Samuel Giovanny García-Castaño
Luis Eliécer Oviedo Zumaque
Ana Milena Vásquez-Bettin
Marvin José Perneth-Montaño
Andrés José Betin Ruiz

PLANTS PHISIOLOGY

Bromeliad Conservation, Stress Signaling, and Postharvest Innovation

Edited by Marcelo F. Pompelli and Miguel P. Guerra

científica digital

EDITORA CIENTÍFICA DIGITAL LTDA
Guarujá - São Paulo - Brasil
www.cientificadigital.org - contato@cientificadigital.org

Editor
Reinaldo Cardoso
Diagramação
João Quintela
Imagens da Capa
Adobe Stock - 2025

Edição © 2025 Editora Científica Digital
Texto © 2025 Os Autores
1ª Edição - 2025
Acesso Livre - Open Access

Dados Internacionais de Catalogação na Publicação (CIP)

P716

Plant Physiology: Bromeliad Conservation, Stress Signaling, and Postharvest Innovation [recurso eletrônico] / Organização de Samuel Giovanny García-Castaño, Luis Eliécer Oviedo Zumaque, Ana Milena Vásquez-Bettin, Marvin José Perneth-Montaño, Andrés José Betin Ruiz./ Editado por Marcelo F. Pompelli e Miguel P. Guerra. – Guarujá-SP: Científica Digital, 2025.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui Bibliografia
ISBN 978-65-5360-838-2
DOI 10.37885/978-65-5360-838-2

1. Botânica. I. García-Castaño, Samuel Giovanny II. Zumaque, Luis Eliécer Oviedo. III. Vásquez-Bettin, Ana Milena. IV. Perneth-Montaño, Marvin José. V. Ruiz, Andrés José Beti. VI. Título

CDD 580

Elaborado por Janaína Ramos – CRB-8/9166

Índice para catálogo sistemático:
I. Botânica

E-BOOK
ACESSO LIVRE ON LINE - IMPRESSÃO PROIBIDA
2025

Samuel Giovanny García-Castaño
Luis Eliécer Oviedo Zumaque
Ana Milena Vásquez-Bettin
Marvin José Perneth-Montaño
Andrés José Betin Ruiz

PLANT PHISIOLOGY:
Bromeliad Conservation, Stress Signaling, and Postharvest Innovation

Edited by Prof. Dr. Marcelo F. Pompelli and Prof. Dr. Miguel P. Guerra

1ª EDIÇÃO

científica digital

2025 - GUARUJÁ - SP

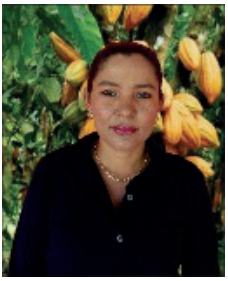
Review and Peer Evaluation

The texts that comprise this work have undergone evaluation by the Editorial Board and external peer review, receiving due recommendation for publication.

Note: This book is the result of a collaborative process, and is configured as a collection in which copyright remains with the respective authors. Some chapters may originate from works previously presented at academic events or scientific journals; however, the authors were instructed to adopt due rigor in preventing self-plagiarism. The responsibility for the content of each chapter, as well as for the originality and integrity of the information published, lies entirely with the respective authors. The content of the work does not necessarily reflect the opinion of the publisher, the organizers or the members of the editorial board.

AUTHOR'S BIOGRAPHIES

Prof. M.Sc. Luis Eliécer Oviedo Zumaqué is an Agricultural Engineer and holds a degree in Biology and Chemistry, with a Master's degree in Microbiology. Currently, Director of the Biotechnology Group of the Department of Chemistry and Department of Biology – GRUBIODEQ, and is Coordinator of the Master's Degree in Biotechnology at the University of Córdoba. His career has established him as an Associate Researcher at the Ministry of Science, Technology, and Innovation of Colombia, actively participating in projects related to agricultural microbiology, environmental biotechnology, and integrated natural resource management.



Ana Milena Vasquez-Bettin, is an agronomist with a master's in Agronomic Science, focusing on plant physiology. She works with cocoa plant physiology and its plant interaction with biotic and abiotic responses to this culture. She is a junior researcher in Agrosavia, Colombia.

Prof. M. Sc. Marvin José Perneth-Montaño is an Agricultural Engineer with expertise in Pastures and Forages from the University of Antioquia. He had a Master's in Agricultural Sciences, emphasizing Plant Physiology. Currently, he is studying for a Ph.D. in Agricultural Sciences with an emphasis on Crop Physiology and Plant Genetic Improvement at the University of Cordoba. His areas of expertise are plant physiology under abiotic stress conditions, seed physiology, tropical crop production, and plant genetic improvement.

Samuel Giovanny García-Castaño is an Agricultural Engineer, a Professional in Territorial Development, and Masters in Crop Science. He has experience in Colombia's agro-export industry, particularly in the plantain sector. His expertise lies in optimizing production chains, sustainability, and supporting small producers. He has contributed to scientific publications related to sustainability and crop production by seeds. His research focuses on plant ecophysiology, abiotic stress, and seed physiology in crop production.

Prof. Andrés José Betin Ruiz, M.Sc., is a Chemist and Master of Biotechnology graduate from the University of Córdoba, Colombia. His work focuses on agricultural and microbial biotechnology, where he has conducted research focused on the use of microorganisms for crop improvement, biofertilization, and soil bioremediation. He currently participates in interdisciplinary projects that promote the sustainable use of biotechnology in agroecological systems.

Collaborators:

Franciscleudo B. Costa is an Agronomist, PhD in Plant Physiology, active in undergraduate and graduate programs at the Federal University of Campina Grande (UFCG). Coordinates the Laboratory of Food Analysis and Food Chemistry and Biochemistry, focusing on postharvest physiology of perishable organs, crop production, minimal processing, and food preservation technologies.

Edited by:

Marcelo F. Pompelli is actually a senior researcher at the Universidad de Córdoba, Colombia. Ph.D. in Plant Physiology. He has supervised several Scientific Initiation students, master's and doctoral students and participated as a Post-Doctoral Supervisor. He has more than 90 scientific publications, books, and book chapters. His interest is in Plant Physiology and stress interactions involving metabolism and biochemical characteristics. Proline biosynthesis and metabolism are your recent focus.

Miguel P. Guerra Full Professor of the Postgraduate Programs in Plant Genetic Resources at the Federal University of Santa Catarina, Florianópolis, SC, of the Postgraduate Program in Agricultural and Natural Ecosystems, UFSC, Curitibanos Campus. Member the National Biodiversity Commission (CONABIO) of the Ministry of the Environment. National Director and Binational Director of the Brazilian-Argentine Center for Biotechnology (CBAB-CABBIO) linked to the MCT - Brazil and MINCyT – Argentina. Research and teaching in plant biotechnologies for characterization, conservation, improvement and sustainable use of plant genetic resources.

PREFACE

Plant physiology is a dynamic and fundamental field for understanding the internal functioning of plants, their interactions with the environment, and their adaptive capacity in the face of diverse challenges. It is both a foundational and an applied science, whose advances are essential for the conservation of native species, the management of agricultural crops, the mitigation of abiotic stresses, and the reduction of losses along the production chain. In this context, the present book – PLANT PHYSIOLOGY: *Bromeliad Conservation, Stress Signaling, and Postharvest Innovation* – brings together a series of applied studies that clearly illustrate the breadth and depth of plant physiology's contributions to solving real-world problems, both in natural environments and productive systems.

The opening chapters focus on the conservation of endangered bromeliads, particularly *Dyckia distachya and Dyckia encholirioides*, endemic species of the Brazilian Atlantic Forest. The destruction of their natural habitat, exacerbated by large infrastructure projects such as hydroelectric dams, underscores the urgency of implementing ex situ conservation strategies. The studies presented herein demonstrate how seed germination under different environmental conditions (temperature, light, salinity, sulfuric acid, and gibberellic acid) can be modulated by physiological factors, and how in vitro cultivation, combined with the use of growth regulators, enables the multiplication and preservation of these species' genetic resources. Micropropagation using seeds and tissues, with protocols based on MS media supplemented with specific phytohormones, highlights the role of physiological technologies in rescuing and maintaining threatened plant populations.

The book also thoughtfully explores issues related to water use and management in agriculture. In a global scenario of increasing water scarcity and intensifying climate change, the rational use of water resources becomes imperative. One chapter addresses the availability of freshwater worldwide, emphasizing the role of agriculture in global water demand and discussing the benefits and risks associated with the use of treated wastewater for irrigation. Water reuse practices are analyzed from both physiological and environmental perspectives, considering their effects on soil, plants, and biogeochemical cycles. In addition,

hands-on experiments are proposed to help visualize physiological phenomena such as xylem transport and osmotic potential, contributing to the formation of students with a critical and applied understanding of plant water physiology.

Among the plant defense mechanisms against abiotic stresses, the use of amino acids as mitigating agents for salinity damage stands out. One chapter is dedicated to the foliar application of proline in maize plants under salt stress, presenting consistent results regarding its effects on growth, ion composition, and membrane stability. The osmoprotective role of proline, combined with its ability to act as a scavenger of reactive oxygen species, reveals its potential as a complementary tool in improving salt stress tolerance, particularly within plant breeding programs focused on developing more resilient cultivars.

Subsequently, the book addresses postharvest physiological processes in fruits and vegetables, a topic of great relevance to food security and the sustainability of production systems. Aspects such as respiration, ethylene production, water loss, and biochemical changes occurring after harvest are discussed alongside strategies for their control. Techniques such as refrigeration, modified atmospheres, edible coatings, and the use of visual indicators are addressed with technical rigor, illustrating how physiological knowledge can be applied to extend shelf life, reduce waste, and preserve nutritional quality. A chapter dedicated to the drying of *pequi* (*Caryocar* spp.) is particularly noteworthy, as it bridges postharvest conservation concepts with the valorization of native species from the Cerrado and Caatinga biomes, with promising potential for incorporation into functional food and phytotherapeutic markets.

Finally, the book concludes with practical activities designed for teaching plant physiology, including experiments that enhance understanding of water transport and balance in plants. These hands-on approaches not only enrich academic training but also stimulate students' interest in the critical importance of physiological processes for plant life and ecosystem sustainability.

This work reflects the effective contribution of plant physiology to biodiversity conservation, technological advancement in agriculture, and the response to global change challenges. By integrating basic and applied science, the book offers readers a broad and critical perspective on the physiological mechanisms that govern plant life. It is, therefore,

an essential read for plant physiologists, agronomists, biologists, environmental engineers, students, and professionals engaged in plant science and agricultural production.

Toshik Iarley da Silva is an Agronomist, holds a DSc. in Crop Science from the Federal University of Viçosa, and is an Assistant Professor at the Federal University of Recôncavo da Bahia. His research focuses on plant physiology applied to abiotic stress mitigation, protected cultivation, edible flowers, and postharvest physiology of vegetables and fruits.

CONTENTS

SECTION I

MICROPROPAGATION AND CONSERVATION OF BROMELIADS

This section introduces the complex and vital topic of preserving and propagating bromeliads, specifically focusing on *Dyckia distachya* and *Dyckia encholirioides*. These species are exemplary members of the genus Dyckia, belonging to the family Bromeliaceae, which thrives predominantly in the unique biome of the Brazilian Atlantic Forest. This forest, renowned for its unparalleled biodiversity, faces severe threats due to human activities, leading to fragmentation and a significant loss of endemic species like *D. distachya*, which is officially recognized as endangered. Misidentification with its relative, *D. brevifolia*, further complicates conservation efforts, as the former's striking rosette structure and branching inflorescences distinguish it. *D. distachya*, a rupicolous and rheophytic plant, has become emblematic of the challenges in *ex situ* and *in vitro* conservation. Restricted to rocky riverbanks, primarily along the Uruguay River, its populations have declined precipitously due to hydroelectric projects like the Itá dam. This section delves into pioneering methods for preserving its genetic diversity through germplasm banks and tissue culture techniques. Integrating in vitro propagation highlights the adaptability of conservation strategies to mitigate habitat destruction, offering insights into ex vitro acclimatization and germination behaviors.

Similarly, *D. encholirioides*, an endemic bromeliad of the Atlantic Forest coastline, faces vulnerabilities exacerbated by its slow growth and sporadic seed germination. Studies included here explore environmental factors affecting its reproductive success, such as salinity tolerance, photoperiods, and the efficacy of plant growth regulators like gibberellic acid. These findings underscore the interplay between ecological conditions and genetic resilience, emphasizing practical applications for restoring degraded ecosystems.

This section also addresses the methodological intricacies of conservation, blending field studies with laboratory-based propagation to strike a balance between scientific precision and ecological restoration. From understanding seed physiology to employing cryopreservation for long-term genetic safeguarding, the chapters provide a comprehensive overview of strategies tailored to the specific challenges posed by these endangered bromeliads.

The chapters collectively represent a holistic approach to bromeliad conservation, aiming to inform and inspire stakeholders in botanical research, horticulture, and environmental management. The fusion of traditional botanical studies with

innovative biotechnological tools demonstrates the potential for reversing biodiversity loss while offering scalable models for global application.

Dive into this section to uncover nature's remarkable resilience, bolstered by human ingenuity. Each page promises to deepen your understanding of bromeliad conservation and these plants' critical role in preserving our planet's ecological balance. Let the journey inspire your commitment to biodiversity!

The authors

CHAPTER I

EX SITU CONSERVATION OF *Dyckia distachya* ON SOUTH BRAZIL

Prof. Dr. Marcelo F. Pompelli

M.Sc. Marvin José Perneth-Montaño

M.Sc. Ana Milena Vásquez-Bettin

Prof. Dr. Miguel P. Guerra

10.37885/250619542

ABSTRACT

The Brazilian Atlantic Forest biome contains a high level of genetic diversity. Historically, several factors have contributed to the drastic reduction of its coverage, which is now restricted to less than 7%. Large forest areas have been cut down in Santa Catarina and Paraná states to make way for hydroelectric power plant dams, and many plant species are threatened to various degrees, especially the endemic. This is the case of the *Dyckia distachya*, bromeliad native to the rocky banks of the River Uruguay, which is threatened with extinction because of the construction of a dam for the Itá Hydroelectric power plant. The present study describes the plant's most relevant characteristics and the results of *ex-situ* and in vitro conservation. Plants and seeds were collected and established *ex-situ* in the bromeliad germplasm bank. Seeds were germinated in the laboratory, and the resulting plants were conserved *in vitro* using the growth reduction technique. Both methodologies were considered adequate for germplasm conservation.

Keywords: atlantic forest; bromeliads; germplasm conservation; tissue culture.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

INTRODUCTION

The devastation of the Atlantic Forest, power plant constructions, and sharp endemism have restricted the *Dyckia distachya* bromeliad to a few remaining populations on the rocky banks of the Uruguay River in the west of Santa Catarina (SC) State and the south of Paraná (PR) State, and in restricted areas in Paraguay and Argentina. This species is commonly mistaken for *D. brevifolia* from which it differs mainly in the greater number of leaves, the more attractive rosette, and a more robust and ramified inflorescence (Reitz 1983, Strehl 1994). *Dyckia distachya* is on the official list of endangered Brazilian plant species [1].

Tissue culture techniques have been used for the germplasm conservation of a wide range of plant species [2]. In Brazil, the use of these techniques for bromeliads has allowed the mass micropropagation of Vriesea, Tillandsia, Guzmania, Neoregelia, and other species to supply the landscape gardening market, preventing extractivism [3].

Bromeliad's history and decline

Bromeliads are typical plants of the American continent and probably originated in the Andean region. The species spread out throughout the millennia and reached the tropical forest about 200,000 years ago. In this taxonomic group, 2700 species were described, all native to the American continent except for a single species, *Pitcairnia feliciana*, which is found in West Africa [4]. Both terrestrial and epiphyte bromeliads are found even in most desert and arid regions of the world [5].

Bromeliaceae is subdivided into three sub-families: Bromelioideae, Tillandsioideae and Pitcairnioideae. The first sub-family is composed of 536 species [6]. They are predominantly epiphytes and seed dispersal occurs through animals [7]. The subfamily Tillandsioideae consists > 1500 species [8]. *Tillandsia*, *Vriesea*, and *Guzmania* are the most common genera for commercial and ornamental purposes. Their seeds are wind-dispersed [9]. Pitcairnioideae comprise 750 species [6] and include the genera *Puya*, *Dyckia*, and *Pitcairnia* [10]. This subfamily is considered the most primitive (Medina and Troughton 1974, Galetto and Bernardello 1992), but recent molecular studies have suggested that Tillandsoideae are the basal derived species [11].

The family has three dispersal centers: one in the Andean region stretching to Mexico and the Antilles, another on the Guiana Plateau, and a third in Brazil [12] (Figure 1). It is estimated that about 40% of the species dwell in Brazil, many of which are endemic and concentrated mainly in the Atlantic Forest biome [11].

Figure 1. Bromeliaceae distribution around the world. Note the endemism of the American region (Reproduced from Benzing [13]).

