, but the agronomic effectiveness is strongly dependent on water availability, with maximal benefits occurring under moderate irrigation. These findings emphasize the need to integrate microbial inoculation with optimized water management to enhance productivity and resilience in semiarid agroecosystems.

REFERENCES

ABDALLAH, M. A.; METHENNI, K.; NOUAIRI, I.; ZARROUK, M.; YOUSSEF, N. B. Drought priming improves subsequent more severe drought in a drought-sensitive cultivar of olive cv. Chétoui. **Scientia Horticulturae**, v. 221, p. 43-52, 2017.

ALI, S.; KHAN, N. Delineation of mechanistic approaches employed by plant growth promoting microorganisms for improving drought stress tolerance in plants. **Microbiological Research**, v. 249, p. 126771, 2021.

ALLEN, R.G., PEREIRA, L.S., RAES, D., SMITH, M. **Crop evapotranspiration: guidelines for computing crop water requirements.** Rome: FAO Irrigation and Drainage (Paper 56). 1998. 300 p.

ALVARES, C. A.; STAPE, J. L.; SENTELHAS, P. C.; GONÇALVES, J. L. M.; SPAROVE, G. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, p. 711-728, 2013.

ANDRADE, L. A.; SANTOS, C. H. B.; FREZARIN, E. T.; SALES, L. R.; RIGOBELO, E. C. Plant growth-promoting rhizobacteria for sustainable agricultural production. **Microorganisms**, v. 11, p. 1088, 2023.

ANTUNES, J. E. L.; LYRA, M. C. C. P.; OLLERO, F. J.; FREITAS, A. S.; OLIVEIRA, L. M. S.; ARAÚJO, A. S. F.; FIGUEIREDO, M. V. B. Diversity of plant growth promoting bacteria associated with sugarcane. **Genetics and Molecular Research**, v. 16, p. 16029662, 2017.

AQUINO, J. P. A.; ANTUNES, J. E. L.; BONIFÁCIO, A.; ROCHA, S. M. B.; AMORIM, M. R.; ALCÂNTARA NETO, F.; ARAÚJO, A. S. F. Plant growth-promoting bacteria improve growth and nitrogen metabolism in maize and sorghum. **Theoretical and Experimental Plant Physiology**, v. 33, p. 249-260, 2021.

AQUINO, J. P. A.; NETO, V. P. C.; ANDRADE, M. R. S.; OLIVEIRA, L. M. S.; ANTUNES, J. E. L.; FREITAS, A. S.; NETO, F. A.; ARAÚJO, A. S. F. Plant growth-promoting bacteria increase the yield of green maize and sweet sorghum. **Journal of Plant Nutrition**, v. 46, p. 58-68, 2022.

ARAÚJO JÚNIOR, G. N.; SILVA, T. G. F.; SOUZA, L. S. B.; ARAÚJO, G. G. L.; MOURA, M. S. B.; ALVES, C. P.; SALVADOR, K. R.; SILVA, C. A.; MONTENEGRO, A. A. A.; SILVA, M. J. Phenophases, morphophysiological indices and cutting time in clones of the forage cacti under controlled water regimes in a semiarid environment. **Journal of Arid Environments**, v. 190, p. 104510, 2021.

ARAÚJO JÚNIOR, G. N.; SOUZA, L. S. B.; JARDIM, A. M. R. F.; SOUZA, C. A. A.; ALVES, C. P.; SILVA, M. C.; FREITAS, E. V.; NETO, A. J.; BRUNINI, O.; MORAIS, J. E. F.; NETO, J. F.; SILVA, T. G. F. Forage cactus clones suggested for cultivation in arid areas of the world: agronomic performance and water requirement by phenophase. **Irrigation Science**, v. 43, p. 1169-1195, 2025.

BATOOL, T.; ALI, S.; SELEIMAN, M. F.; NAVEED, N. H.; ALI, A.; AHMED, K.; ABID, M.; RIZWAN, M.; SHAHID, M. R.; ALOTAIB, M.; AL ASHKAR, I.; MUBUSHAR, M.

Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. **Scientific Reports**, v. 10, p. 16975, 2020.

BATRA, N. G.; SHARMA, V.; KUMARI, N. Drought-induced changes in chlorophyll fluorescence, photosynthetic pigments, and thylakoid membrane proteins of *Vigna radiata*. **Journal of Plant Interactions**, v. 9, p. 712-721, 2014.

BITTENCOURT, P. P.; ALVES, A. F.; FERREIRA, M. B.; SILVA, I. L. E. S.; PINTO, V. B.; OLIVARES, F. L. Mechanisms and applications of bacterial inoculants in plant drought stress tolerance. **Microorganisms**, v. 11, p. 502, 2023.

CASTRO, I. N.; SANTOS, S. R.; SANTOS, M. R.; OLIVEIRA, P. M.; ASPIAZÚ, I.; GODINHO, F. L. Root distribution of cactus pear genotypes under different soil water replacement levels. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 25, p. 819-825, 2021.

CAVALCANTE, L. A.; SANTOS, G. R. A.; SILVA, L. M.; FAGUNDES, J. L.; SILVA, M. A. Respostas de genótipos de palma forrageira a diferentes densidades de cultivo. **Pesquisa Agropecuária Tropical**, v. 44, p. 424-433, 2014.

CHIEB, M.; GACHOMO, E. W. The role of plant growth promoting rhizobacteria in plant drought stress responses. **BMC Plant Biology**, v. 23, p. 407, 2023.

CORTÁZAR, V.; NOBEL, P. S. Prediction and measurement of high annual productivity for *Opuntia ficus-indica*. **Agricultural and Forest Meteorology**, v. 56, p. 261-272, 1991.