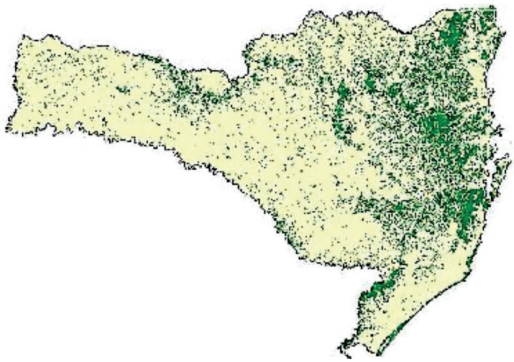
The bromeliads, being mostly epiphytes, depend on forest conservation. The devastation process of the Atlantic Forest biome is the main cause of the genetic erosion of the bromeliad species. Unfortunately, Brazilian megalopolises' devastation and urban development have resulted in the loss of more than 1,105,896 hectares of this precious biome [14], consisting of mature fragments exceeding three hectares with a closed canopy or showing no degradation detectable by satellite image. Regrettably, most of these remnants are small, exhibit high fragmentation, possess limited connectivity, and are characterized by secondary vegetation or non-forest structures. Additionally, the most critical fragments face the threat of further forest degradation, and their distribution is uneven across the biome's different phytophysiognomies [15,16]. The escalating deforestation of the Brazilian Atlantic Forest has resulted in a significant loss of biodiversity. On a more positive note, collaborative efforts between the scientific community and ecologically motivated stakeholders have generated restoration initiatives that promise to mitigate the adverse effects of this degradation.

As if the vast devastation of the Atlantic Forest in Brazil and SC State (Figure 2) were not enough, the bromeliads were also identified as hosts of the Anopheles mosquitoes in the middle of the last century. Thus, millions of bromeliads were pulled up and/or burnt with the justification of eradicating the flies that reproduced there. In Florianópolis, the capital of SC State alone, 15,981,431 bromeliads were collected and destroyed between 1944 and 1950, which was associated to a drop in the city's malaria cases. The total value of the plants

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

eradicated in SC alone was estimated at 319,900,314 units. This was the price paid to control malaria in several cities in southern Brazil [17].

Figure 2. Forest remnants in the Brazilian State of Santa Catarina.



Distribution of the bromeliad genera in south Brazil and in Santa Catarina

The most frequent genera in SC are *Vriesea*, *Dyckia*, *Tillandsia*, *Bromelia*, *Aechmea*, and *Billbergia* with a wide dispersal from Rio Grande do Sul (RS) to Rio de Janeiro (RJ) and Minas Gerais (MG) States. *Catopsis*, *Nidularium*, *Wittockia*, and *Canistrum* species are also present predominantly in the east of this region. *Pitcairnia* and *Ananas* are distributed in the northern coastal region and Itajaí valley in SC and the coastal region of PR [18]. The genera *Hohembergia* and Neoregelia are also present in this region (Figure 3).

Figure 3. Geographic distribution of the genera in the State of Santa Catarina, Brazil.

Dyckia distachya is highly endemic, probably because of the restricted seed dispersion by wind [11]. It is a rupestris and rheophyte flowering plant, 70 to 130 cm in height, with many leaves in a beautiful rosette. The 12-10 cm long leaves are rigid, concave-channeled with many claw-shaped, sharply pointed up-turned thorns on the edges, inflorescence with side exit, not very twisted, 70 to 130 cm tall, generally forming a terminal panicle of 2-8 branches, yellow--orange flowers, very similar to *D. brevifolia*. It is characteristic and exclusive to the islands or along the rocky banks of the Uruguay River and the Paraná River rapids in the south of PR and west of SC.

Dyckia distachya is under serious threat of extinction mainly because the riverbeds and banks of its primary dispersion area have recently been dammed for the construction of hydroelectric power plants at Itaipú (PR), Itá, and Machadinho (SC) [19].

Dyckia distachya Hassler micropropagation and conservation

The conservation of genetic resources of tropical biomes is a very important subject. While many species are at risk of extinction in temperate regions, several tropical species have rapidly disappeared. Therefore, the protection of species threatened with extinction is a priority issue. The conservation and management of biodiversity, even in protected areas in the tropics, consists of complex challenges that require basic knowledge of species distribution and abundance, their mutual interactions, reproductive biology, and the genetic structure of their populations [20].

The *in vitro* conservation techniques can be valuable for conserving plant genetic resources [21]. It has been postulated that the in vitro maintenance of cultures is expensive; nevertheless, this technique holds great potential for application [22]. Vulnerability should also be considered as the main disadvantage of germplasm collections maintained under field conditions. Plants are exposed to pathogen attacks and climatic variations and can be lost by faults in identification or human errors. Therefore, the possibility of *in vitro* conservation is attractive for economic and practical reasons. Even considering these aspects, Hammer and Tekl [22] emphasize that ex-situ conservation is the most efficient form of plant germplasm conservation. *In situ* conservation prioritizes habitat protection, thus allowing the continuous evolution of species and the maintenance of the intra and inter-specific diversities of populations, individuals, and genes.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

The dynamics of tropical forest destruction have led to severe alterations in the ecosystems that compose the respective biomes due to the high fragmentation of the habitats and genetic erosion [23], making establishing germplasm conservation programs necessary and urgent. The present study assessed the potential of applying *ex situ* and in vitro conservation methods to *Dyckia distachya*.

MATERIAL AND METHODS

Plant collection

Fifteen *Dyckia distachya* populations were identified in an approximately 500-meter-long area on the Uruguay Riverbanks in the Chapecó region of west SC before the dam of the Itá hydroelectric power plant was closed in January 2000. A *Dyckia distachya* population was assessed at approximately every 30 meters and three or four plants were removed, packed in cardboard boxes, and transported by road (600 km) to the bromeliad germplasm collection at the Department of Plant Sciences at the Santa Catarina Federal University, Florianopólis, Brazil. All plants were planted in plastic or clay pots containing a mixture of maxim (fibers of *Dycksonia sellowiana*), vermiculite, and sand (2:2:1) and placed on 1.3 m high tables under-screened conditions with 40% photosynthetic active radiation (PAR) reduction.

Germplasm collection

To prepare the material *in vitro*, seed-containing capsules were collected from the populations and cool-stored under refrigeration at 4 ± 2 ºC in polyethylene tubes as recommended by Moncaleano-Escandon [24]. The seeds were immersed in a 70% ethanol solution for 3 min. and then in commercial bleach (5% NaOCl) for 30 min. in an aseptic chamber, followed by five rinses in sterilized water. Seeds were then inoculated in Petri dishes containing 20 mL KC culture medium [25] supplemented with Morel vitamins [26], sucrose (30 g L^{-1}), and gelled with agar (10 g L^{-1}). The pH was adjusted to 4.7 by adding HCl (0.5 N) before being autoclaved for 15 min. at 1.5 atm. Cultures were maintained in a growth room at 25 ± 3ºC, 60 ± 5% relative humidity, and 60 mmol m^{-2} s^{-1} PAR provided by cool white lamps during 16 hours of light regime.

After 30 days, the germinated plantlets were transferred to test tubes (100 x 15 mm) containing 10 mL KC medium, pH adjusted to 4.5, and solidified agar

(10g L^{-1}) or placed in groups of 10 plantlets in 500 mL glass flasks containing 30 ml of liquid KC medium. Cultures were kept under the conditions described above.

RESULTS AND DISCUSSION

Ex vitro conservation

Dyckia distachya seeds are dispersed by wind, forming dense clumps. Many of these clumps derive from the development of the shoots themselves or from the germination of seeds that drop near the mother plant (Figure 4).

The collected plants adapted well to the *ex vitro* germplasm collection conditions. There have been three flowering cycles since the arrival of the plants, with shoot emission comparable to the field behavior of the species (Figures 5 and 6). The formation of capsules with fertile seeds has also been observed, indicating adequate acclimatization of the collected plants to the new environment. Thus, this method is recommended for the conservation of *Dyckia distachya*.

According to Zaniboni-Filho and Ribolli [27], *ex vitro* germplasm conservation should be encouraged since it represents a form of escape from extinction threats to plant species caused by natural and human catastrophes, such as dam building and/or hydroelectric power plant construction. According to Benzing [11], *ex situ* conservation is probably more promising for endangered Bromeliaceae, but only if priorities change. According to this author, about 200 notable collections of bromeliads exist in the USA, of which perhaps two dozen are well-documented. In agreement with Joly [28], it should be stressed that Brazilian legislation enforces the need to establish safeguards for the collection and conservation of endangered plant species, mainly when activities or projects pose risks to biodiversity, as in the case of the construction of hydroelectric power plants.

In vitro conservation

The extrusion of the first leaves was observed three days after inoculating the seeds, and the seedling development was completed after 30 days. The plantlets were then replicated individually. Initially, the transference intervals between the subcultures and fresh culture media lasted 90 days. Cultures could be maintained in test tubes for over a year without subculturing. After this period, a basal shoot was selected, isolated, and re-inoculated in test tubes for *in vitro* conservation.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

The *in vitro* germplasm collection currently consists of 50 test tubes containing one plantlet each and 15 flasks (500 mL) containing ten plantlets each, totaling more than 250 genotypes collected in the region of natural occurrence. They are derived from seeds and not from somatic tissues that would generate a clonal population. This is an essential aspect because the primary purpose of any germplasm collection is to conserve the genetic variability that represents the target species. The cultures are kept under conditions that are easily manipulated and available at any time for mass multiplication, population reintroduction in the regions of origin, and germplasm exchange.

Endangered bromeliads could be inexpensively preserved *in vitro* at low temperatures, but at this point, it is not without risk. Cryopreservation is still experimental, and few taxa have been tested after long-term storage [11]. However, recent advances in general procedures and methodologies are honing these techniques for conserving endangered plant species. The results here showed the potential use of ex-situ and in vitro techniques for *D. distachya* conservation, an endangered bromeliad species from the Atlantic Forest biome.

Acknowledgements

The authors thank PADCT/CNPq for financial support and CAPES and CNPq for fellowships to the authors. The authorities of the Itá Hydroelectric power plant are specially acknowledged for the permission to collect biological material.

DIRECT STUDY

Bertsouklis, K.; Panagaki, K.-P. 2022. *In vitro* germination and propagation of *Dyckia brevifolia*, an ornamental and endangered bromeliad. **Horticulturae** 8: 390. doi: 10.3390/horticulturae8050390.

Corredor-Prado, J.P.; De Conti, D.; Guerra, M.P.; Dal Vesco L.L.; Pescador, R. 2020. **Acta Sci Agron** 42: e42448. doi: 10.4025/actasciagron.v42i3.42448.

Garrido-Aguilar, D.C.; Villanueva-Couoh, E.; Pinzón-López, L.L.; Reyes-Ramírez, A. 2022. *In vitro* establishment and multiplication of *Aechmea fasciata* (Lindl.) Baker, a bromeliad of commercial interest. **Agro Productividad**. 15: 85-91. doi: 10.32854/agrop.v15i5.2139.

Montoya-Serrano, F.S.; Dal Vesco, L.L. and Pescador, R. 2022. Seeds cryopreservation of *Vriesea reitzii* Leme & A.F. Costa endemic bromeliad from Atlantic Rainforest. **Hoehnea** 49: e082021. doi: 10.1590/2236-8906-08/2021e082021.

Pompelli, M.F.; Fernandes, D.; Guerra, M.P. 2005. Somatic embryogenesis in *Dyckia distachya* Hassler (bromeliaceae) – an endangered bromeliad from south Brazil. **Propag Ornam Plants** 5: 192-198.

Pompelli, M.F.; Guerra, M.P. 2004. *Ex situ* conservation of *Dyckia distachya*: an endangered bromeliad from South Brazil. C**rop Breed Appl Biotechnol** 4: 273-279.

Pompelli, M.F.; Guerra, M.P. 2005. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. **Crop Breed Appl Biotechnol** 5: 117-126.

Pompelli, M.F.; Fernandes, D.; Guerra, M.P. 2006. Germination of *Dyckia encholirioides* (Gaudichaud) Mez var. encholirioides under saline conditions. **Seed Sci & Technol** 34: 759-763. doi: 10.15258/sst.2006.34.3.24

REFERENCES

1. Zanella, C.M.; Janke, A.; Paggi, G.M.; Goetze, M.; Reis, M.S.; Bered, F. Microsatellites in the endangered species *Dyckia distachya* (Bromeliaceae) and cross-amplification in other bromeliads. **Int J Mol Sci** 2012, 13, 15859-15866, doi:10.3390/ijms131215859.

2. Khalil, M.; Sarwar, M.S. A new method for plant breeding through the use of plant tissue culture. **J Life Soc Sci** 2023, 2, 1-9.

3. Lima, A.P.P.S.; Bastos, F.J.O.; Lima-Brito, A.; Fernandes, G.B.; Santana, J.R.F. Modulation of culture medium on the ex situ conservation of *Neoregelia mucugensis* leme (bromeliaceae). **Rev Caatinga** 2021, 34, 763-771, doi:10.1590/1983-21252021v34n403rc.

4. Zizka, A.; Azevedo, J.; Leme, E.; Neves, B.; Caceres, D.; Zizka, G. Biogeography and conservation status of the pineapple family (Bromeliaceae). **Divers Distrib** 2020, 26, 183-195, doi:10.1111/ddi.13004.

5. Reyes-García, C.; Mejia-Chang, M.; Griffiths, H. High but not dry: Diverse epiphytic bromeliad adaptations to exposure within a seasonally dry tropical forest community. *N***ew Phytol** 2012, 193, 745-754, doi:10.1111/j.1469-8137.2011.03946.x.

6. Rundel, P.W.; Dillon, M.O. Ecological patterns in the Bromeliaceae of the lomas formations of Coastal Chile and Peru. **Plant Syst Evol** 1998, 212, 261-278, doi:10.1007/BF01089742.

7. Rocha, J. Neotropical bromeliads as food sources for birds: A systematic review and perspectives on the management of ecological interactions. **Ibis 2023**, *165*, 17-33, doi:10.1111/ibi.13138.

8. Vera-Paz, S.I.; Contreras Díaz, D.D.D.; Jost, M.; Wanke, S.; Rossado, A.J.; Hernández-Gutiérrez, R.; Salazar, G.A.; Magallón, S.; Gouda, E.J.; Ramírez-Morillo, I.M.; *et al.* New plastome structural rearrangements discovered in core Tillandsioideae (Bromeliaceae) support recently adopted taxonomy. **Front Plant Sci** 2022, 13, 924922, doi:10.3389/fpls.2022.924922.

9. Fischer, E.A.; Araujo, A.C. Spatial organization of a bromeliad community in the Atlantic rainforest, southeastern Brazil. **J Trop Ecol** 1995, 11, 559-567.

10. Mercier, H.; Kerbauy, G.B. Microprogation of ornamental bromeliads. In **Biotechnology in Agriculture and Forestry - Serie High-Tech and Microprogation**, Bajaj, Y.P.S., Ed.; Springer-Verlag: New York, 1997; Volume 40, pp. 43-57.

11. Benzing, D.H. Bromeliaceae: a brief profile and some topics that warrant further inquiry. **Selbyana** 2023, 34, 1-79.

12. Ballego-Campos, I.; Forzza, R.C.; Paiva, É.A.S. Extranuptial nectaries in bromeliads: a new record for Pitcairnia burchellii and perspectives for Bromeliaceae. **Sci Nat** 2022, 109, 28, doi:10.1007/s00114-022-01799-5.

13. Benzing, D.H. *B***romeliaceae: Profile of An Adaptive Radiation**; Cambridge University Press: Cambridge, U.K., 2005.

14. RBMA. A Mata Atlântica. Reserva da Biosfera da Mata Atlântica. Available online: https://rbma.org.br/n/a-mataatlantica/ (accessed on 27 June 2024) 2024.

15. Marques, M.C.M.; Trindade, W.; Bohn, A.; Grelle, C.E.V. The Atlantic Forest: An Introduction to the Megadiverse Forest of South America. In **The Atlantic Forest**, Marques, M.C.M., Grelle, C.E.V., Eds.; Springer, Cham: 2021; Volume Berlim.

16. SOS_Mata_Atlântica. **Atlas dos Remanescentes Florestais da Mata Atlântica período 2021-2022**. Available on https://acervo.socioambiental.org/sites/default/files/documents/13d00112.pdf. Acessed in Oct *10, 2024*; SOS Mata Atlântica: São Paulo, 2023.

17. Oliveira, E.S.D.A. O combate à malária em Florianópolis e suas implicações ambientais. **Tempos Histórico** 2011, 15, 405-429.

18. Reitz, R. **Bromeliáceas e a malária-Bromélia endêmica: Flora ilustrada Catarinense**; Herbário Barbosa Rodrigues: Itajaí, 1983.

19. Rogalski, J.M.; Berkenbrock, I.S.; Vieira, N.K.; Reis, A. Demographic structure of clonal, endemic, and endangered rheophyte bromeliad *Dyckia ibiramensis*: asexual vs sexual reproduction. **Rodriguésia** 2021, 72, e00752020, doi:10.1590/2175-7860202172116.

20. Barbieri, R.L.; Costa Gomes, J.C.; Alercia, A.; Padulosi, S. Agricultural biodiversity in southern Brazil: Integrating efforts for conservation and use of neglected and underutilized species. **Sustainability** 2014, 6, 741-757, doi:10.3390/su6020741.

21. Salgotra, R.K.; Chauhan, B.S. Genetic diversity, conservation, and utilization of plant genetic resources. **Genes** 2023, 14, 174, doi:10.3390/genes14010174.

22. Hammer, K.; Tekl, Y. Plant genetic resources: Selected issues from genetic erosion to genetic engineering. **J Agric Rural Dev Trop Subtrop** 2008, 109, 15–50.