DAUALA, G. A.; FONSECA, B. S. F. D.; SOUSA, A. B. D.; PAULA-MARINHO, S. O.; MORAIS, P. G. C.; LEITE, M. R. L.; SILVA, R. F.; AVIZ, R. O.; DUARTE, M. H. F.; … NETO, F. A.; MIRANDA, R. S. Plant growth-promoting bacteria modulate metabolism and nitrogen accumulation to counteract drought damage in cactus pear plants. **Scientific Reports**, v. 15, p. 36432, 2025.

DHAMI, N.; CAZZONELLI, C. I. Environmental impacts on carotenoid metabolism in leaves. **Plant Growth Regulation**, v. 92, p. 455-477, 2020.

DUBEUX JR., J. C. B.; SANTOS, M. V. F.; CUNHA, M. V.; SANTOS, D. C.; SOUZA, R. T. A.; MELLO, A. C. L.; SOUZA, T. C. Cactus (*Opuntia* and *Nopalea*) nutritive value: a review. **Animal Feed Science and Technology**, v. 275, p. 114890, 2021.

EDVAN, R. L.; BARROS, D. M.; NASCIMENTO, R. R.; ARAÚJO, M. J.; SOUSA, H. R.; NERIS, L. M. L.; BEZERRA, L. R.; SILVA-FILHO, E. C. Potential use of hydrogel from natural fiber in the initial growth of *Nopalea cochenillifera* under water stress: water source of slow release. **Communications in Soil Science and Plant Analysis**, v. 54, p. 3063-3075, 2023.

EDVAN, R. L.; CARNEIRO, M. S. S. **Palma forrageira: cultivo e uso na alimentação animal**. Appris: Curitiba, Brazil, 2019. 93 p.

EDVAN, R. L.; MOTA, R. R. M.; DIAS, S. T. P.; NASCIMENTO, R. R.; SOUSA, S. V.; SILVA, A. L.; ARAÚJO, J. S. Resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. **Scientific Reports**, v. 10, p. 10040, 2020.

FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Brazilian Journal of Biometrics**, v. 37, p. 529-535, 2019.

GILLER, K. E.; JAMES, E. K.; ARDLEY, J.; UNKOVICH, M. J. Science losing its way: examples from the realm of microbial N_2-fixation in cereals and other non-legumes. **Plant and Soil**, v. 511, p. 1-24, 2025.

GUPTA, A.; MISHRA, R.; RAI, S.; BANO, A.; PATHAK, N.; FUJITA, M.; HASANUZZAMAN, M. Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. **International Journal of Molecular Sciences**, v. 23, p. 3741, 2022.

IILYAS, M.; NISAR, M.; KHAN, N.; HAZRAT, A.; KHAN, A. H.; HAYAT, K.; FAHAD, S.; KHAN, A.; ULLAH, A. Drought tolerance strategies in plants: a mechanistic approach. **Journal of Plant Growth Regulation**, v. 40, n. 3, p. 926-944, 2021.

KHAN, I.; ULLAH, N.; MAQBOOL, H.; HASHMI, A.; KHAN, B.; KHALID, A.; NAZ, I.; PARKER, N. P.; MUNIS, M. F. H.; CHAUDHARY, H. J. Holistic review on *Paenibacillus*: the multifunctional genus in heavy metal detoxification, pesticide degradation, and abiotic stress mitigation for sustainable agriculture. **Plant and Soil**, v. 518, p. 1245-1280, 2026.

LAHBOUKI, S.; BEN-LAOUANE, R.; ANLI, M.; BOUTASKNIT, A.; AIT-RAHOU, Y.; AIT-EL-MOKHTAR, M.; EL GABARDI, S.; DOUIRA, A.; WAHBI, S.; OUTZOURHIT, A. Arbuscular mycorrhizal fungi and/or organic amendment enhance the tolerance of prickly pear (*Opuntia ficus-indica*) under drought stress. **Journal of Arid Environments**, v. 199, p. 104703, 2022.

LAHBOUKI, S.; FERNANDO, A. L.; RODRIGUES, C.; BEN-LAOUANE, R.; AIT-EL-MOKHTAR, M.; OUTZOURHIT, A.; MEDDICH, A. Effects of humic substances and mycorrhizal fungi on drought-stressed cactus: focus on growth, physiology, and biochemistry. **Plants**, v. 12, p. 4156, 2023.

MANTOVANI, A. A method to improve leaf succulence quantification. **Brazilian Archives of Biology and Technology**, v. 42, p. 9 -14, 1999.

MIRANDA, J. R.; FURTADO, D. A.; SILVA, V. C.; NETO, D. J.; SOUZA, J. T. A.; ARAÚJO, J. S. Physiology of the forage cactus cultivar *Opuntia stricta* (Haw.) Haw. under different irrigation frequencies in the semiarid. **Revista Ceres**, v. 70, p. 24-31, 2023a.

MIRANDA, R. S.; FONSECA, B. S. F.; PINHO, D. S.; BATISTA, J. Y. N.; BRITO, R. R.; SILVA, E. M.; FERREIRA, W. S.; COSTA, J. H.; LOPES, M. S.; SOUSA, R. H. B.; NEVES, L. F.; PENHA, J. A. F.; SANTOS, A. S.; LIMA, J. J. P.; PAULA-MARINHO, S. O.; ALCÂNTARA NETO, F.; AGUIAR, E. S.; SANTOS, C. P.; GOMES-FILHO, E. Selection of soybean and cowpea cultivars with superior performance under drought using growth and biochemical aspects. **Plants**, v. 12, p. 3134, 2023b.

NEFZAOUI, A. *Opuntia ficus-indica* productivity and ecosystem services in arid areas. **Italus Hortus**, v. 25, p. 29-39, 2018.