23. Phattaraphakin, K.; Vejwitayaklung, Y.; Jueajinda, S.; Phopli, W.; Chaiwiwat, U.; Jirayukul, S. Tropical forest destruction versus reforestation strategies. **J Mod Learning Dev** 2023, 8, 449–453.

24. Moncaleano-Escandon, J.; Silva, B.C.F.; Silva, S.R.S.; Granja, J.A.; Alves, M.C.J.L.; Pompelli, M.F. Germination responses of *Jatropha curcas* L. seeds to storage and aging. **Ind Crops Prod** 2013, 44, 684-690, doi:doi: 10.1016/j.indcrop.2012.08.035.

25. Knudson, L. A New nutrient soluction for the germination of orchid seed. **Am Orch Soc Bull** 1946, 15, 214-217.

26. Morel, G.M.; Wetmore, R.H. Fern callus tissue culture. **Am J Bot 1951**, *38*, 141-143.

27. Zaniboni-Filho, E.; Ribolli, J. Potential use of germplasm banks of South American neotropical species in environmental conservation in Brazil. In **Cryobiology for South American neotropical fish species,** Streit Jr., D.P., Zhang, T., Paredes, E., Eds.; CRC Press: Boca Raton, 2024; pp. 241-255.

28. Joly, C.A.; Scarano, F.R.; Bustamante, M.; Gadda, T.M.C.; Metzger, J.P.W.; Seixas, C.S.; Ometto, J.P.H.; Pires, A.P.F.; Boesing, A.L.; Sousa, F.D.R.; *et al.* Brazilian assessment on biodiversity and ecosystem services: summary for policy makers. **Biota Neotrop** 2019, 19, e20190865.

CHAPTER II

A ROLE OF NACL, LOW TEMPERATURES, GIBBERELLIC ACID, AND SULPHURIC ACID ON GERMINATION OF *Dyckia encholirioides* (*Gaudichaud*) Mez var. *encholirioides*

M.Sc. Ana Milena Vásquez-Bettin

Prof. Dr. Luis Eliécer Oviedo Zumaque

M.Sc. Andrés José Betin Ruiz

Prof. Dr. Miguel P. Guerra

10.37885/250619543

ABSTRACT

Dyckia encholirioides (Gaudichaud) Mez var *encholirioides* is an endangered endemic bromeliad in the Brazilian Atlantic Forest running along the Atlantic Ocean. It is considered vulnerable because it has restricted, discontinuous clusters dispersed throughout central-southern Brazil and is listed as endemic to these regions. In this study, we investigated the role of seed storage in low-temperature gibberellic acid (GA_3), as well as the effect of sulphuric acid (H_2SO_4), to enhance seed germination. Also, it was tested different salt levels (0, 100, 200, 300, 400, and 500 mmol L_{-1} NaCl) under various temperature regimes (10-20°C, 15-25°C, 20-30°C, and 25-35°C), both in 12 hours of dark and 12 hours of light photoperiod and complete darkness on seed germination. We found that GA_3 has little or no effect on seed germination promotion. In contrast, storage of the seeds at low temperatures efficiently promoted seed germination since its germination was delayed. The seed germination was almost 100% in all treatments with GA_3 or lower temperatures and 80% with H_2SO_4 as a promoter. The mean germination time (MGT) in response to GA_3 or cold ranged from 6 to 14 days, increasing as cold time increased. Under H_2SO_4, the MGT ranged from 4 to 9 days, with higher values in intermediate H_2SO_4 levels. Time exposure to H_2SO_4 progressively reduced seed germination. Under NaCl as a negative promotor, the highest seed germination rate was obtained at 0, 100, and 200 mmol L^{-1} NaCl at 20-30°C, and a further increase in salinity resulted in a gradual decrease in germination. Less than 5% of seeds germinated at 500 mmol L^{-1} NaCl. Salinity treatment at 15°-25°C was slightly inhibitory to the seed germination than the optimal temperature regime, whereas under both 10°-20ºC and 25°-35°C temperature regimes, seed germination was substantially reduced. Few seeds germinated at concentrations higher than 200 mmol L^{-1} NaCl. Germination rate was fastest at 20°-30°C and slowest at 10°-20°C. A low seed germination rate was obtained in the dark as compared to seeds germinated in a 12-hour photoperiod under saline conditions. Recovery experiments showed that exposure of seeds to various salinity and temperature regimes had little effect on viability, and seeds could successfully germinate after salt stress relief in *D. encholirioides* seeds.

Keywords: germination dynamics; seed dormancy; desert adaptation; ecological restoration; seed banks.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

INTRODUCTION

The Atlantic Rain Forest is a Brazilian biome containing high biological diversity and endemism [1,2]. The impressive fragmentation of this biome and its associated ecosystems resulted in a genetic erosion of its components. *Dyckia encholirioides* is a not halophyte bromeliad native to this biome. Nevertheless, it is distributed at long seaboard under rocks, vulnerable to water with low osmotic potential. Possible sources of moisture are the ocean water or summer rains. *D. encholirioides* flowers once a year, from September to November, and a large number of seeds are produced from October to December [3]. After dispersal, seeds become part of the seed bank and rarely germinate. *Dyckia encholirioides* var *encholirioides*, an endangered bromeliad, belongs to the Bromeliaceae family, subfamily Pitcairnioideae. Popularly known as bromeliad or gravatá (in the native language), it is an endemic species of the Brazilian Atlantic coast, occurring in discontinuous bands from the States of Rio de Janeiro to Santa Catarina. It presents characteristics of slow growth, with abundant production of stolons and fruits.

In recent years, interest in the propagation of native forest species has intensified due to the current emphasis on environmental problems, highlighting the need to recover degraded areas and restore the natural landscape [4-9]. However, the knowledge available for managing and analyzing the seeds of most species to characterize their physical and physiological attributes is limited. There is also a need to obtain basic information about the germination, cultivation, and potential of these native species, with the aim of using them for the most diverse purposes. Currently, several studies have been carried out to promote the germination of seeds of native forest species.

It is recognized that water, light, and temperature, as well as the plant growth regulators (PGR) such as ethylene, abscisic acid, and gibberellins, can interfere with the metabolic processes that trigger seed germination. In this sense, the gibberellic acid (GA_3) acts as a mediator of the *de novo* synthesis and release of hydrolytic enzymes of the amylase type in the starchy endosperm, which are responsible for the degradation of long starch chains, thus generating free sugars which will be used to provide energy for the germinating embryo [10-13].

Seeds have the ability to germinate within a certain temperature range, characteristic for each species, which is a determining factor for the speed and maximum percentage of germination [14]. These authors observed a close relationship between temperature and the germination of *Acacia polyphylla*

seeds. In this sense, Okusanya [15] refers to the sensitivity of *Celosia cristata* L. seeds to low temperatures, which have lower limits between 15-21ºC and a maximum of 31ºC. However, only 16% of the seeds exposed to 21ºC germinated, a factor that was reversed when the seeds were exposed to higher temperatures, which indicates, according to the author, that low temperatures inhibit germination.

Despite this information regarding seed germination and the ecological potential of native species, to date, no works describing the germination potential of *Dyckia encholirioides* seeds are known. This species is rarely multiplied from seed ensuring the genetic diversity of local ecotypes is maximized [16]. However, each species has particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments [17,18]. Soil salinity is an important constraint to plant fitness affecting about 95 million hectares worldwide [19]. Although some plants are moderately tolerant to saline conditions, many others are adversely affected. Salinity impairs seed germination, reduces nodule formation, plant development and reduces crop yield [20].

Halophytes are distributed in coastal and inland saline habitats throughout the world [21]. This species are vulnerable because of the direct action of high--salinity stress or other associated abiotic factors [22]. Perennial halophytes vary in their ability to tolerate salinity, and this variation is attributed to a number of factors such as light, temperature, and moisture stresses [23]. Tolerance to salinity during germination is critical for the establishment of plants growing in saline soil of arid regions [24]. In these regions, germination occurs during rainy seasons when soil salinity levels are usually reduced [25]. Even in the halophytic species, best germination is obtained under non-saline conditions and their germination decreases with increases in salinity [26].

Temperature and salinity can interact in determining salinity tolerance during germination. Although higher salinity decreases germination, the detrimental effect is generally less severe at optimum temperature [26]. The adverse effect of high salinity is further aggravated by either an increase or decrease in temperature [26,27]. Also, in many plant species light is an important environmental regulatory signal that interact with temperature to regulate seed germination [28]. Seed germination in hypersaline conditions is affected by the temperature regime to which seeds are exposed [2,29,30]. Halophytic species show a range of responses from partial to complete germination recovery when salinity stress is alleviated [30].

The present study focuses on *in vitro* requirements for seed germination of *Dyckia encholirioides* by analyzing the response to promotors and

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

inhibitors. As promoter, we tested the vernalization by cold temperatures, addition of GA_3 in culture medium and chemical scarification under H_2SO_4. To study the inhibitors, we tested different NaCl concentrations, promoting low osmotic potentials under different incubation temperature. To study the photoblastism, the seeds were incubated under a regime of 12h light/12h darkness and full darkness.

MATERIAL AND METHODS

Local description and seed collection

Seeds of *Dyckia encholirioides* were collected from natural population in the Santinho beach, Florianópolis, Santa Catarina State, South Brazil (27º26'48" S; 48º22'09"W; 20 m. asl). Seed-containing capsules were sealed in polyethylene bags and transported to the Laboratory of Plant Developmental Physiology and Genetics, Department of Plant Sciences, of the Federal University of Santa Catarina, Florianópolis, Brazil. After collected the fruits were dried outdoors, in a shaded place, for seven days, to complete their dehiscence and facilitate seed extraction and then the seeds were stored in polyethylene flasks as recommended by Moncaleano-Escandon*, et al.* [31] until use.

Disinfection of seeds

Before inoculation in culture media, the seeds were immersed in ethanol (70%) during 3 minutes followed by a 5% of sodium hypochlorite solution for 30 min and finally five times with sterilized water using the methodology described by Pompelli and Guerra [32].

Effect of cold stratification under seed incubation on gibberellic acid (GA_3)

The seeds were stored in refrigerator at 4ºC by 48 and 168 hours, plus control, without stratification. After each cold stratification, the seeds were transferred to Petry dishes disposed by 15 mL of Murashige and Skoog [33] (Basal Media, MS plus Gamborg Modified, Sigma Aldrich, St. Louis, MO, USA, part number M0404), in addition of 0, 100, and 200 mM L^{-1} of gibberellic acid (GA_3, Sigma Aldrich, part number G7645), sucrose (Sigma Aldrich, part number PHR1001), and 6 g L^{-1} agar base (w/v) (Sigma Aldrich, part number A1296), pH 5.8.

Effect of chemical stratification by sulphuric acid (H_2SO_4)

The seeds were dipped in sulphuric acid (H_2SO_4, Sigma Aldrich, part number 258105) by 0, 10, 30, and 60 seconds. In time zero, the seeds were very fast deep (less than 2 seconds) in H_2SO_4. After that the seeds were disinfected as described above. Then, all seeds were transferred to Petry dishes disposed with the same media described above.

Effect of different concentration of NaCl and light

The seeds were disinfected as described above and then transferred to Petry dishes disposed with the same media described above. Seeds were germinated in Biological Organisms Development (BOD) incubators at four alternating temperature regimes of 10-20ºC, 15-25ºC, 20-30ºC, and 25-35°C. A 24-hour cycle was employed where the temperatures of 20ºC, 25ºC, 30ºC, and 35°C were associated with 12-hour light period provided by Sylvania cool white, fluorescent lamps, resulting in a 40 mmol m^{-2} s^{-1} light intensity. The temperatures of 10ºC, 15ºC, 20ºC, and 25°C coincided with a 12-dark period. Seeds were germinated at six salt levels (0, 100, 200, 300, 400, and 500 mmol L^{-1} NaCl). In seeds germinated in complete darkness, the Petri dishes evolved by two layers of aluminum foil.

Germination conditions and evaluation

All experiments were conducted in 100 mm-Petry dishes containing MS culture media described above. Ten replicates with 25 seeds each were used in each treatment. All Petry dishes were transferred to Biological Organisms Development (BOD) incubators under 25-20ºC except for seed germination under saline conditions and different temperatures experiments. A 24-hour cycle was employed where the temperatures of 25ºC were associated with a 12-hour light period provided by Sylvania cool white fluorescent lamps, resulting in a 40 mmol m^{-2} s^{-1} light intensity. The temperature of 20ºC coincided with a 12-dark period.

Seeds were considered to be germinated at the emergence of the apical shoot following the description of Pompelli and Guerra [32]. The germination rate was recorded every two days for 20 days. After 20 days, the germination was computed and uploaded in GerminaR R package [34] to compute seed germination percentages, germination in time, mean germination time (MGT), and synchrony (SYN) in seed germination in accordance with formulas stablished by Maguire [35], coupled to GerminaR [34].

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Only in experiments evolving NaCl in different incubation temperatures, after 20 days all non-germinated seeds were transferred to distilled water to study the recovery of germination, which was also recorded at two-day intervals for 20 days. The recovery rate was scored by the formula *Recovery rate*= $\frac{(a-b)}{(c-b)} x\ 100$, where a is the total number of germinated seeds after their transference to distilled water, b is the total number of seeds germinated in saline solution, and c is the total number of seeds.

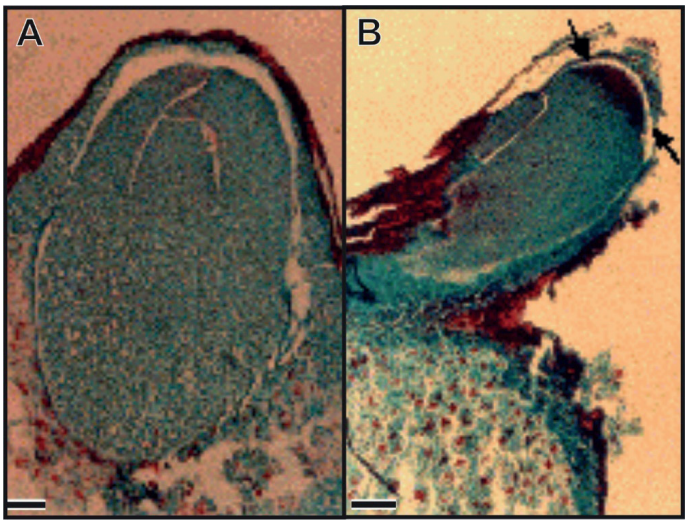
Statistical analysis

When necessary, the data were transformed into ArcSine $\sqrt[2]{\frac{x}{100}}$, or in $\log_{10}(x+2)$ [36]. All data were subsequently subjected to two-way ANOVA and mean separation test (SNK $p \leq 0.05$) using the SigmaPlot for windows v. 15.1 (Systat Software, Inc., San Jose, CA, USA).

RESULTS

Regardless of the evaluated treatment, all germinated seeds were assessed considering the emission of aerial part distinct from the majority of studies that assess the root protrusion, confirmed by anatomical and ontogenetic studies of germination (Fig. 1)

Figure 1. The first stages of germination of *Dyckia distachya* evaluated as ontogenetic procedures. In A, the embryo is visualized into the seed and in B the shoot protrusion of the seed coat. Bars 66.7 mm and 88 mm, respectively.



Effect of cold stratification under seed incubation on gibberellic acid (GA_3)

In the presence of GA3 the vast majority of *Dyckia encholirioides* seeds showed higher germination rates without, however, differing from the control treatment (Fig. 2). The concentration of 100 μM of GA_3 promoted a slightly higher germination rate when compared to the rate of 200 μM L^{-1}. A plausible explanation for this fact may be due to a phytotoxic effect of the higher concentration on the embryos; however, this evidence cannot be confirmed since the values were very similar and did not show statistical significance. Seeds previously vernalized at 4ºC had delayed germination (Fig. 2, evaluation at 5 days). However, at the end of the evaluation time, all treatments showed similar germination rates with little or no significance between treatments (Fig. 2). Delayed seed germination was shown in time (Fig. 3) or after analysis of mean germination time (MGT). In a global evaluation, seeds vernalized by 168 hours at 4ºC increased their MGT by 75.2% from 7.41 days to 12.99 days (Fig. 4A). The regression curve between the time of vernalization and MGT was positive (R^2 = 0.949), referring to the negative effect of vernalization on first germination. Besides, the synchrony, which expresses the synchronization of germination, does not present the same pattern of MGT because the SYN values sometimes increased or decreased in the results of GA_3 concentrations (Fig. 4B), and the vernalization shows little or no effect. The regression between the time of vernalization and SYN was positive (R^2 = 0.963) but non-significative.

Figure 2. Effect of cold (0, 48, and 168h) and gibberellic acid (GA_3; 0, 100, and 200 mM L^{-1}) on germination of *Dyckia encholirioides* seeds. All evaluation was performed under 12h light/12h dark. All bars denote a median of 10 repetitions. Means followed by different lowercase letters denote statistical significance between GA_3 concentrations in the same time of cold and evaluation data and different uppercase letters denote statistical differences between times of cold in the same GA_3 concentration and evaluation data (Newman-Keuls $P \leq 0.01$).