NETO, D. J.; MATOS, R. M.; SILVA, P. F.; LIMA, A. S.; AZEVEDO, C. A.; SABOYA, L. M. Growth and yield of cactus pear under irrigation frequencies and nitrogen fertilization. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 24, p. 664-671, 2020.

PEREIRA, J. D. S.; CAVALCANTE, A. B.; SIMÕES, W. L.; VOLTOLINI, T. V. Forage yield, structural responses and chemical composition of spineless cactus 'Orelha de Elefante Mexicana' in different water depths and irrigation frequencies. **Ciência Rural**, v. 51, p. e20200324, 2021.

R CORE TEAM. **R:** a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2016. Disponível em: http://www.R-project.org. Acesso em: nov. 2025.

RAMOS, J. P. F.; MACÊDO, A. J. S.; SANTOS, E. M.; EDVAN, R. L.; SOUSA, W. H.; PERAZZO, A. F.; SILVA, A. S.; CARTAXO, F. Q. Forage yield and morphological traits of cactus pear genotypes. **Acta Scientiarum Agronomy**, v. 43, p. 51214, 2021.

SALEM, M. A.; ISMAIL, M. A.; RADWAN, K. H.; ABD-ELHALIM, H. M. Unlocking the potential of plant growth-promoting rhizobacteria to enhance drought tolerance in Egyptian wheat (*Triticum aestivum*). **Sustainability**, v. 16, p. 4605, 2024.

SCALISI, A.; MORANDI, B.; INGLESE, P.; BIANCO, R. L. Cladode growth dynamics in *Opuntia ficus-indica* under drought. **Environmental and Experimental Botany**, v. 122, p. 158-167, 2016.

SELEIMAN, M. F.; AL-SUHAIBANI, N.; ALI, N.; AKMAL, M.; ALOTAIBI, M.; REFAY, Y.; DINDAROGLU, T.; ABDUL-WAJID, H. H.; BATTAGLIA, M. L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. **Plants**, v. 10, p. 259, 2021.

SINGH, D.; THAPA, S.; SINGH, J. P.; MAHAWAR, H.; SAXENA, A. K.; SINGH, S. K.; MAHLA, H. R.; CHOUDHARY, M.; PARIHA, M.; CHOUDHARY, K. B.; CHAKDA, H. Prospecting the potential of plant growth-promoting microorganisms for mitigating drought stress in crop plants. **Current Microbiology**, v. 81, p. 81-84, 2024.

SOUSA, A. B.; LEITE, M. R. L.; DAUALA, G. A.; MORAIS, P. G. C.; PAULA-MARINHO, S. O.; MONTEIRO, G. N.; DUARTE, M. H. F.; OLIVEIRA, R. I.; SILVA, R. F.; AVIZ, R. O.; MIRANDA, R.; SOUZA; BOECHAT, C. L.; ARAÚJO, A. S. F.; EDVAN, R. L.; COSTA, E. M. *Bacillus* and *Paenibacillus* strains enhance nitrogen metabolism and increase biomass production of *Nopalea cochenillifera* grown in semi-arid soil. **Journal of Soil Science and Plant Nutrition**, v. 25, p. 10782-10798, 2025.

SOUSA, D. M. G.; LOBATO. E. **Cerrado: Correção do Solo e Adubação**. Brasília: Embrapa Informação Tecnológica, 2004. 416 p.

VURUKONDA, S. S. K. P.; VARDHARAJULA, S.; SHRIVASTAVA, M.; ALI, S. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. **Microbiological Research**, v. 184, p. 13-24, 2016.

WANG, D. D.; ZHAO, W.; REYILA, M.; HUANG, K. C.; LIU, S.; CUI, B. K. Diversity of microbial communities of *Pinus sylvestris* var. *mongolica* at spatial scale. **Microorganisms**, v. 10, p. 371, 2022.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of Plant Physiology**, v. 144, p. 307-313, 1994.

7 CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Os resultados obtidos demonstram que a intensidade dos danos causados pelo déficit hídrico no desempenho da palma forrageira depende do ambiente de cultivo. Em todos os casos, o déficit hídrico afeta negativamente os processos fisiológicos, bioquímicos e o crescimento de plantas. Esses efeitos são mais pronunciados em condições de campo para ambas as variedades estudadas, Doce Miúda e Baiana, com reduções marcantes no acúmulo de biomassa e na produtividade de cladódios.

Nos ensaios em casa de vegetação, a estirpe IPACC29 destacou-se como a mais promissora para inoculação em palma forrageira, ativando importantes respostas de defesa, especialmente relacionadas ao acúmulo de nitrogênio e compostos orgânicos, o que resultou em maior desenvolvimento radicular e crescimento das plantas. Os dados sugerem que metabólitos como ácido cítrico, ácido málico, ácido 4-aminobutírico, inositol, sacarose, frutose, ácido piroglutâmico, glicina e prolina estão associados à tolerância ao estresse. As vias de defesa induzidas pela IPACC29 foram associadas ao (i) aumento dos teores de nitrogênio, que estimulou o acúmulo de solutos compatíveis como prolina, carboidratos solúveis, proteínas solúveis e ácidos orgânicos, contribuindo para o melhor equilíbrio hídrico; (ii) maior capacidade antioxidante, pela ação coordenada de antioxidantes enzimáticos (SOD e APX) e não enzimáticos (prolina, proteínas solúveis e ácidos orgânicos), resultando em menores níveis de H_2O_2 e peroxidação lipídica; e (iii) regulação de metabólitos-chave envolvidos no ajuste osmótico e balanço energético.

Em condições de campo, os benefícios da inoculação com IPACC29 foram mais evidentes sob disponibilidade hídrica adequada (8,5 mm a cada 7 dias), com incremento no desempenho das plantas de ambas as variedades, refletido no maior acúmulo de água e biomassa fresca e seca dos cladódios, característica relevante para a alimentação animal. Esse benefício, entretanto, não ficou evidente sob déficit hídrico severo (8,5 mm a cada 30 dias), pois, embora as plantas inoculadas com as estirpes IPACC29 e IPACC55 tenham ativado respostas de defesa relevantes, os ajustes não foram suficientes para aumentar a tolerância ao déficit hídrico, pelo menos durante o período avaliado.

Os dados demonstram que a inoculação com BPCPs constitui estratégia promissora para o manejo da palma forrageira em ambientes sujeitos à limitação hídrica. O uso estratégico de estirpes bacterianas poderá contribuir para maior resiliência da cultura em condições adversos, com potencial redução do uso de fertilizantes químicos e aumento da sustentabilidade agrícola. Esses achados fornecem subsídios para o desenvolvimento de ferramentas biotecnológicas

sustentáveis voltadas ao cultivo dessa cultura em regiões semiáridas, especialmente no semiárido brasileiro.

Mais estudos são necessários para investigar o papel das BPCPs no desempenho produtivo da palma forrageira em longo prazo (acima de dois anos de cultivo), bem como seu impacto na qualidade nutricional e regulação a nível molecular, incluindo a expressão gênica. Esses avanços poderão ampliar o conhecimento sobre o potencial da estirpe IPACC29 como agente mitigador do déficit hídrico e regulador de crescimento da cultura, tanto em condições controladas quanto em campo, visando a alimentação animal e resiliência de sistemas produtivos.

Adicionalmente, para estudos futuros, recomenda-se a incorporação da curva de retenção de água do solo (CRA), a fim de estimar o potencial matricial (Ψm) correspondente aos níveis de umidade de 25 e 50% da capacidade de campo (CC), bem como caracterizar a umidade do solo ao longo do perfil, tanto em ensaios de casa de vegetação quanto em campo. Também se sugere a realização de análises econômicas para avaliar a viabilidade dos custos de irrigação na cultura da palma forrageira.

APPENDICES

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Plant growth-promoting bacteria modulate metabolism and nitrogen accumulation to counteract drought damage in cactus pear plants

Gonçalves Albino Dauala[1], Bruno Sousa Figueiredo da Fonseca[2], Acacyara Batista de Sousa[1], Stelamaris de Oliveira Paula-Marinho[3], Pâmalla Graziely Carvalho Morais[1], Marcos Renan Lima Leite[3,4], Rodrigo Fonseca da Silva[1], Rhaiana Oliveira de Aviz[4], Maria Helena Ferreira Duarte[1], Romilda Rodrigues Nascimento[5], Elaine Martins da Costa[1], Cácio Luiz Boechat[1], Ademir Sérgio Ferreira de Araújo[1,4], Renato Oliveira de Sousa[1], José Hélio Costa[3], Ricardo Loiola Edvan[6], Francisco de Alcântara Neto[4,7] & Rafael de Souza Miranda[1,7]✉

Searching sustainable management techniques to enhance plant resistance to drought is crucial for agriculture in stressful scenarios. This study aimed to investigate the role of Plant Growth-Promoting Bacteria (PGPB) strains on forage cactus pear performance under drought. Trials were conducted in greenhouse using forage cactus plants in randomized block experimental design, 8×2 factorial scheme, consisting of eight inoculation treatments [inoculation with *Paenibacillus* sp. IPACC38 and IPACC55, and *Bacillus subtilis* IPACC29 strains combined with and without nitrogen (N) fertilization] and two water regimes [control at 50% field capacity (FC) and drought at 25% FC]. Drought significantly decreased plant growth across all treatments, regardless of N/inoculation treatment. The best performance under drought was observed in plants grown without N and inoculated with IPACC29 and IPACC38 strains. Improved performance under drought was closely related to PGPB's capacity to provide N accumulation for strategic photosynthetic pigments content, resulting in better root growth and plant development. Our findings reveal that IPACC29 and IPACC38 are the most promising PGPB strains for inoculating forage cactus pear, activating important defense responses against drought. This system offers alternative for cultivating cactus under water restriction and reduced N availability to increase green forage biomass production in dry regions.

Keywords Nitrogen availability, *Opuntia cochenillifera*, Semiarid, Stress tolerance, PGPB inoculation, Water deficit

Drought is a global phenomenon that affects crops growth and productivity in arid and semi-arid ecosystems worldwide, due to low rainfall and high temperatures throughout a year[1,2]. Water limitation negatively influences biochemical and physiological processes in plants, depending on intensity and duration of stress, well as plant developmental stage, genotypes and species[3–5].

[1]Postgraduate Program in Agricultural Sciences, Campus Professora Cinobelina Elvas, Federal University of Piauí, 64900-000 Bom Jesus, Brazil. [2]Agronomic Engineering Course, Campus Professora Cinobelina Elvas, Federal University of Piauí, 64900-000 Bom Jesus, Brazil. [3]Department of Biochemistry and Molecular Biology, Federal University of Ceará, 60440-554 Fortaleza, Brazil. [4]Postgraduate Program in Agronomy, Campus Ministro Petrônio Portella, Federal University of Piauí, 64048-550 Teresina, Brazil. [5]Department of Animal Science, Federal University of Campina Grande, 58708-110 Campina Grande, Brazil. [6]Department of Animal Science, Federal University of Piauí, 64048-550 Teresina, Brazil. [7]Department of Plant Science, Federal University of Piauí, 64048-550 Teresina, Brazil. ✉email: rsmiranda@ufpi.edu.br

Appendix 1 – Paper published in Scientific Reports journal.