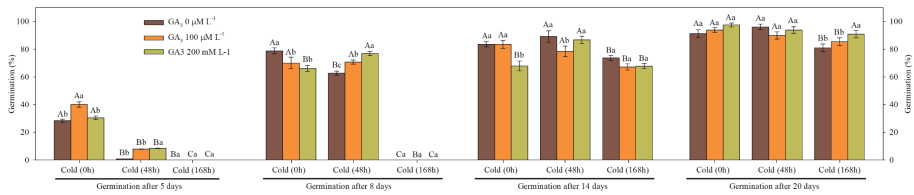


Figure 3. Effect of cold (0, 48, and 168h) and gibberellic acid (GA_3; 0, 100, and 200 mM L^{-1}) on germination in time of *Dyckia encholirioides* seeds. All evaluation was performed under 12h light/12h dark. All symbols denote a median of 10 repetitions.

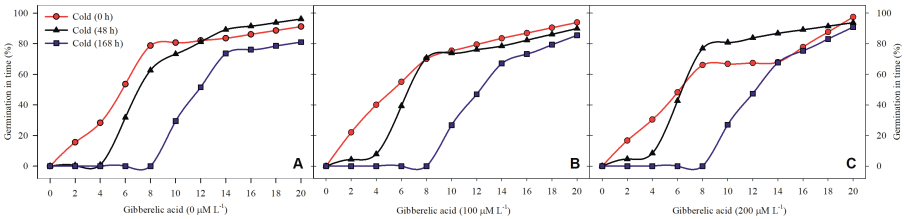
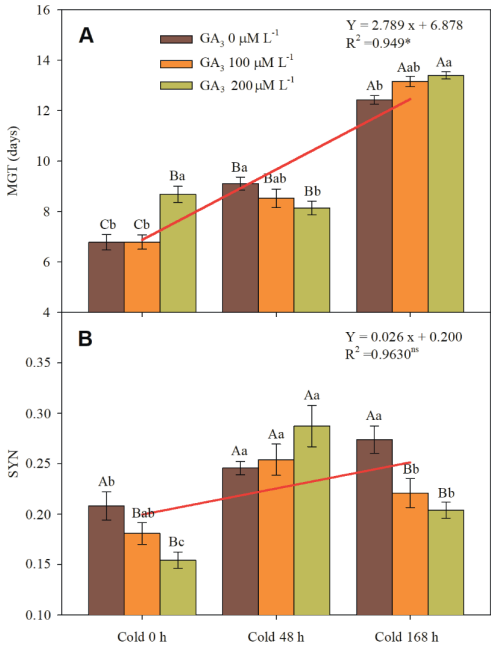


Figure 4. Effect of cold (0, 48, and 168h) and gibberellic acid (GA_3; 0, 100, and 200 mM L^{-1}) on mean germination time (MGT) and synchrony (SYN) of *Dyckia encholirioides* seeds. All evaluation was performed under 12h light/12h dark. All bars denote a median of 10 repetitions. Means followed by different lowercase letters denote statistical significance between GA_3 concentrations at the same time of cold and evaluation data. Different uppercase letters denote statistical differences between cold times in the same GA_3 concentration and evaluation data (Newman-Keuls $P \leq 0.01$). In the regression curve, the asterisks (*) denote a significance at $P \leq 0.05$, and ns denote non-significant.



Effect of chemical stratification by sulphuric acid (H_2SO_4)

Scarification with a solution of H_2SO_4 at 30% (v/v) was significantly more effective in promoting the germination of *D. encholirioides* seeds than scarification at other concentrations, regardless of the exposure time (Fig. 5). The exposure time of 0, 10, 30, and 60 seconds under H_2SO_4 at 30%, promote the germination

of *D. encholirioides* in about 3.9-, 7.1-, 5.6-, and 64.2-fold higher than H_2SO_4 at 100%. In response to H_2SO_4 exposure time, the H_2SO_4 at 30% at 60 minutes reduces the seed germination in 14.4%, in a distinct manner of H_2SO_4 at 50% at 60 minutes that promote the seed germination in 31%. Also, the germination decreases as H_2SO_4 concentration or H_2SO_4 exposure time increases (Fig. 5A).

Seeds treated with H_2SO_4 at 30% and 100% did not result in a statistically different germination rate. However, the first concentration allowed the average germination of 72.2% of seeds with the regeneration of 50.7% of complete seedlings containing both aerial and root parts. In comparison, the 100% concentration allowed the germination of only 14.4% of seeds with the regeneration of only 7.1% of seedlings with complete formation. It was also found that the time of exposure to the H_2SO_4 acid was not relevant, as within each concentration this factor did not show statistical significance; but at 100% concentration, the seeds did not resist the acid treatment, as they disintegrated when exposed for 60 seconds at that concentration and then rinsed with distilled water, which made the seeds unviable.

Effect of different concentration of NaCl and light

The statistical analysis showed significant effects of salinity, temperature, and their interaction on germination rate and recovery rate of *D. encholirioides* seeds (data not showed). The highest germination rate in light was observed in the non-saline control under all temperature regimes (Fig. 6). Different salinity levels resulted in a gradual decrease in the germination rate, and this reduction ranged with the changes in the temperature regime (Fig. 6). Highest germination rate under saline conditions was observed at 20-30°C treatment, where germination in 0 and 100 mmol L^{-1} NaCl levels was at least 95% and 2.5%, respectively in seed germinated on light and 92% and 0% on dark. However, in the presence of 100 mmol L^{-1} NaCl the germination rate was highest than 80% especially in seeds germinated in light conditions (Fig. 1). Seed germination in the distilled water was not affected by darkness under any temperature regime. However, seed maintained in salt treatments and complete darkness greatly reduced the germination, this reduction being affected by temperature. Temperature also influenced germination rate. At low and high temperatures, seeds showed the lowest germination rate from 100 to 300 mmol L^{-1} NaCl than the values observed at 20-30°C and 15-25°C (Fig. 6).

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 5. Effect of sulphuric acid (H_2SO_4; 30%, 50%, and 100%) and exposure time (0s, 10s, 30s, and 60s) on seed germination (A), mean germination time (MGT; B) and plantlets survival of *Dyckia encholirioides* seeds. All evaluation was performed under 12h light/12h dark. All bars denote a median of 10 repetitions. Means followed by different lowercase letters denote statistical significance between exposure time on H_2SO_4 in the same H_2SO_4 concentration and different uppercase letters denote statistical differences between H_2SO_4 concentrations in the same H_2SO_4 exposure time (Newman-Keuls $P \leq 0.01$).

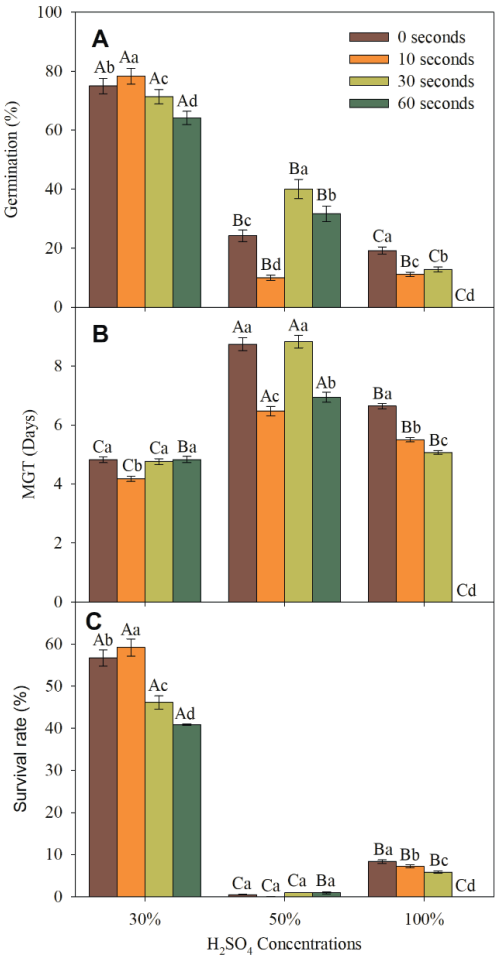
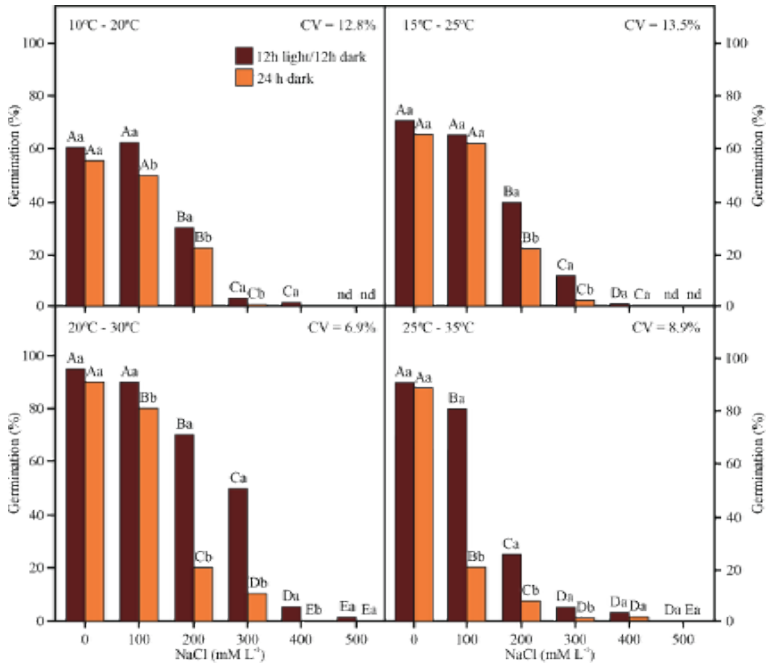


Figure 6. Effect of NaCl, light, and temperature on seed germination of *Dyckia encholirioides*. All bars denote a median of 5 repetitions. Means followed by different uppercase letters denote statistical differences between NaCl concentration in the same light regime and means followed by different small case letters denote statistical differences between light regimes in the same NaCl concentration (Newman-Keuls $P \leq 0.01$).

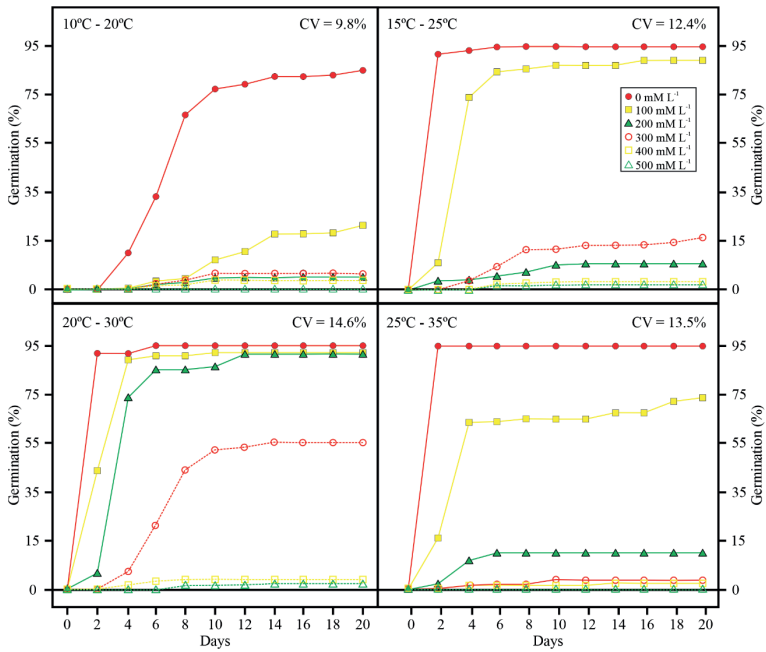


Temperature also affected germination speed under both saline and non--saline conditions (Fig. 7). Highest seed germination in the distilled water was obtained after two days under all temperature regimes except for 10-20°C (Fig. 7). In saline solutions highest germination ranged from eight 8 to 20 days. Seed germination peaked at 10 days for all salt concentrations at 15-25°C, except in 300 mmol L^{-1} (20 days) (Fig. 7).

When light-treated, non-germinated seeds from salt treatments were transferred to distilled water, they recovered completely under all salinity and temperature regimes (Figure 8). The sum of seed germination with seed germination after salt stress relief is almost 100% confirming the high viability of *D. encholirioides* if all procedures descripted in Pompelli and Guerra [32] and Moncaleano-Escandon, Silva, Silva, Granja, Alves and Pompelli [31] was referred as pattern.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 7. Cumulative mean percent germination of *Dyckia encholirioides* seeds over time in 0, 100, 200, 300, 400 and 500 mmol L^{-1} NaC1 in 12 hours light and 12 hours dark photoperiod.

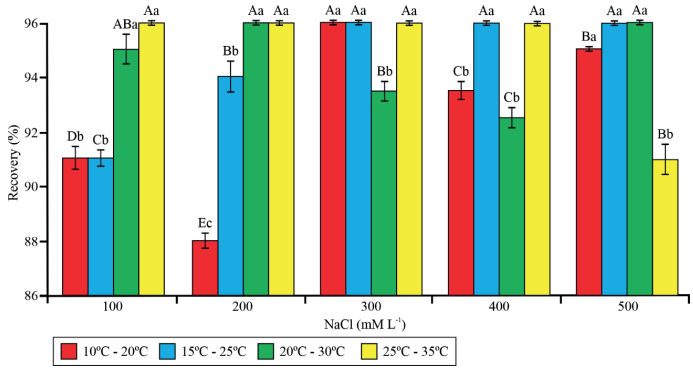


DISCUSSION

The seed germination results from a series of temperature-dependent biochemical reactions, since hydrolytic enzymes are involved in this process, which have optimal conditions for action [37,38]. Therefore, in the present study, it is plausible that with the reduction in temperature, germination was delayed but not blocked, since when the seeds were transferred to higher temperatures, the germination potential was restored, as already described by Araújo-Neto, Aguiar and Ferreira [14], Okusanya [15], Lozano-Isla, *et al.* [39], and Bhatt, *et al.* [40] for seeds of *Acacia polyphylla*, *Celosia cristata, Jatropha curcas, and Rumex obtusifolius* respectively. Seeds can germinate within a specific temperature range, which is characteristic of each species and is a determining factor for the speed and maximum percentage of germination [14,40]. In this sense, Okusanya [15] refers to the sensitivity of *Celosia cristata* L. seeds to low temperatures, which have lower limits between 15-21ºC and a maximum of 31ºC. However, only 16% of the seeds exposed to 21ºC germinated, a factor that was reversed when the seeds were exposed to higher temperatures, which indicates, according to the author, low temperatures inhibit germination. Also, in accordance with this author,

the temperature proved to be more important than light in the germination of seeds of that species.

Figure 8. Germination rate of *Dyckia encholirioides* in distilled water under different temperature regimes in various salinity treatments. Means followed by different small case letters denote statistical differences between incubation temperatures in the same NaCl concentration and means followed by upper case letters denote statistical differences between NaCl concentration in the same incubation temperature. CV = 18.7%.



Another factor that may have delayed seed germination during the cooling period may have been darkness, as some seeds, predominantly positive photoblastic ones, depend on exposure to light to begin their germination process [41-43]. This fact may be involved in phytochrome reactions, the pigment responsible for capturing light signals and essential for the germination of these seed species. In our experiment, where the effect of cold vernalization was evaluated, the germination in the dark was not assessed. However, in the experiment in the presence of NaCl at different temperatures, it was clear that depending on the incubation temperature, D. encholirioides seeds were entirely positive photoblastic with germination twice to three times greater in the light than in the dark, under the same osmotic condition (Fig. 6). Recently Bhatt [6] and Bhatt [40] described a similar effect in different invasive species in China, where the different incubation temperatures resulted in different photoblastism. Also, cold stratification is regarded as an important factor to release dormancy when dry storage cannot release dormancy completely, especially in alpine plants [44-46]. Storage seeds at -18°C to -20°C with 5-10% moisture content is recommended for long term storage of orthodox seeds [47]. However, the successful of the seed storage is depends on ability of species to maintain their viability under optimal environmental conditions; however, storage under unfavorable conditions causes precocious germination or deterioration [48,49].

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Ideal storage conditions involve reducing seed metabolism by reducing seed moisture contents or lowering their storage temperatures [39,50].

It is well known that temperatures lower or higher than the ideal temperature for each species tend to reduce germination speed, exposing seedlings for a longer period to adverse factors, which can lead to a reduction in total germination [51]. In the positive photoblastic species, light is important for germination events, as described for *Acacia polyphylla* [14], and there was a considerable reduction in the percentage and speed of germination when the seeds were incubated in the dark. Although the light quality was not evaluated in this experiment, it is clear that the change in light conditions was favorable to germination, as evidenced in *Celosia cristata* seeds [15], in Rumex obtusifolius [40], Arthrocnemum macrostachyum and Suaeda vermiculata [52]. However, as reported by Bhatt, Chen, Pompelli, Jamal, Mancinelli and Radicetti [6], the light requirement is temperature-dependent. In this sense, Okusanya [15] refers to the sensitivity of *Celosia cristata* L. seeds to low temperatures, which have lower limits between 15-21ºC and a maximum of 31ºC. However, alternating periods of light and dark-induced germination more quickly than continuous light and promoted higher seedling regeneration than the continuous dark treatment. In accordance with Bhatt, Chen, Gallacher, Phartyal, Rodriguez-Paez, Pineda-Rodriguez, Pompelli, Jamal, Mancinelli and Radicetti [40] a thermal shock may be necessary for the seeds to germinate since the percentage of germination was significantly higher when the temperature was first lowered and then raised, the values were significantly higher than when the temperature was maintained stable throughout the period; results that are corroborated by our data. A similar effect was described by Araújo-Neto, Aguiar and Ferreira [14] in *Acacia polyphylla* seeds germinated at a temperature of 15ºC, which were heavily damaged by the lack of growth of the primary root of the seedlings. However, at 20ºC the percentage and speed of germination were lower than at 25ºC, also resulting in a longer average germination time.