Appendix 2 - Chemical analysis of soil collected the 0-20 cm layer for growing cactus pear plants in greenhouse

pH	Ca	Mg	Al	H+Al	K	SB	T	P	V	OM	Clay	Silt	Sand
H_2O	----------$cmol_c\ dm^{-3}$----------							$mg\ dm^{-3}$	%	$g\ kg^{-1}$	$g\ kg^{-1}$		
5.03	1.42	0.35	0.00	0.76	0.29	2.07	2.83	9.00	73.2	11.60	111	50	839

Note: pH – Potential of hydrogen; Ca (calcium), Mg (magnesium), Al (exchangeable acidity), H+Al (potential acidity at pH 7.0), K (potassium), SB (total exchangeable bases), T (cation-exchange capacity at pH 7.0), P (ascilable phosphorus), V (base saturation), and OM (organic matter).

Appendix 3 - Absolute values and statistical details for biometric (plant height; cladode number for plant; cladode total area; root length; thickness, length and width of primary cladode) and biomass parameters (fresh and dry mass of cladodes and roots, and total dry mass) of cactus pear plants (*Nopalea cochenillifera* Salm-Dyck, var. Doce miúda) submitted to no inoculation (UI), and inoculation with *Bacillus subtilis* (IPACC29 – IC29) and *Paenibacillus* sp. (IPACC38 – IC38 and IPACC55 – IC55) strains with 0 or 50% nitrogen, 150 days after imposition to different irrigation levels (control and drought). Means followed by different capital letters (in the same line) indicate significant differences due to inoculation treatment, and different lowercase letters (in the same column) represent significant difference between irrigated condition (control or drought), using Scott-Knott test ($p \leq 0.05$)

Variables	Treatments	UI	IC29	IC38	IC55	UI	IC29	IC38	IC55
		50% nitrogen				0% nitrogen			
		Biometric parameters							
Plant height (cm)	Control	47.70 Aa	49.05 Aa	45.13 Aa	46.63 Aa	42.13 Ba	43.63 Ba	44.73 Ba	46.70 Ba
	Drought	32.93 Bb	34.63 Bb	34.60 Bb	33.68 Bb	34.75 Bb	36.03 Bb	40.18 Ab	35.00 Bb
Cladode number for plant	Control	6.25 Aa	5.50 Aa	6.00 Aa	6.25 Aa	4.25 Ba	4.50 Ba	5.25 Aa	5.50 Aa
	Drought	4.50 Ab	3.25 Ab	4.25 Ab	5.25 Aa	4.75 Aa	4.75 Aa	4.25 Aa	5.50 Aa
Cladode total area (cm^2 per plant)	Control	592.22 Ba	555.71 Ca	582.85 Ba	643.59 Aa	532.65 Ca	493.52 Da	525.41 Ca	483.33 Da
	Drought	405.80 Ab	338.35 Bb	405.34 Ab	438.38 Ab	440.67 Ab	430.85 Ab	424.17 Ab	423.20 Ab
Root lenght (cm)	Control	51.00 Aa	55.75 Aa	46.00 Ba	58.00 Aa	49.67 Ba	60.00 Aa	49.00 Ba	45.25 Ba
	Drought	27.35 Ab	37.65 Ab	31.25 Ab	34.35 Ab	35.92 Ab	39.85 Ab	30.15 Ab	36.82 Ab
Primary cladode thickness (mm)	Control	12.18 Aa	12.29 Aa	11.72 Aa	12.37 Aa	12.65 Aa	12.41 Aa	12.50 Aa	12.61 Aa
	Drought	8.00 Ab	7.53 Ab	8.60 Ab	8.42 Ab	7.99 Ab	8.90 Ab	8.70 Ab	8.34 Ab

Primary cladode lenght (cm)	Control	22.31 Aa	20.69 Aa	21.15 Aa	21.68 Aa	23.70 Aa	22.41 Aa	22.05 Aa	21.16 Aa
	Drought	17.55 Ab	18.20 Ab	17.79 Ab	18.22 Ab	19.08 Ab	19.11 Ab	19.23 Ab	17.70 Ab
Primary cladode width (cm)	Control	9.17 Aa	9.32 Aa	9.09 Aa	9.55 Aa	9.41 Aa	9.22 Aa	9.47 Aa	8.99 Aa
	Drought	7.45 Ab	7.52 Ab	7.44 Ab	7.61 Ab	7.04 Ab	7.77 Ab	7.16 Ab	7.69 Aa
Biomass parameters									
Cladode fresh mass (g per plant)	Control	624.15 Aa	597.66 Ba	619.73 Aa	652.25 Aa	592.36 Ba	570.43 Ba	574.97 Ba	535.02 Ba
	Drought	238.49 Bb	306.83 Ab	276.61 Bb	318.34 Ab	311.24 Ab	340.56 Ab	320.47 Ab	261.95 Bb
Root fresh mass (g per plant)	Control	35.02 Aa	29.20 Ca	30.13 Ca	36.34 Aa	14.28 Da	37.69 Aa	22.38 Ca	18.31 Da
	Drought	8.38 Bb	9.19 Bb	9.61 Bb	7.16 Bb	10.03 Aa	12.01 Ab	15.18 Ab	14.94 Aa
Cladode dry mass (g per plant)	Control	110.61 Aa	110.57 Aa	110.14 Aa	108.31 Aa	103.44 Ba	95.33 Ba	97.61 Ba	101.26 Ba
	Drought	69.61 Bb	62.98 Bb	81.36 Ab	78.17 Ab	81.17 Ab	76.91 Ab	82.74 Ab	73.04 Bb
Root dry mass (g per plant)	Control	10.73 Aa	9.01 Ba	11.72 Aa	10.20 Aa	8.59 Ba	10.81 Aa	8.68 Ba	6.94 Ca
	Drought	2.49 Ab	3.57 Ab	3.67 Ab	3.18 Ab	3.08 Ab	4.17 Ab	2.96 Ab	3.96 Ab
Total dry mass (g per plant)	Control	120.65 Aa	119.59 Aa	125.08 Aa	119.45 Aa	111.48 Ba	112.61 Ba	105.73 Ba	109.65 Ba
	Drought	63.16 Bb	66.32 Bb	78.64 Ab	80.54 Ab	84.75 Ab	80.37 Ab	76.89 Ab	76.74 Ab

Appendix 4 - List of metabolites detected in cactus pear cladodes, including their compound classification, retention time, mass fragment used for relative quantitative, and Kyoto Encyclopedia of Genes and Genomes (KEGG ID)[a] identifier

	Name of metabolite	Compound type	Retention time (min)	Mass fragment (m/z)	Compound ID[a]
1	Pyruvic acid	Organic acid	7.12	174	C00022
2	Oxalic acid	Organic acid	7.32	190	C00209
3	Glycolic acid	Organic acid	7.64	161	C00160
4	Alanine	Amino acid	8.25	116	C00041
5	3-hydroxy Butyric acid	Organic acid	9.36	233	C01089
6	Valine	Amino acid	10.41	144	C00183
7	Serine	Amino acid	11.12	116	C00065
8	Glycine	Amino acid	11.24	174	C00037
9	Isoleucine	Amino acid	11.35	158	C00407
10	Glycerol	Sugar alcohol	11.41	205	C00116
11	Phosphoric acid	Inorganic acid	11.44	314	C00009
12	Proline	Amino acid	11.75	142	C00148
13	Maleic acid	Organic acid	11.85	245	C01384
14	Succinic acid	Organic acid	11.96	247	C00042
15	Glyceric acid	Organic acid	12.29	189	C00258
16	Fumaric acid	Organic acid	12.45	143	C00122
17	Malic acid	Organic acid	14.51	233	C00149
18	Erythritol	Sugar alcohol	14.75	217	C00503
19	Aspartic acid	Amino acid	14.88	202	C00049
20	Pyroglutamic acid	Amino acid	14.95	156	C01879
21	4-amino Butyric acid	Organic acid	15.00	174	C00334
22	Lactic acid dimer	Organic acid	15.12	117	523342*
23	Erythronic acid	Organic acid	15.45	292	2781043*
24	Oxo-Glutaric acid	Organic acid	15.56	198	C00026
25	Lyxose	Carbohydrate	16.63	307	C00476
26	Xylose	Carbohydrate	16.69	160	C00181
27	Arabinose	Carbohydrate	16.87	217	C00216
28	Ribose	Carbohydrate	17.35	217	C00121
29	Ribitol[#]	Sugar alcohol	17.4	319	C00474
30	Valeric acid	Organic acid	17.45	174	C00803
31	Nicotinamide	Vitamin	17.71	193	C00153
32	Citric acid	Organic acid	18.38	273	C00158
33	Dehydroascorbic acid	Organic acid	18.73	173	C00425
34	Adenine	Nitrogen base	18.88	264	C00147
35	Quinic acid	Organic acid	18.85	345	C06746
36	Fructose	Carbohydrate	19.12	307	C02336

	Name of metabolite	Compound type	Retention time (min)	Mass fragment (m/z)	Compound ID[a]
37	Galactose	Carbohydrate	19.23	160	C00984
38	Glucose	Carbohydrate	19.47	160	C00031
39	Glucoheptonic acid	Carbohydrate	19.99	333	101713*
40	Galactonic acid	Carbohydrate	20.47	319	C00880
41	Saccharic acid	Organic acid	20.61	373	C00818
42	Inositol - allo	Sugar alcohol	21.17	305	892*
43	Glucoheptose	Carbohydrate	23.61	259	219662*
44	Sucrose	Carbohydrate	25.73	437	C00089
45	Cellobiose	Carbohydrate	25.89	204	C06422
46	Maltose	Carbohydrate	26.27	204	C00208
47	Trehalose	Carbohydrate	26.52	191	C01083
48	Isomaltose	Carbohydrate	27.45	204	C00252
49	Galactinol	Sugar alcohol	28.4	191	C01697
50	Palatinose	Carbohydrate	29.59	204	C01742
51	Unknown	-	30.59	361	-
52	Melezitose	Carbohydrate	33.36	271	C08243
53	Maltotriose	Carbohydrate	33.56	204	C01835