In relation to the seed germination after exposure to sulfuric acid, it was verified that the exposure time to sulfuric acid was not relevant, as within each concentration this factor did not show any significance; but at 100% concentration, the seeds did not resist the treatment, as they disintegrated when exposed for 60 seconds at that concentration, making their use unfeasible. Generally, the first structure to emerge from the seed is the radicle, followed by the aerial part. However, in this study it was observed that the first structure to emerge

from the seed of *Dyckia encholirioides* was the aerial part (Fig. 1), later emitting the root part. This fact may have been preponderant in the non-significant or significant negative effect on germination, since sulfuric acid may have damaged the fragile structure of the aerial part of the germinating seedlings.

Plants native to the subtropical maritime ecosystems are exposed to various moisture and salinity stress because of an unpredictable raining period. These conditions lead to different strategies that allow the plants to maximize their fitness [53]. Coastal areas of Pakistan, for example, are reported to have around 100 species of halophytes [54], and salinity tolerance of only a small number of these species is known. The available reports indicate that dicotyledonous species range in their tolerance during germination, including species such as Arthrocnemum macrostachyum and Cressa cretica [30], Suaeda fruticosa, and *Urochondra setulosa* [2,55], *Prosopis juliflora* [56], *Jatropha curcas* [39]. These species could grow from 500 to 1000 mmol L^{-1} NaCl environments. For D. encholirioides increase in salinity resulted in lower and slower seed germination. This study describes that D. encholirioides is a moderately salt-tolerant halophyte. This is supported by the fact that the seeds did not germinate in saline concentrations higher than 500 mmol L^{-1} NaCl.

It is well-known that temperature and salinity affect the germination of halophytes [57]. The detrimental effect of NaCl at high temperatures is attributed to Na^+ toxicity [38,39]. Some species are more sensitive to changes in temperature (Cressa cretica and *Zygophyllum simplex* L.) than others (Arthrocnemum macrostachyum and Suaeda fruticosa) [30,58]. In the present study seed germination was also influenced by temperature. We found 20-30°C to be the optimal temperature for germination and any increase or decrease in temperature inhibited germination. Miranda, Correia, Almeida-Cortez and Pompelli [56] describes that the seed germination of *Prosopis juliflora* was reduced from 100% to 4% at −0.75 MPa promoved by PEG when seeds were incubated in 30ºC. In this condition, mean germination time was 9 days or 7.1-fold lower than non-salt-stressed seeds. This inhibition was progressively increased by salinity, suggesting that this is responsible for the absence of seed germination of this species in its natural sites. In agreement with Khan and Ungar [55] germination of halophyte seeds in subtropical coastal and inland salt marshes usually occurs after monsoon rains, which causes a reduction in temperature and reduces the soil salinity. Several reports showed that seed germination rate is more sensitive to salinity than germination rate [59,60]. Fasted germination was reported for

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Haloxylon recurvum and H. salicornicum [61] and *Limonium axillare* [62], and this was considered to be a strategy to utilize the brief period of water availability after rainfall. Rogers, *et al.* [63] suggested that fast germination ensures rapid seedling establishment which can minimize competition. Recently, Bhatt, Chen, Pompelli, Jamal, Mancinelli and Radicetti [6] showed mean germination rime ranged from 3 to 11 days to complete germination of Plantago virginica, Solanum americanum, and Veronica persica seeds in the other temperature conditions (i.e., 10/20ºC, 20/30ºC, 25/35ºC). Seeds of Lepidium virginicum from 6 to 7 days to germinate, on average, under 20/30ºC and 25/35ºC, while Phytolacca americana seeds lasted twice the time (from 12 to 14 days) at the same temperature regimes. At 35/40ºC, MGT tended to be faster (e.g., 3 to 4 days), but followed by low germination percentage. In the present study, seeds of D. encholirioides germinated rapidly in the control treatment and in up to 200 mmol L^{-1} NaCl at 20-30°C, a temperature regime similar to the average early summer period in south of Brazil. The pattern of germination of D. encholirioides is very similar to the pattern described by Rubio-Casal, Castillo, Luque and Figueroa [57] for Arthrocnemum macrostachyum and Salicornia ramosissima or Triglochin bulbosa and T. striata [64].

In this study, darkness decreased germination in all temperature and salinity treatments, and Zia and Khan [65] observed the same in *Limonium stocks* seed. However, Navarro and Guitián [66] described a contrary pattern for *Petrocoptis* sp. and Martin, *et al.* [67] for high mountain Mediterranean species.

Dyckia encholirioides seeds displayed a great tolerance to high-salinity and temperature stress before germination. Seeds germinated within two days when transferred to non-saline media from various salinity treatments and temperature regimes. Similar results were obtained by Mahmoud, El Sheikh and Baset [62] for *Limonium axillare*, which showed 95% recovery for 60%-100% seawater treatments. Khan and Ungar [55] also observed a quick recovery in Suaeda fruticosa seeds at all temperature regimes. The ability of halophyte seeds to survive in hypersaline conditions and germinate when salinity is reduced provides them with multiple opportunities for their establishment in unpredictable saline environments [30].

Dyckia encholirioides usually grows in coastal regions under saline conditions and their seeds remain in the soil as seed banks. These seeds are exposed to high-temperature stress (around 40°C or higher) while imbibed in saline seawater during some months of the year. The dormancy reduces the risk of

seedling mortality when moisture is limited, and salinity is increased during summer. For example, in the desert annual Zygophyllum simplex, germination occurs only when temperature and salinity were reduced, but this event, just occur during winter or after monsoon rain [30]. The inhibitory effect of salinity at higher temperatures has ecological significance since it prevents seeds from germinating in salt-affected habitats and consequently avoids seedling mortality during this period [55]. This could be true if the damage caused by Na^+ toxicity is not irreversible [26].

Although seeds could germinate in up to 500 mmol L^{-1} NaCl, which is much lower in comparison to other associated halophytic species, their salinity tolerance during storage in the seed bank confers a successful reproductive strategy in these unpredictable conditions. Seeds of some species such as Salicornia *ramosissima* and *Arthrocnemum macrostachyum* lose its viability when exposed to high temperature and salinity stress [57]. Survival of seeds under extreme conditions provides this species with a strategy for successful recruitment in a harsh environment.

An alternative strategy for the low germination rate of *D. encholirioides* plants in situ is the asexual reproduction through offshoot. This strategy allows the continuous colonization of new areas and the perpetuation of the species. Even considering a low germination rate plants derived from seeds contain new allelic combinations that fit to this harsh environment. These reproductive strategies are responsible to the enhanced fitness of this species in this environment.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

CONCLUSION

In all the treatments described in this study, considerable percentages of germination were achieved, because except when the seeds were damaged, never less than 30% of them germinated and this value could even reach close to 90%, when gibberellic acid (200 μM) was used combined with 48 hours of cold at 14 days of germination. Therefore, it can be concluded that the objectives were achieved. However, it is clear that the seeds of *Dyckia encholirioides* var encholirioides require a scarification process, as when is allowed to germinate without such a process or without the use of a germination inducer, they did not germinate. To confirm this procedure, in addition to the control test carried out *in vitro*, another test was carried out under field conditions and no seeds showed signs of germination, up to 120 days after inoculation.

The longevity of *Dyckia encholirioides* seeds can be considered high, as even after one year of storage, within the conditions established for *Dyckia distachya* [68], the seeds still showed a germination potential of up to 70%. Knowing that the seeds do not germinate under *ex vitro* conditions, and that the species is listed as vulnerable, the procedure used in this study is very applicable as plant tissue culture techniques of great applicability, especially if the species is threatened with extinction.

The results of the present work are relevant when considering the importance or the Atlantic Forest Biome, which is considered the fourth biodiversity hotspot of the planet. Deepen on the studies of its plant endemic species may help in the conservation of this hotspot.

Acknowledgements

The authors wish to thank to PADCT/CNPq for its financial support and CAPES and CNPq for providing fellowships to the authors.

DIRECT STUDY

Bhatt, A., W. Batista-Silva, D.J. Gallacher and M.F. Pompelli. 2020. Germination of *Cenchrus ciliaris*, *Pennisetum divisum*, and *Panicum turgidum* are seasonally dependent. **Botany** 98: 449-458. doi:10.1139/cjb-2019-0194.

Bhatt, A., N.R. Bhat, F. Lozano-Isla, D. Gallacher, A. Santo, W. Batista-Silva, *et al.* 2019. Germination asynchrony is increased by dual seed bank presence in two desert perennial halophytes. **Botany** 97: 639-649. doi:10.1139/cjb-2019-0071.

Bhatt, A., I.F. Daibes, D. Gallacher, A. Jarma-Orozco and M.F. Pompelli. 2022. Water stress inhibits germination while maintaining embryo viability of subtropical wetland seeds: a functional approach with phylogenetic contrasts. **Front Plant Sci** 13: 906771. doi:10.3389/fpls.2022.906771.

Bhatt, A., P.C. Phondani and M.F. Pompelli. 2018. Seed maturation time influences the germination requirements of perennial grasses in desert climate of Arabian Gulf. **Saudi J Biol Sci** 25: 1562-1567. doi:10.1016/j.sjbs.2016.02.004.

Lozano-Isla, F., M.L.O. Campos, E. L. and M.F. Pompelli. 2018. Effects of seed storage time and salt stress on the germination of *Jatropha curcas* L. **Ind Crop Prod** 118: 214-224. doi:10.1016/j.indcrop.2018.03.052.

Murru, V., A. Santo, C. Piazza, L. Hugot and G. Bacchetta. 2015. Seed germination, salt-stress tolerance, and the effect of nitrate on three Tyrrhenian coastal species of the *Silene mollissima* aggregate (Caryophyllaceae). **Botany** 93: 881–892. doi:10.1139/cjb-2015-0148.

Podda, L., A. Santo, C. Leone, O. Mayoral and G. Bacchetta. 2017. Seed germination, salt stress tolerance and seedling growth of *Opuntia ficus-indica* (Cactaceae), invasive species in the Mediterranean Basin. **Flora** 229: 50-57. doi:10.1016/j.flora.2017.02.002.

Research Agreement: This chapter was written based on its original form published in Seed Science and Technology. Pompelli, M.F.; Fernandes, D.; Guerra M.P. Germination of *Dyckia encholirioides* (Gaudichaud) Mez var encholirioides under saline conditions. **Seed Science and Technology**, 34: 759-763, 2006. doi: https://doi.org/10.15258/sst.2006.34.3.24. Under the permission of the authors. Free access.

REFERENCES

1. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Fonseca, G.A.B.; Kent, J. Biodiversity hotspots for conservation priorities. **Nature** 2000, 403, 853-858.

2. Gulzar, S.; Khan, M.A.; Ungar, I.A. Effect of salinity and temperature on the germination of *Urochondra setulosa* (Trin.) C.E. Hubbard. **Seed Sci Tech** 2001, 29.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

3. Reitz, R. **Bromeliáceas e a malária-Bromélia endêmica: Flora ilustrada Catarinense. [Bromeliaceae and Endemic malaria-Bromeliad: Illustrated Flora of Santa Catarina State]**; Barbosa Rodrigues Herb: Itajaí, 1983.

4. Bhatt, A.; Phondani, P.C.; Pompelli, M.F. Seed maturation time influences the germination requirements of perennial grasses in desert climate of Arabian Gulf. **Saudi J Biol Sci** 2018, 25, 1562-1567, doi:10.1016/j.sjbs.2016.02.004.

5. Bhatt, A.; Gallacher, D.; Jarma-Orozco, A.; Pompelli, M.F. Seed mass, dormancy and germinability variation among maternal plants of four Arabian halophytes. **Seed Sci Res** 2022, 32, 53-61, doi:10.1017/S0960258522000083.

6. Bhatt, A.; Chen, X.; Pompelli, M.F.; Jamal, A.; Mancinelli, R.; Radicetti, E. Characterization of invasiveness, thermotolerance and light requirement of nine invasive species in China. **Plants** 2023, 12, 1192, doi:10.3390/plants12051192.

7. Bhatt, A.; Bhat, N.R.; Lozano-Isla, F.; Gallacher, D.; Santo, A.; Batista-Silva, W.; Fernandes, D.; Pompelli, M.F. Germination asynchrony is increased by dual seed bank presence in two desert perennial halophytes. **Botany** 2019, 97, 639-649, doi:10.1139/cjb-2019-0071.

8. El-Keblawy, A.; Gairola, S.; Bhatt, A. Maternal salinity environment affects salt tolerance during germination in *Anabasis setifera*: A facultative desert halophyte. **J Arid Land** 2016, 8, 254-263, doi:10.1007/s40333-015-0023-2.

9. El-Keblawy, A.; Bhatt, A.; Gairola, S. Perianth colour affects germination behavior in the wind pollinated *Salsola rubescens* in the Arabian Deserts. **Botany** 2013, 92, 69-75, doi:10.1139/cjb-2013-0183.

10. Cruz, V.M.V.; Romano, G.; Dierig, D.A. Effects of after-ripening and storage regimens on seed-germination behavior of seven species of *Physaria*. **Ind Crops Prod** 2012, 35, 185-191.

11. Gupta, R.; Chakrabarty, S.K. Gibberellic acid in plant: Still a mystery unresolved. **Plant Signal & Behav** 2013, 8, e25504 doi:10.4161/psb.25504.

12. Marth, P.C.; Audia, W.V.; Mitchell, J.W. Effects of gibberellic acid on growth and development of plants of various genera and species. **Bot Gaz** 1956, 118, 1-5, doi:10.1086/335932.

13. Shah, S.H.; Islam, S.; Mohammad, F.; Siddiqui, M.H. Gibberellic acid: a versatile regulator of plant growth, development and stress responses. **J Plant Growth Regul** 2023, 42, 7352–7373, doi:10.1007/s00344-023-11035-7.

14. Araújo-Neto, J.C.; Aguiar, I.B.; Ferreira, V.M. Efeito da temperatura e da luz na germinação de sementes de *Acacia polyphylla* DC. **Rev Bras Bot** 2003, 26, 249-256.

15. Okusanya, O.T. Germination and growth of *Celosia cristata* L., under various light and temperature regimes. **Am J Bot** 1980, 67, 854-858, doi:10.2307/2442425.

16. Fay, M.F. Conservation of rare and endangered plants using in vitro methods. ***In Vitro* Cell Dev Biol Plant** 1992, 28, 1-4, doi:10.1007/BF02632183.

17. Harper, J.L.; Lovell, P.H.; Moore, K.G. The shapes and sizes of seeds. **Ann Rev Ecol Evol Syst** 1970, 1, 327-356, doi:10.1146/annurev.es.01.110170.001551.

18. Schütz, W.; Milberg, P. Seed dormancy in *Carex canescens*: regional differences and ecological consequences. **Oikos** 1997, 78, 420-428, doi:10.2307/3545604.

19. Szabolcs, I. Soils and salinization. In **Handbook of plant and crop stress**, Pessarakli, M., Ed.; Marcel Dekker: New York, 1994; pp. 3-11.

20. Greenway, H.; Munns, R. Mechanisms of salt tolerance in nonhalophytes. **Annu Rev Plant Physiol Plant Mol Biol** 1980, 31, 149-190.

21. Adam, P. **Saltmarsh ecology** Cambridge University Press: Cambridge, U.K., 1990.

22. Huang, Z.; Zhang, X.; Zheng, G.; Gutterman, Y. Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*. **J Arid Environ** 2003, 55, 453-464.

23. Noe, G.B.; Zedler, J.B. Differential effects of four abiotic factors on the germination of salt marsh annuals. **Am J Bot** 2000, 87, 1679-1692.

24. Schabes, F.I.; Sigstad, E.E. Calorimetric studies of quinoa (*Chenopodium quinoa* Willd.) seed germination under saline stress conditions. **Termochim Acta** 2005, 428, 71-75.

25. El-Keblawy, A. Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*. **Seed Sci Tech** 2004, 32, 943-948, doi:10.15258/sst.2004.32.3.24.

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

27. Khan, M.A.; Gul, B. High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. **Int J Plant Sci** 1998, 159, 826-832.

28. Baskin, C.C.; Baskin, J.M. *Seeds:* **Ecology, Biogeography and Evolution of Dormancy and Germination**, 2nd Edition; Academic Press: London, 2014.

29. Woodell, S.R.J. Salinity and seed germination patterns in coastal plants. **Vegetatio** 1985, 61, 223-229, doi:10.1007/BF00039828.

30. Khan, M.A. Comparative influence of salinity and temperature on the germination of subtropical halophytes. In **Halophyte uses in different climates. I: ecological and ecophysiological studies**, Lieth, H., Moschenko, M., Lohman, M., Koyro, H.W., Hamdy, A., Eds.; Backhuys Publishers: Leiden, Netherlands, 1999; pp. 77-88.

31. Moncaleano-Escandon, J.; Silva, B.C.F.; Silva, S.R.S.; Granja, J.A.; Alves, M.C.J.L.; Pompelli, M.F. Germination responses of *Jatropha curcas* L. seeds to storage and aging. **Ind Crops Prod** 2013, 44, 684-690, doi:doi: 10.1016/j.indcrop.2012.08.035.