*PubChem CID (Compound Identifier); #Internal standard

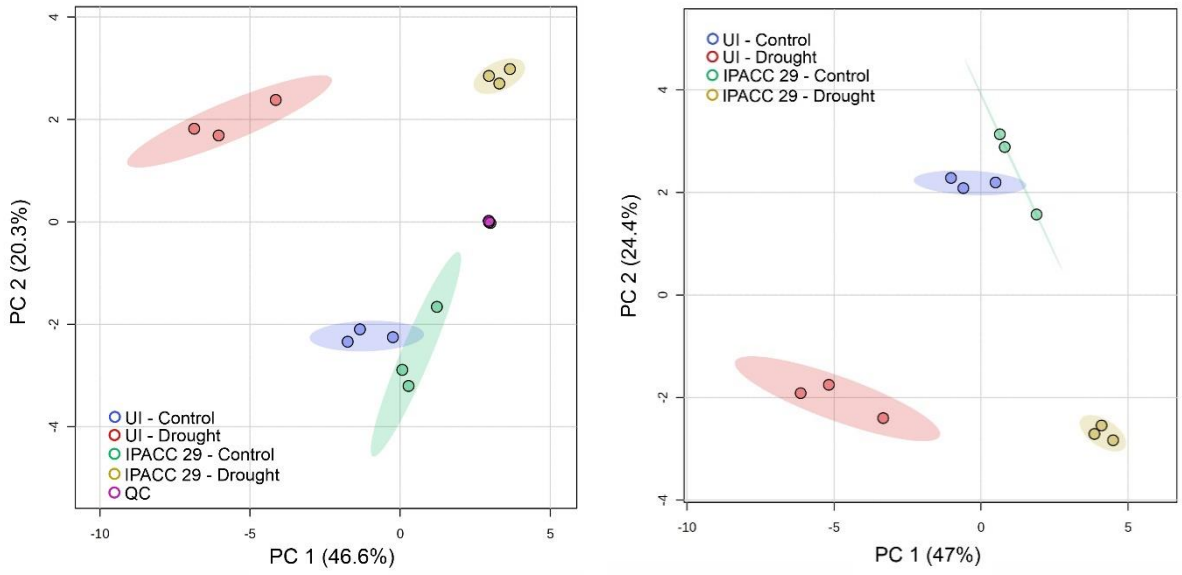
Appendix 5 - Values of relative concentration for cladode metabolites of cactus pear. (var. Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC],150 days after drought imposition. Values represent the mean of three repetitions. Different letters indicate significant difference among the treatments, according to Tukey test $p \leq 0.05$* and $p \leq 0.01$**

	Relative concentration[a]				
	Control		Drought		
Metabolite	UI	IPAC-C29	UI	IPAC-C29	F value
Sugar					
Lyxose	6.85	7.55	6.01	7.48	0.243
Xylose	53.20 a	78.36 a	3.97 b	34.03 a	10.99**
Arabinose	34.14 b	47.35 a	1.61 c	25.91 b	483.65**
Ribose	420.46 a	339.30 a	48.07 b	27.57 b	11.36**
Fructose	3636.29 a	2724.45 a	8.86 b	1620.84 a	53.70**
Galactose	335.44 a	623.82 a	1.86 b	208.47 a	61.83**
Glucose	916.07 a	1061.18 a	2.21 b	455.84 a	82.47**
Glucoheptonic acid	10.17	9.42	3.41	10.90	37.84
Galactonic acid	5.37 b	19.71 a	3.95 b	28.12 a	16.71**
Glucoheptose	1.06	1.32	0.85	1.08	25.81

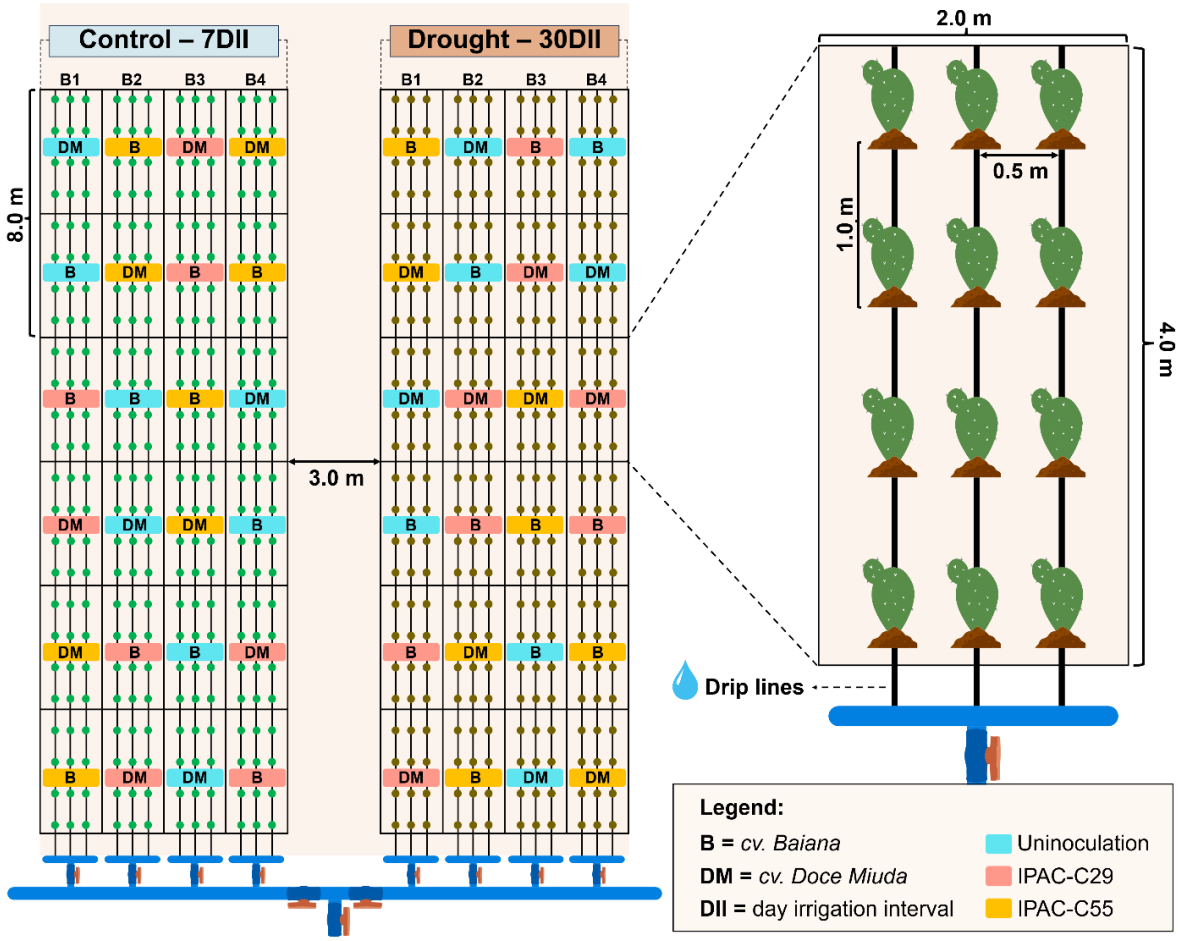
	Relative concentration[a]				
	Control		Drought		
Metabolite	UI	IPAC-C29	UI	IPAC-C29	F value
Sucrose	64.29 b	178.54 b	153.83 b	1807.84 a	32.68**
Cellobiose	46.51 a	37.42 a	3.38 b	33.97 a	17.85**
Maltose	13.37 ab	25.57 a	3.20 b	9.17 ab	82.85*
Trehalose	54.63 b	45.90 b	440.39 a	12.59 b	91.31*
Isomaltose	36.71 a	84.38 a	3.45 b	63.17 a	12.33**
Palatinose	8.91 b	14.26 b	11.15 b	73.42 a	12.29**
Melezitose	1.39	2.68	4.03	4.05	0.957
Maltotriose	2.40 b	7.38 a	2.80 b	6.09 a	14.81**
Sugar alcohol					
Glycerol	3731.17	5382.63	1285.62	956.54	34.07
Erythritol	749.63 a	366.12 a	80.41 b	15.13 c	34.02**
Inositol - allo	2308.90 a	2088.33 ab	1054.12 b	1977.38 ab	49.73*
Galactinol	23.21	60.35	45.03	43.55	11.14
Organic acid					
Pyruvic acid	7.19	4.16	6.57	8.00	11.37
Oxalic acid	2.51	2.14	1.59	2.17	0.179
Glycolic acid	1.22 ab	0.79 b	0.58 b	2.04 a	58.01**
3-hydroxy Butyric acid	0.43	0.57	1.18	1.29	24.00
Maleic acid	8.98 b	16.02 a	8.14 b	29.63 a	84.02*
Succinic acid	8.83 a	2.20 b	7.34 a	5.94 a	14.10**
Glyceric acid	1.52	2.25	1.28	3.46	21.19
Fumaric acid	57.89	60.14	36.11	33.23	22.96
Malic acid	143.48 b	419.13 b	140.19 b	2161.63 a	23.87**
4-amino Butyric acid	2.68 b	4.17 b	1.46 b	157.86 a	16.96**
Lactic acid dimer	25.69 a	3.23 b	5.74 b	39.29 a	18.91**
Erythronic acid	4.05 a	6.35 a	0.41 b	4.60 a	42.91**
Oxo-Glutaric acid	3.02	2.62	2.28	0.85	3.35
Valeric acid	5.09	5.91	3.21	15.16	31.35
Citric acid	45.97 b	1924.64 a	66.18 b	6994.78 a	41.94**
Dehydroascorbic acid	0.45	1.87	0.93	1.35	31.79
Quinic acid	2.82 a	3.56 a	0.98 b	2.34 a	25.71**
Saccharic acid	23.73 a	21.70 a	3.78 b	43.24 a	12.98**
Amino acid and nitrogen compounds					
Alanine	3.66 b	1.21 b	3.33 b	42.58 a	78.85*
Valine	2.61 b	1.74 b	0.90 c	25.64 a	37.74**
Serine	9.37	2.56	2.86	3.42	22.10