32. Pompelli, M.F.; Guerra, M.P. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. **Crop Breed Appl Biotechnol** 2005, 5, 117-126.

33. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiol Plant** 1962, 15, 473-497, doi:10.1111/j.1399-3054.1962.tb08052.x.

34. Lozano-Isla, F.; Alfaro, O.B.; Pompelli, M.F. GerminaR: an R package for germination analysis with the interactive web application 'GerminaQuant for R'. **Ecol Res** 2019, 34, 339-346, doi:10.1111/1440-1703.1275.

35. Maguire, J.D. Speed of germination - aid in selection and evaluation for seedling emergence and vigor. **Crop Sci** 1962, 2, 176-177.

36. Sokal, R.R.; Rohlf, F.J. **Biometry**, 3rd Edition; W.H. Freeman and Co: New York, 1995.

37. Spera, M.R.N.; Cunha, R.; Teixeira, J.B. Quebra de dormência, viabilidade e conservação de sementes de buriti (*Mauritia flexuosa*). **Pesq Agropec Bras** 2001, 36, 1567-1572, doi:10.1590/S0100-204X2001001200015.

38. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. **Seeds: Physiology of Development, Germination and Dormancy**. 3rd Edition; Springer-Verlag: New York, 2013.

39. Lozano-Isla, F.; Campos, M.L.O.; L., E.; Pompelli, M.F. Effects of seed storage time and salt stress on the germination of *Jatropha curcas* L. **Ind Crop Prod** 2018, 118, 214-224, doi:10.1016/j.indcrop.2018.03.052.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

40. Bhatt, A.; Chen, X.; Gallacher, D.J.; Phartyal, S.S.; Rodriguez-Paez, L.A.; Pineda-Rodriguez, Y.Y.; Pompelli, M.F.; Jamal, A.; Mancinelli, R.; Radicetti, E. Storage on maternal plants affects temperature requirements during germination in *Rumex obtusifolius*. **Plants 2023**, *12*, 2403, doi:10.3390/plants12132403.

41. Bhatt, A.; Batista-Silva, W.; Gallacher, D.J.; Pompelli, M.F. Germination of *Cenchrus ciliaris*, *Pennisetum divisum*, and *Panicum turgidum* are seasonally dependent. **Botany** 2020, 98, 449-458, doi:10.1139/cjb-2019-0194.

42. Bhatt, A.; Bhat, N.R.; Suleiman, M.K.; Santo, A. Effects of storage, mucilage presence, photoperiod, thermoperiod and salinity on germination of *Farsetia aegyptia* Turra (Brassicaceae) seeds: implications for restoration and seed banks in Arabian Desert. **Plant Biosyst** 2019, 153, 280-287, doi:10.1080/11263504.2018.1473524.

43. Bhatt, A.; Carón, M.M.; Gallacher; Souza-Filho, P.R.M. Storage duration, light, temperature and salinity exposure influence germination of the glycophyte *Rhanterium epapposum*. **Botany** 2021, 99, 2-12, doi:10.1139/cjb-2020-0153.

44. Schwienbacher, E.; Navarro-Cano, J.A.; Neuner, G.; Erschbamer, B. Seed dormancy in alpine species. **Flora: Morphol Distrib Funct Ecol Plants** 2011, 206, 845-856, doi:10.1016/j.flora.2011.05.001.

45. Rayburn, A.P.; Davidson, J.B.; Schupp, E.W. Effect of storage time, site and floral morph on seed germination of the threatened distylous primrose *Primula cusickiana* var. maguirei. **Plant Spec Biol** 2013, 28, 101-108, doi:10.1111/j.1442-1984.2011.00362.x.

46. Tian, M.H.; Tang, A.J. Effects of storage temperature and pre-treatments on seed germination of *Primula mallophylla* and *P. sertulum* (Primulaceae), two narrowly-distributed endemic species from southwest China. **Seed Sci Tech** 2023, 51, 371-377, doi:10.15258/sst.2023.51.3.09.

47. Hong, T.D.; Linington, S.; Ellis, R.H. **Seed storage behavior: a compendium. Handbooks for genebanks***:* n. 4; International Plant Genetic Resources Institute: Rome, Italy, 1996.

48. El-Keblawy, A. Effects of seed storage on germination of desert halophytes with transient seed bank. In *Sabkha Ecosystems.* **Tasks for Vegetation Science***,* vol 47, Khan, M.A., Böer, B., Öztürk, M., Al Abdessalaam, T.Z., Clüsener-Godt, M., Gul, B., Eds.; Springer: Dordrecht, The Netherlands, 2014.

49. Suleiman, M.K.; Bhatt, A.; Jacob, S.; Thomas, R.R.; Sivadasan, M.T. Seed longevity in desert species and the possibility of forming a persistent soil seed bank. **Sustainability** 2023, 15, 15904, doi:10.3390/su152215904.

50. Kohoma, S.; Maluf, A.M.; Bila, D.A.C.; Barbedo, C.J. Secagem e armazenamento de sementes de *Eugenia brasiliensis* Lam. (Grumixameira). **Rev Bras Sementes** 2006, 28, 72-78, doi:10.1590/S0101-31222006000100010.

51. Carvalho, N.M.; Nakagawa, J. **Sementes: ciência, tecnologia e produção**. 3. ed.; Fundação Cargill: Campinas, 1988.

52. Bhatt, A.; Gallacher, D.; Jarma-Orozco, A.; Fernandes, D.; Pompelli, M.F. Seed provenance selection of wild halophyte seeds improves coastal rehabilitation efficiency. **Estuar Coast Shelf Sci** 2022, 265, 107657, doi:10.1016/j.ecss.2021.107657.

53. Kigel, J. Seed germination in arid and semiarid regions. In **Seed development and germination**, Kigel, J., Galili, G., Eds.; Marcel Dekker: New York, 1995; pp. 645-697.

54. Khan, M.A.; Gul, B. Salt tolerant plants of coastal sabkhas of Pakistan. In **Sabkha ecosystems**, Barth, H., Boer, B., Eds.; Kluwer Academic Press: Netherlands, 2002; pp. 123-129.

55. Khan, M.A.; Ungar, I.A. Germination of salt tolerant shrub *Suaeda fruticosa* from Pakistan: salinity and temperature responses. **Seed Sci Tech** 1998, 26, 657-667.

56. Miranda, R.Q.; Correia, R.M.; Almeida-Cortez, J.S.; Pompelli, M.F. Germination of *Prosopis juliflora* (Sw.) D.C seeds at different osmotic potentials and temperatures. **Plant Species Biol** 2014, 29, e9-e20.

57. Rubio-Casal, A.E.; Castillo, J.M.; Luque, C.J.; Figueroa, M.E. Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. **J Arid Environ** 2003, 53, 145-154.

58. Sheikh-Mohamadi, M.-H.; Etemadi, N.; Nikbakht, A.; Farajpour, M.; Arab, M.; Majidi, M.M. Wheatgrass germination and seedling growth under osmotic stress. **Agron J** 2018, 110, 572-585, doi:10.2134/agronj2017.06.0364.

59. West, D.W.; Francois, L.E. Effects of salinity on germination, growth and yield of cowpea. *Irrig Sci* 1982, 3, 169-175, doi:10.1007/BF00446005.

60. Marcar, N.E. Salt tolerance in the genus *Lolium* (ryegrass) during germination and growth. **Aust J Agric Res** 1987, 38, 297-307, doi:10.1071/AR9870297.

61. Sharma, T.P.; Sen, D.N. A new report on abnormally fast germinating seeds of *Haloxylon* spp.: an ecological adaptation to saline habitat. **Curr Sci** 1989, 58, 382–385.

62. Mahmoud, A.; El Sheikh, A.M.; Baset, S.A. Germination of two halophytes: *Halopeplis perfoliata* and *Limonium axillare* from Saudi Arabia. **J Arid Environ** 1983, 6, 87–98, doi:10.1016/S0140-1963(18)31520-9.

63. Rogers, M.E.; Noble, C.L.; Halloran, G.M.; Nicolas, M.E. The effect of NaCl on germination and early seedling growth of white clover (*Trifolium repens* L.) population selected for high and low salinity tolerance. **Seed Sci Tech** 1995, 23, 277–287.

64. Naidoo, G.; Naicker, K. Seed germination in the coastal halophytes *Triglochin bulbosa* and *Triglochin striata*. **Aquat Bot** 1992, 42, 217–229, doi:10.1016/0304-3770(92)90023-C.

65. Zia, S.; Khan, M.A. Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*. **Can J Bot** 2004, 82, 151-157, doi:10.1139/B03-118.

66. Navarro, L.; Guitián, J. Seed germination and seedling survival of two threatened endemic species of the northwest Iberian peninsula. **Biol Conserv** 2003, 109, 313-320.

67. Martin, A.; Grzeskowiak, V.; Puech, S. Germination variability in three species in disturbed Mediterranean environments. **Acta Oecol** 1995, 16, 479-490.

68. Pompelli, M.F.; Fernandes, D.; Guerra, M.P. Somatic embryogenesis in *Dyckia distachya* Hassler (bromeliaceae) – an endangered bromeliad from south Brazil. **Propag Ornam Plants** 2005, 5, 192-198.

CHAPTER III

Dyckia distachya Hassler: AN ENDANGERED BROMELIAD MICROPROPAGATION BY SEED GERMINATION AND TISSUE CULTURE

M.Sc. Marvin José Perneth-Montaño

M.Sc. Ana Milena Vásquez-Bettin

M.Sc. Samuel Giovanny García-Castaño

Prof. Dr. Miguel P. Guerra

10.37885/250619544

ABSTRACT

The Atlantic Forest has a rich biological diversity and elevated levels of endemism, in which bromeliads are frequent but endangered. Tissue culture techniques comprises efficient tools to promote mass propagation of elite individuals for commercial aims, or the conservation of natural populations threatened with extinction. The present study evaluated aspects of seed-based *Dyckia distachya* micropropagation. A high rate of *in vitro* regeneration (78.93 shoots per gram) was obtained in response to liquid MS culture medium supplemented with 2 μM of 6-Benzylaminopurine (BA) after 120 days in culture or supplemented with 2 μM of 1-Naphthaleneacetic acid (NAA), 4 μM of BA, and 6 μM of Paclobutrazol (133.58 shoots per explant) after 142 days in culture. All shoots were grown until 1.5 cm long before acclimatization in *ex-vitro* conditions with 92.6% survival. *D. distachya* is responsive to applying tissue culture techniques for mass propagation and conservation purposes. Using seeds as initial explants allows the maintenance of the genetic diversity observed in the natural populations. Mass regeneration is a promising technique for efficiently conserving this endangered bromeliad.

Keywords: atlantic forest; deforestation; organogenesis; tissue culture; bromeliads.

INTRODUCTION

The Brazilian Atlantic Forest encompasses diverse ecosystems, including mangroves and rainforests. This biome's structure and floristic composition are notably distinct and influenced by several factors, including soil diversity, topography, and climates [1]. Atlantic Forest is a biome listed as a biodiversity hotspot, showing elevated levels of endemism [2]. This diverse biome, spanning across Brazil, Paraguay, and Argentina, has captured the attention of scientists, conservationists, and policymakers alike. With its exceptional range of species found nowhere else on Earth, the Atlantic Forest has rightfully earned its status as one of the world's most crucial ecological hotspots [2]. However, the destruction of this biome and its associated ecosystems caused an impressive reduction in its biodiversity. This impact also cut back the epiphytic flora rich in bromeliad species. The devastation of the Atlantic Forest, hydroelectric plant constructions and expressive endemism have restricted the *Dyckia distachya* bromeliads to a few subsisting along the rocky banks of the Uruguay River in the west of Santa Catarina and the south of Paraná state and in restricted areas of Paraguay and Argentina. This species is similar to *Dyckia brevifolia*, from which it differs mainly in the number of leaves, the more appealing rosette, and the robust and ramified inflorescence [3].

Conventional propagation methods for bromeliads are not appropriate for large-scale plant production, as required for ornamental or endangered species [4]. In bromeliads, seed propagation methods do not allow for the production of a homogenous population [5], leading to variabilities in colorful and plant architecture [6,7]. Furthermore, cutting propagation is constrained due to the limited quantity of individual plants that can be acquired simultaneously from a single mother-plant [6,8,9]. Moreover, micropropagation techniques are considered viable for small to large-scale production over a short period [9-12].

Tissue culture-based techniques are up-and-coming to overcome these constraints. However, the consistency of the culture medium influences micropropagated tissue culture. Jellified and liquid culture media are used in different in vitro protocols. Liquid culture medium allows the best contact of the culture with the medium [13], enhancing the induction and development of adventitious shoots in the bromeliads *Quesnelia quesneliana, Vriesea poelmanii,* and *Aechmea fasciata* [14-18]. The present study investigated inductive conditions for the in

vitro morphogenesis of *Dyckia distachya*, aiming at the conservation and mass propagation of this endangered Brazilian bromeliad.

MATERIAL AND METHODS

Direct shoot induction from seeds

The seed collection process was meticulous and followed the recommended procedures by Moncaleano-Escandon, *et al.* [19]. Mature seeds were collected from plants of natural populations in the Uruguay River basin, western Santa Catarina State. These seed-containing capsules were carefully sealed in polyethylene bags to ensure their safe transportation. The seeds were then transported to the Laboratory of Developmental Physiology and Genetics of the Federal University of Santa Catarina, Florianópolis, Santa Catarina, in the south of Brazil, where the study was conducted.

The seeds were immersed in a solution of 70% (v/v) ethanol for 3 min under aseptic conditions and then in a 5% NaOCl solution for 30 min and rinsed five times with sterilized water. Ten seeds were inoculated in each Petri dish (100 mm x 20 mm) containing 30 mL MS culture medium [20], Morel and Wetmore [21] vitamins, 30 g L^{-1} of sucrose (Sigma Aldrich, St. Louis, MO, USA, part number S1174), 6 g L^{-1} agar base (w/v) (Sigma Aldrich, part number A1296), and supplemented with two plant growth regulators (PGR) such 6-Benzylaminopurine (BA, Sigma Aldrich, part number B3408) or Kinetin (Kin, Sigma Aldrich, part number K3378). The concentration of both PGR was 0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, and 30 μM L^{-1} of both PGR without interaction between them, where zero denotes a free-PGR treatment.

Each treatment consisted of three replicates in four Petri dishes arranged in a completely randomized design. After 60 days of culture the fresh weight and multiplication rates were recorded. The cultures were subsequently reinoculated in 250 mL flasks containing 25 mL liquid culture medium. The formation of shoot direct from *D. distachya* seeds were evaluated for the fresh weight and multiplication rates.

The data of fresh weight were transformed by $\sqrt[2]{x + 0.5}$ and the number of shoots by $\log_2 x+1$ and then submitted to ANOVA and SNK test of mean separation (Newman-Keuls test; SNK at $P \leq 0.05$).

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Indirect shoot induction

Seeds were collected and disinfected as described above and inoculated in Petri dishes containing 30 mL KC culture medium [22] jellified with 10 g L^{-1} agar base (w/v) (Sigma Aldrich, part number A1296). Each Petri dish was inoculated with ten seeds. After germination the plantlets were individually transferred to test tubes (150 mm x 25 mm) containing 10 mL of the same culture medium as described above. Plantlet roots of at least 1.5 cm length were excised and inoculated in 250 mL flasks containing 30 mL of culture medium as described below.

Thirteen treatments with a combination of BA, 2-Naphthaleneacetic acid (NAA, Sigma Aldrich, part number N4002) or α-tert-Butyl-β-(4-chlorobenzyl)--1H-1,2,4-triazole-1-ethanol (Paclobutrazol, PBZ, Sigma Aldrich, part number 19847). The basal medium composition was based on liquid or jellified (agar base 6 g L^{-1}) MS culture medium as described in Table 1. All culture media was supplemented with 30 g L^{-1} sucrose (Sigma Aldrich, part number S9378).

Table 1. Composition of KC culture medium supplemented with different PGR for the micropropagation of *Dyckia distachya* Hassler.

Media consistency	NAA (μM L^{-1})	BA (μM L^{-1})	PBZ (μM L^{-1})	Abbreviation
Liquid	0	0	0	PGR-free liquid
Jellified	0	0	0	PGR-free jellified
Liquid	2	4	0	N2B4 liquid
Jellified	2	4	0	N2B4 jellified
Liquid	2	4	3	N2B4P3 liquid
Jellified	2	4	3	N2B4P3 jellified
Liquid	2	4	6	N2B4P6 liquid
Jellified	2	4	6	N2B4P6 liquid
Liquid	0	0	5	PBZ5 liquid
Jellified	0	0	5	PBZ5 liquid
Liquid	1	1	0	N1B1 liquid
Liquid	1	2	0	N1B2 liquid
Liquid	1	2	3	N1B1P3 liquid

Each treatment consisted of three replicates in four flasks or Petri dishes arranged in a completely randomized design. Subcultures were established in fresh medium every 30 days. The data of fresh weight were transformed by x + 0.5 and the number of shoots by $\log_2 x$ +2, then submitted to ANOVA and the mean separation test (SNK $P \leq 0.05$) by Statistic version 14.0 (StatSoft, Tulsa, OK, USA).

Acclimatization

Shoots of at least 1.5 cm length were acclimatized in trays containing a substrate mixture of peat, vermiculite and sand (2:2:1) and maintained in a greenhouse under 50% light intensity promoted by black nylon net.

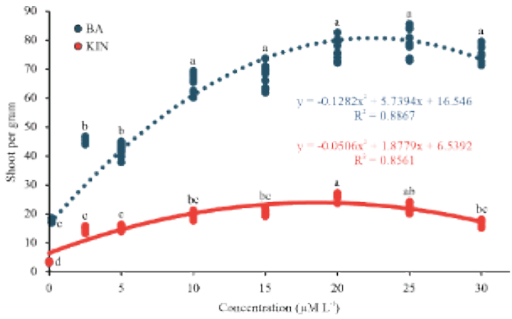
RESULTS AND DISCUSSION

Direct organogenesis from seeds

After 30 days in culture all viable seeds germinated, most of them presenting the formation of adventitious shoots induced by the PGR in the culture medium. Similarly, Fischer and Zimmer [23] reported that bromeliad seeds germinated in cytokinin supplemented culture medium originated more than one shoot.

Both BA and KIN increased the shoot regeneration under increased levels of this PCR. Under BA, the highest shoot regeneration (77.1 ± 3.5 shoot per gram) occurred in response to 20 μM L^{-1} (Fig.1), then decreased in response to 30 μM L^{-1}. The shoot regeneration values were not statistically significant in BA between 10 μM L^{-1} to 30μM L^{-1}. The highest shoot regeneration in response to KIN occurred in 20 μM L^{-1}, the same concentration verified as BA. Levels of KIN ranging from 20 μM L^{-1} to 25 μM L^{-1} did not show differences in regenerative rate; levels of 30 μM L^{-1} KIN showed toxicity (Fig. 1). In comparison, BA induced 3-fold more shoot per gram than KIN at the same concentration. In the micropropagation of apricot, TDZ attained best results [24]. This cytokinin was however less effective than BA in the micropropagation of *Cryptanthus sinuosus*, a Brazilian bromeliad threatened with extinction [25]. The regeneration rate in *Centella* asiatica micropropagation was enhanced in response to 22 μM L^{-1} BA [26].

Figure 1. *In vitro* regeneration rate of *Dyckia distachya* in response to 6-Benzylaminopurine (BA) and/or Kinetin (KIN), after 120 days in culture.



ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Shoot multiplication

The highest in vitro regeneration rate occurred in the liquid culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (6 μM L^{-1}). This treatment resulted in the induction rate of 21.31, 80.96, 121.93, and 133.58 shoots per explant after 45, 75, 112, and 142 days in culture, respectively (Fig. 2). However, the best multiplication rate (72.94) was observed in response to the jellified MS culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (6 μM L^{-1}) after 112 days in culture (Fig. 3).

Figure 2. Evolution of number of shoots per explant of *Dyckia distachya* cultivated in jellified (J) or liquid (L) MS culture medium supplemented with 0, 1 and 2 μM L^{-1} 2-Naphthaleneacetic acid (NAA, N), 0, 1, 2 and 4 μM L^{-1} 6-Benzylaminopurine (BA, B) and 0, 3, 5 and 6 μM L^{-1} (Paclobutrazol, PBZ, P). Means followed by the same letter are not statistically different (SNK $P \leq 0.05$)

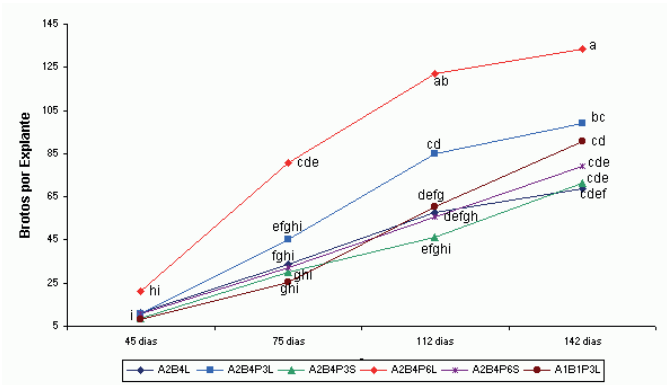
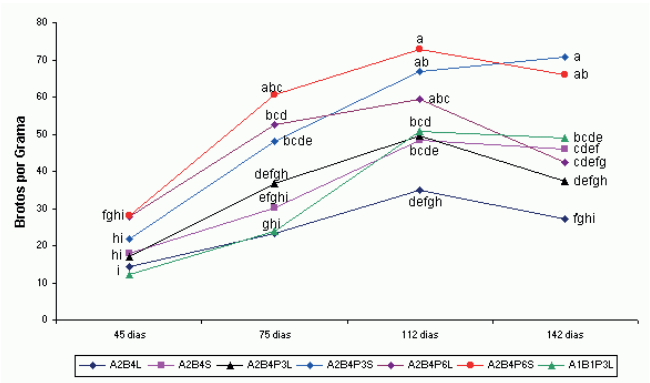


Figure 3. Evolution of number of *Dyckia distachya* shoots per gram of fresh mass in jellified (J) or liquid (L) MS culture medium supplemented with 0, 1 and 2 μM 2-Naphthaleneacetic acid (NAA, N), 0, 1, 2 and 4 μM 6-Benzylaminopurine (BA, B) and 0, 3, 5 and 6 μM PBZ (Paclobutrazol, PBZ, P). Means followed by the same letter are not statistically different (SNK $P \leq 0.05$).



Compared to the multiplication rates commonly observed in response to different micropropagation protocols the regenerative rates observed in the present study were high, revealing high efficiency of this protocol. For example, for the endemic bromeliad of the Atlantic Forest *Aechmea fasciata* the best multiplication rate for the was 4.53 shoots per explant in response to the culture medium supplemented with 2.0 mg L^{-1} KIN and IAA [27]. Similar results were reported by Hosoki and Asahira [14] in the micropropagation of *Vriesea poelmanii, Aechmea fasciata* and *Quesnelia quesneliana* where 4.4 and 8.0 adventitious shoots were induced, respectively, in response to 1.0 mg L^{-1} NAA and BA. The highest multiplication rate in pineapple micropropagation was 13.5 shoots per explant in response to the culture medium supplemented with 2 μM NAA L^{-1} and 4 μM BA L^{-1} [28]. For Tillandsia cyanea, 14.6 shoots per explant were obtained in response to the culture medium supplemented with 0.2 mg L^{-1} BA [29]. Also, the highest regeneration rate (22.5 shoots per explant) in *Vriesea fosteriana* was observed in response to the KC culture medium supplemented with 2.7 μM NAA L^{-1} and 8.9 μM L^{-1} BA [30]. The micropropagation of Vriesea friburgensis achieved 231.86 shoots starting from 0.10 g microbuds after 60 days in culture medium supplemented with 2 μM NAA L^{-1} and 4 μM^{-1} BA [31]. Only one study on Dyckia was found in the revised literature. *Dyckia macedoi* showed a multiplication rate of 9 shoots per explant in response to the culture medium supplemented with 0.1 mg L^{-1} NAA and 5.0 mg L^{-1} BA [32].

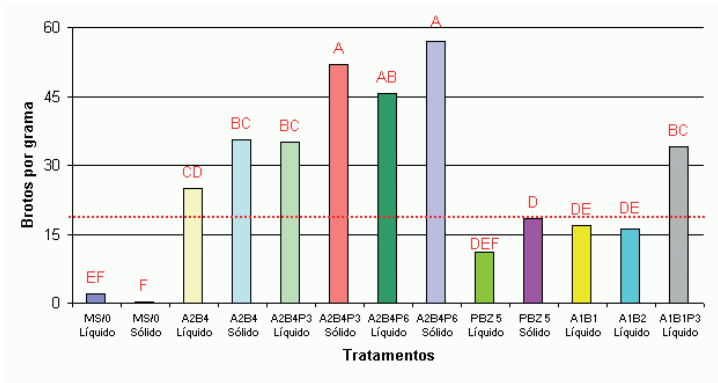
In the present study, the MS liquid culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}), and PBZ (6 μM L^{-1}) resulted in the regeneration of 89.44 shoots per explant. However, jellified MS culture media supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}), and PBZ (3 and 6 μM L^{-1}) resulted in mean values of 56.88 shoots per gram of fresh mass, respectively. These values did not show differences from those observed in response to the MS liquid medium supplemented with 2 μM L^{-1} NAA , 4 L^{-1} μM BA and 6 μM L^{-1} PBZ but were statistically different from those observed in the other treatments.

The treatments with a 1/3 higher regeneration in response to the culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (6 μM L^{-1}) were selected for a more detailed study into the subculture effects (dotted line in Fig. 4 and 5 shows the limit to be used). Six treatments were chosen for the parameter shoots per explants: liquid MS culture medium supplemented with NAA (2 μM L^{-1}) and BA (4 μM L^{-1}); jellified and liquid MS culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (3 and 6 μM L^{-1}),

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

and; liquid MS culture medium supplemented with NAA (1 μM L^{-1}), BA (1 μM L^{-1}) and PBZ (3 μM L^{-1}). Considering the parameter shoots per inoculum seven treatments were selected: jellified and liquid MS culture medium supplemented with NAA (2 μM L^{-1}) and BA (4 μM L^{-1}); jellified and liquid MS culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (3 and 6 μM L^{-1}), and; liquid MS culture medium supplemented with NAA (1 μM L^{-1}), BA (1 μM L^{-1}) and PBZ (3 μM L^{-1}). Among these it is possible to show the efficiency of the liquid MS culture medium supplemented with NAA (2 μM L^{-1}), BA (4 μM L^{-1}) and PBZ (6 μM L^{-1}) (Fig. 2).

Figure 4. Shoot number per explant of *Dyckia distachya* cultivated in jellified or liquid MS [20] culture medium supplemented with 2-Naphthaleneacetic acid (NAA, N), 6-Benzylaminopurine (BA, B) and Paclobutrazol (PBZ, P) after 142 days in culture. PGR-free = culture medium without plant growth regulator. Means followed by the same letter are not statistically different (SNK $P \leq 0.05$).

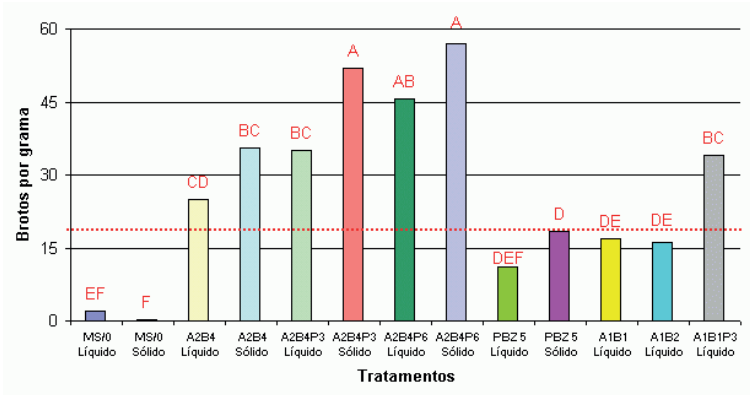


The highest multiplication media was generally observed in the third subculture, with a reduction in the fourth subculture. The highest value in the third subculture (72.94 shoots per gram of fresh mass) was observed in the jellified MS culture medium supplemented with 2 μM L^{-1} NAA, 4 μM L^{-1} BA and 6 μM L^{-1} PBZ (Fig. 3). The shoot regeneration obtained in the present work was too high if is considered the 2.7 or 2.3 regeneration rate in *Dyckia brevifolia* under 1 mg L^{-1} of BA or N6-(2-Isopentenyl)adenine (2-iP) [33].

The presence of hyperhydric shoots was observed in most of the treatments in the fourth subculture (Fig. 6-H and I). Hyperhydric tissue cells show alterations in the cytoplasm with consequent loosening of the cell walls and a low photosynthetic rate responsible for the translucent aspect of hyperhydric plants [34]. Hyperhydric shoots present less apical dominance and damage in the cell wall [34]. In tissue culture, cytokinins can lead to a hyperhydricity

of shoots with restrictive effects in the micropropagation efficiency [35]. This occurrence was also reported for *Fragaria x ananassa* [36] and *Vriesea friburgensis* [31] in vitro cultures.

Figure 5. Shoot number per gram of fresh mass of *Dyckia distachya* cultivated in jellified or liquid MS [20] culture medium supplemented with 2-Naphthaleneacetic acid (NAA, N), 6-Benzylaminopurine (BA, B) and Paclobutrazol (PBZ, P) after 142 days in culture. PGR-free = culture medium without plant growth regulator. Means followed by the same letter are not statistically different (SNK $P \leq 0.05$).



Liquid medium enhanced the multiplication rate and effectively reduced the explant oxidation and improved the in vitro regeneration rates of *Neoregelia cruenta*, a Brazilian endangered bromeliad [4]. It was also observed that lateral or axillary?? Buds grew faster in liquid than in jelled medium [14]. It is well documented that the nutrient uptake in bromeliads occurs not only through the roots but the leaves as well [37]. This fact could explain the efficiency of the liquid mediumÏ observed in the present study. It should be stressed that agar is one of the most expensive components of the culture medium and consequently increases micropropagation costs [38].

The present study also observed that cultures maintained in the presence of PBZ formed thick and short roots. Conversely, long and thin roots were induced in a PGR-free culture medium. Cultures maintained in PBZ-supplemented culture medium frequently showed toxic effects ranging from a yellow coloring to the culture's death (Fig. 6-G). PBZ affects the gibberellin synthesis, resulting in reduced growth in *in vitro* cultures. These effects were also observed in sugarcane [39] and pineapple [40]. In the treatments with low PBZ levels, an enhancement in the shoot development with normal features was observed (Fig. 6-J). When cultured in the absence of PBZ, the shoots were often etiolated (Fig. 6-K). All

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

data described here agreed with a recently published study with *D. brevifolia*, where rooted plantlets produced 2.1 and 2.3 roots per plantlets of 1.2 and 1.1 cm in length [33].

Elongated shoots (at least 1.5 cm) cultured in MS culture medium free of PGR were successfully transferred and acclimatized to ex vitro conditions. Shoots shorter than 1.5 cm were not able to survive ex vitro. Consequently, the shoots must be maintained in vitro until they reach this minimum size. A survival rate of 92.6% was observed after 120 days of ex vitro conditions (Fig. 7). The acclimatized plants showed regular morphological features after two years in a greenhouse. This survival rate is in accordance with studies of *D. brevifolia*, where rooted micro shoots were acclimatized in pots where they grew with a survival rate of 100% after two months [33].

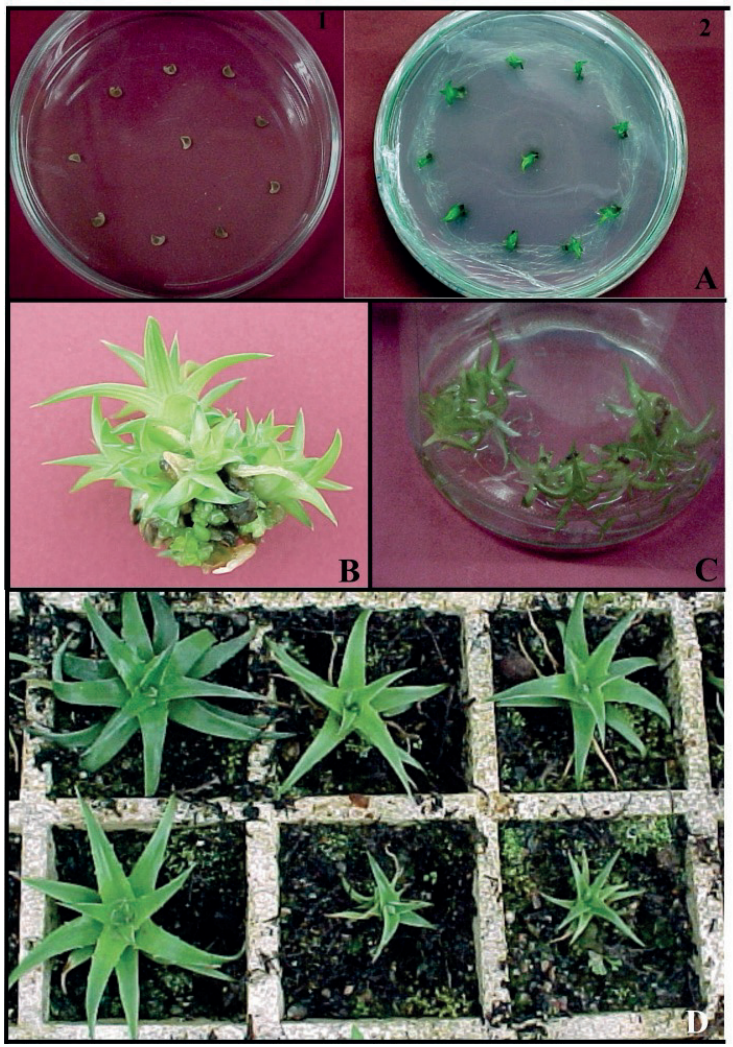
In conclusion, the results of the present study demonstrated that tissue culture techniques are valuable tools for D. distachya mass propagation. High rates of in vitro regeneration were obtained in response to liquid MS culture medium supplemented with BA 2 $\mu M\ L^{-1}$ after 120 days in culture, or supplemented with NAA 2 $\mu M\ L^{-1}$, BA 4 $\mu M\ L^{-1}$ and PBZ 6 $\mu M\ L^{-1}$ after 142 days in culture. Using seeds as initial explants allows the maintenance of the genetic diversity observed in the natural populations. This is an essential approach to the conservation of this endangered bromeliad.