	Relative concentration[a]				
	Control		Drought		
Metabolite	UI	IPAC-C29	UI	IPAC-C29	F value
Glycine	10.74 b	8.83 b	1.49 c	112.37 a	70.89**
Isoleucine	0.41	1.08	0.48	0.43	43.91
Proline	1.34 b	1.72 b	1.40 b	89.86 a	55.51**
Aspartic acid	5.99	7.24	8.05	11.52	0.411
Pyroglutamic acid	5.03 b	5.41 b	5.52 b	117.40 a	104.02**
Adenine	6.85	3.47	6.70	15.06	26.77
Other compounds					
Phosphoric acid	2.25 c	7.96 b	1.70 c	18.91 a	24.34**
Nicotinamide	1.69	2.28	1.56	1.85	35.51
Unknown -1	13.57 c	44.21 a	27.88 b	57.73 a	41.57**

[a] Metabolites were quantified by GC-MS using ribitol as internal standard.



Appendix 6 - Principal Component Analysis-PCA with and without quality control (QC) of the metabolic in cladodes of cactus pear (var. Doce Miúda) subjected to inoculation treatments [uninoculated (UI) and inoculated with *Bacillus subtilis* strain (IPACC29)] and water levels [control - 50% of soil field capacity (FC) and drought - 25% FC], 150 days after drought imposition.



Appendix 7 - Experimental field layout with cactus pear varieties (Baina and Doce Miúda) subjected to two irrigation intervals (control – irrigation every 7 days and drought - irrigation every 30 days) and three inoculation levels (*Bacillus subtilis* IPACC29, *Paenibacillus* sp. IPACC55, and uninoculation).

Appendix 8 - Chemical analysis of soil collected the 0-20 cm layer for growing cactus pear plants in field

pH	Ca	Mg	Al	H+Al	K	SB	T	P	V	OM	Clay	Silt	Sand
H_2O	----------$cmol_c\ dm^{-3}$----------							$mg\ dm^{-3}$	%	$g\ kg^{-1}$	$g\ kg^{-1}$		
6.6	3.45	0.87	0.00	1.48	0.25	4.57	6.06	17.4	75.5	10.54	172	54	774

Note: pH – Potential of hydrogen; Ca (calcium), Mg (magnesium), Al (exchangeable acidity), H+Al (potential acidity at pH 7.0), K (potassium), SB (total exchangeable bases), T (cation-exchange capacity at pH 7.0), P (ascilable phosphorus), V (base saturation), and OM (organic matter).