The Uruguay River is a major river in South America. It flows from north to south and forms parts of the boundaries of Brazil, Argentina, and Uruguay. Due to the hydroelectric ventures in its basin, eight of the nine strictly endemic *Dyckia distachya* populations became locally extinct. To avoid local extinction of this species, a conservation plan was drawn up, including activities to rescue natural populations, relocation to tributaries of the Barra Grande AHE (inter situ conservation), maintenance of a reference population in a nursery [41-44] as well as the development of the protocol for mass propagation here described. In agreement of efforts of the company "Meio Biótico Serviços Ambientais" [45] D. distachya population could be saved, regenerated, and available to the future generations.

Figure 6. Micropropagation of *Dyckia distachya.* A. Seed inoculation (left) and germination after 30 days (right). B. Plantlets elongated individually in test tubes. C. Subculture in liquid medium, and D. jellified. Bar - 1 cm. E. Cluster of shoots (arrows). F. Elongation of shoots in liquid MS culture medium free of PGR. Bar – 1 cm. G. Phytotoxicity caused by PBZ. H, I. Hyperhydricity (see arrows) of shoots in liquid culture medium. Bar 1 cm and 5mm. J. Normal shoots formed in PBZ-supplemented culture medium. Bar 1 cm. K. Etiolated shoots. Bar - 1 cm.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 7. Micropropagation of *Dyckia distachya*. A. Seeds inoculation (1) and seed germination after 30 days (2). B. Clusters of multiple cultivation cycle. C. Individual plantlets after elongation. D. 6-months *Dyckia distachia* acclimatized plants.



DIRECT STUDY

Bertsouklis, K. and K.P. Panagaki. 2022. In vitro germination and propagation of *Dyckia brevifolia*, an ornamental and endangered bromeliad. **Horticulturae** 8: 390. doi:10.3390/horticulturae8050390.

Biótico, M. 2024. Manutenção e Monitoramento de Reófitas Relocadas (Reintrodução de *Dyckia distachya* Hassler). Meio Biótico.

Pompelli, M.F., D. Fernandes and M.P. Guerra. 2005. Somatic embryogenesis in *Dyckia distachya* Hassler (bromeliaceae) – an endangered bromeliad from south Brazil. **Propag Ornam Plants** 5: 192-198.

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

Pompelli, M.F. and M.P. Guerra. 2004. Ex situ conservation of *Dyckia distachya*: an endangered bromeliad from South Brazil. **Crop Breed Appl Biot** 4: 273-279.

Pompelli, M.F. and M.P. Guerra. 2005. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. **Crop Breeding and Applied Biotechnology** 5: 117-126.

Bertsouklis, K. and K.P. Panagaki. 2022. In vitro germination and propagation of *Dyckia brevifolia*, an ornamental and endangered bromeliad. **Horticulturae** 8: 390. doi:10.3390/horticulturae8050390.

Biótico, M. 2024. Manutenção e Monitoramento de Reófitas Relocadas (Reintrodução de *Dyckia distachya* Hassler). Meio Biótico.

Research Agreement: This chapter was written based on its original form published in Crop Breeding and Applied *Biotechnology. Pompelli*, M.F.; Guerra M.P. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. **Crop Breeding And Applied Biotechnology**, 5: 117-126, 2005. Available at https://www.proquest.com/docview/1936391547?pq-origsite=gscholar&fromopenview=true&sourcetype=Scholarly%20Journals. Under the permission of authors. Free access.

REFERENCES

1. Patricia, L.; Morellato, C.; Haddad, C.F.B. Introduction: The Brazilian Atlantic Forest. **Biotropica** 2000, 32, 786-792, doi:10.1111/j.1744-7429.2000.tb00618.x.

2. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Fonseca, G.A.B.; Kent, J. Biodiversity hotspots for conservation priorities. **Nature** 2000, 403, 853-858.

3. Strehl, T. Periodically submersed bromeliads. **Bromélia** 1994, 3, 19-21.

4. Carneiro, L.A.; Araújo, R.F.G.; Brito, G.J.M.; Fonseca, M.H.P.B.; Costa, A.; Crocomo, O.J.; Mansur, E. In vitro regeneration from leaf explants of *Neoregelia cruenta* (R. Graham) L.B. Smith, an endemic bromeliad from Eastern Brazil. **Plant Cell Tiss Organ Cult** 1999, 55, 79-83.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

5. Falcioni, R.; Moriwaki, T.; Perez-Llorca, M.; Munné-Bosch, S.G., Mariana Sversut; Sato, Francielle; Pelozo, Andressa; Pattaro, Mariana Carmona; Giacomelli, Marina Ellen; Rüggeberg, Markus; Antunes, Werner Camargos. Cell wall structure and composition is affected by light quality in tomato seedlings. **JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY B-BIOLOGY**, v. 203, p. 111745, 2020.

6. Jitendra, M.; Monika, S.; Ratan, S.D.; Priyanka, G.; Priyanka, S.; D.J., K. Micropropagation of an Anti diabetic Plant - *Stevia rebaudiana* Bertoni, (Natural Sweetener) in Hadoti Region of South-East Rajasthan, India. **J Biol Sci** 2012, 1, 37-42.

7. Ferreira, C.M.; Handro, W. Micropropagation of *0* through leaf explants from adult plants. **Planta Med** 1988, 54, 157-160, doi:10.1055/s-2006-962377. PMID: 17265228.

8. Ahmed, M.B.; Salahin, M.; Karim, R.; Razvy, M.A.; Hannan, M.M.; Sultana, R.; Hossain, M.; Islam, R. An efficient method for in vitro clonal propagation of a newly introduced sweetener plant (*Stevia rebaudiana* Bertoni.) in Bangladesh. **Am Eurasian J Sci Res** 2007, 2, 121-125.

9. Sivaram, L.; Mukundan, U. *In vitro* culture studies on *Stevia rebaudiana*. ***In Vitro*** **Cell Dev Biol Plant** 2003, 39, 520-523, doi:10.1079/IVP2003438.

10. Medorio-García, H.P.; Hernández-Domínguez, E.; Andueza-Noh, R.H.; López-Aguilar, D.R.; Ramírez-Mosqueda, M.A. Micropropagation of Stevia (*Stevia rebaudiana* Bert.) in RITA. In Micropropagation Methods in Temporary Immersion Systems. **Methods in Molecular Biology**, vol 2759, Ramírez-Mosqueda, M.A., Cruz-Cruz, C.A., Eds.; Humana: New York, 2024.

11. Razak, U.N.A.A.; Ong, C.B.; Yu, S.T.; Lau, L.K. In vitro Micropropagation of *Stevia rebaudiana* Bertoni in Malaysia. **Braz Arch Biol Technol** 2014, 57, 23-28, doi:10.1590/S1516-89132014000100004.

12. Pande, S.S.; Gupta, P. Plant tissue culture of *Stevia rebaudiana* (Bertoni): A review. **J Pharmacognosy Phytother** 2013, 5, 26-33, doi:10.5897/JPP13. 0258.

13. Teisson, C.; Alvard, D.; Berthouly, B.; Côte, F.; Escalant, J.V.; Etienne, H.; Lartaud, M. Simple apparatus to perform plant tissue culture by temporary immersion. **Acta Hortic** 1996, 440, 521-526, doi:10.17660/ActaHortic.1996.440.91.

14. Hosoki, T.; Asahira, T. In vitro propagation of bromeliads in liquid culture. **Hort Sci** 1980, 15, 603-604.

15. Guerra, M.P.; Dal Vesco, L.L. Strategies for the micropropagation of bromeliads. In Protocols for in vitro propagation of ornamental plants. **Methods in Molecular Biology**, vol 589, Jain, S., Ochatt, S., Eds.; Humana Press: Totowa, New Jersey, USA, 2010.

16. Dal Vesco, L.L.; Vieira, P.M.; Corredor, J.P.; Pescador, R.; Welter, L.J.; Guerra, M.P. Induction and development of nodular cluster cultures in *Vriesea reitzii* (Leme and Costa), an endangered bromeliad from the Brazilian Atlantic Forest. **J Hortic Sci Biotech** 2014, 89, 542–548, doi:10.108 0/14620316.2014.11513118.

17. Filho, A.; Dal Vesco, L.L.; Nodari, R.; Lischka, R.W.; Müller, C.V.; Guerra, M.P. Tissue culture for the conservation and mass propagation of *Vriesea reitzii* Leme and Costa, a bromeliad threatened of extinction from the Brazilian Atlantic Forest. B**iodivers Conserv** 2005, 14, 1799–1808, doi: 10.1007/s10531-004-0700-5.

18. Martins, J.P.R.; Rodrigues, L.C.A.; Silva, T.S.; Rossini, F.P.; Gontijo, A.B.P.L.; Falqueto, A.R. Morphophysiological responses of *Aechmea blanchetiana* (Bromeliaceae) to excess copper during in vitro culture. **Plant Biosyst** 2020, 153, 447–456, doi:10.1080/11263504.2020.1756976.

19. Moncaleano-Escandon, J.; Silva, B.C.F.; Silva, S.R.S.; Granja, J.A.; Alves, M.C.J.L.; Pompelli, M.F. Germination responses of *Jatropha curcas* L. seeds to storage and aging. **Ind Crops Prod** 2013, 44, 684-690, doi:doi: 10.1016/j.indcrop.2012.08.035.

20. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiol Plant** 1962, 15, 473-497, doi:10.1111/j.1399-3054.1962.tb08052.x.

21. Morel, G.M.; Wetmore, R.H. Fern callus tissue culture. **Am J Bot** 1951, 38, 141-143.

22. Knudson, L. A New nutrient soluction for the germination of orchid seed. **Am Orch Soc Bull** 1946, 15, 214-217.

23. Fischer, G.; Zimmer, K. Regeneration of germinating seeds *in vitro*. **Acta Hortic** 1988, 226, 615-618, doi:10.17660/ActaHortic.1988.226.83.

24. Tornero, O.P.; Egea, J.; Vanoostende, A.; Burgos, L. Assessment of factors effecting adventitious shoot regeneration from in vitro cultured leaves of apricot. **Plant Sci** 2000, 158, 61-70, doi:10.1016/s0168-9452(00)00303-4.

25. Carneiro, L.A.; Cândido, M.D.; Araújo, R.F.G.; Fonseca, M.H.P.B.; Crocomo, O.J.; Mansur, E. Clonal propagation of *Cryptanthus sinuosus* L.B. Smith, an endemic stoloniferous bromeliaceae species from Rio de Janeiro, Brazil. **Plant Tissue Cult Biotechnol** 1998, 4, 152-158.

26. Tiwari, K.N.; Sharma, N.C.; Tiwari, V.; Singh, B.D. Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. P**lant Cell Tiss Org Cult** 2000, 63, 179-185, doi:10.1023/A:1010690603095.

27. Vinterhalter, B.; Vinterhalter, D. True-to-the-type in vitro propagation of *Aechmea faciata* Baker. **Sci Hort Amsterdam** 1994, 57, 253-263, doi:10.1016/0304-4238(94)90145-7.

28. Dal Vesco, L.L.; Pinto, A.A.; Zaffari, G.R.; Nodari, R.O.; dos Reis, M.S.; Guerra, M.P. Improving pineapple micropropagation protocol through explant size and medium composition manipulation. **Fruits** 2001, 56, 143-154.

29. Pierik, R.L.M.; Sprenkels, P.A. Micropropagation of *Tillandsia cyanea*. **J Bromeliad Soc** 1991, 1, 9-12.

30. Mercier, H.; Kerbauy, G.B. In vitro multiplication of *Vriesea fosteriana*. **Plant Cell Tiss Org Cult** 1992, 30, 247-249, doi:10.1007/BF00040029.

31. Alves, G.M.; Guerra, M.P. Micropropagation for mass propagation and conservation of *Vriesea friburgensis* var. paludosa from microbuds. **J Bromeliad Soc** 2001, 51, 202-212.

32. Mercier, H.; Kerbauy, G.B. Micropropagation of *Dyckia macedoi* - an endangered endemic *Brazilian bromeliad*. **Bot Gard Microprog News** 1993, 1, 70-72.

33. Bertsouklis, K.; Panagaki, K.P. In vitro germination and propagation of *Dyckia brevifolia*, an ornamental and endangered bromeliad. **Horticulturae** 2022, 8, 390, doi:10.3390/horticulturae8050390.

34. Kevers, C.; Goldberg, R.; Chu-Ba, J.; Gaspar, T.H. Composition of the walls of stem and leaves of vitrifying carnation. **Biol Plant** 1988, 30, 219-223, doi:10.1007/BF02878763.

35. Nobre, J. *In vitro* cloning and micropropagation of *Lavandula stroechas* from field-grown plants. **Plant Cell Tiss Org** 1996, 46, 151-155, doi:10.1007/BF00034849.

36. Lopez-Aranda, J.M.; Pliego-Alfaro, F.; Lopez-Navidad, I. Micropropagation of strawberry (*Fragaria x ananassa* Duch.). Effect of mineral salts, benzyladenine levels and number of subcultures on the in vitro and field behaviour of the obtained microplants and fruiting capacity of their progeny. **J Hort Sci Biotechnol** 1994, 69, 625-637, doi:10.1080/14620316.1994.11516494.

37. Benzing, D.H. **Bromeliaceae: profile of an adaptive radiation**; Cambridge University Press: Cambridge, 2000; p. 690.

38. George, E.F. **Plant propagation by tissue culture**, part 1; The Technology: Exegetic, London, 1993; p. 574.

39. Lorenzo, J.C.; Blanco, M.A.; Peláez, O.; González, A.; Cid, M.; Iglesias, A.; González, B.; Escalona, M.; Espinosa, P.; Borroto, C. Sugarcane micropropagation and phenolic excretion. **Plant Cell Tiss Org Cult** 2001, 65, 1-8, doi:10.1023/A:1010666115337.

40. Feuser, S.; Nodari, R.O.; Guerra, M.P. Eficiência comparativa dos sistemas de cultura estacionária e imersão temporária para a micropropagação do abacaxizeiro. **Rev Bras Frutic** 2001, 23, 6-10.

41. Pompelli, M.F.; Guerra, M.P. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. **Crop Breed Appl Biotechnol** 2005, 5, 117-126.

42. Pompelli, M.F.; Guerra, M.P. Ex situ conservation of *Dyckia distachya*: an endangered bromeliad from South Brazil. C**rop Breed Appl Biotechnol** 2004, 4, 273-279.

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

44. Pompelli, M.F.; Fernandes, D.; Guerra, M.P. Somatic embryogenesis in *Dyckia distachya* Hassler (bromeliaceae) – an endangered bromeliad from south Brazil. **Propag Ornam Plants** 2005, 5, 192-198.

45. Biótico, M. Manutenção e Monitoramento de Reófitas Relocadas (Reintrodução de *Dyckia distachya* Hassler). Meio Biótico 2024.

SECTION II

CROP PHYSIOLOGY

Water, plant life, and the science behind modern agricultural production form an essential link in understanding the major challenges and possibilities for a sustainable future. In this second section of the book, readers will be guided through reflections that connect water availability, plant defense mechanisms under stress conditions, and the inner workings of photosynthesis. Together, these topics create a comprehensive overview of the relationship between nature, technology, and survival in agriculture. The three chapters presented here are in close dialogue, each revealing crucial aspects of present and future agricultural practices.

In chapter five, the focus shifts to strategies for mitigating salinity stress in maize plants, particularly through foliar application of proline. This experimental study, carried out on two maize cultivars, shows that proline can significantly alleviate the damaging effects of salt stress, helping to maintain photosynthetic activity, chlorophyll levels, and cellular membrane stability. The findings also highlight genotypic differences in response to treatment, suggesting the potential for tailored applications in agricultural settings. This represents a practical and physiological innovation that holds promise for boosting productivity under harsh soil conditions.

Chapter six concludes this journey with an engaging experimental approach to the inner dynamics of photosynthesis. Using the Hill reagent DCPIP and the herbicide Diuron, the chapter explains how interference with the electron transport chain can effectively halt photosynthesis, ultimately causing plant death. Besides presenting these experiments in an accessible way, the text revisits key historical milestones in photosynthesis research, highlighting the roles of chloroplasts and light capture systems. What is often an invisible and abstract process becoming tangible, offering the reader a clear view into the chemistry of light, electrons, and plant survival.

By the end of this section, readers will have explored more than just data and discoveries—they'll have gained a broad understanding of how science, nature, and technology intertwine in the challenge of producing food in a world of finite resources. From water scarcity to biotechnological innovations in plant stress tolerance, each chapter provides a vital piece of a complex puzzle that calls for integrated and sustainable solutions. This journey informs, inspires, and lays

the groundwork for deeper insights into the following sections.
So, grab your scientific explorer's hat and dive into this rich field of knowledge! Section II is like a fertile crop of ideas—full of surprises, rooted in the soil, reaching toward the sun, and pulsing with the heartbeat of science. Between a drop of water, a beam of light, and a resilient maize leaf, you'll discover that the world of plants is more vibrant and fuller of life than you ever imagined. Happy reading—and don't be surprised if you fall in love with plants along the way!

The authors

CHAPTER IV

WATER IN THE WORLD: AVAILABILITY, SHORTAGE AND BENEFITS AND HARMS OF WASTEWATER IN MODERN AGRICULTURE

M.Sc. Ana Milena Vásquez-Bettin

Prof. Dr. Luis Eliécer Oviedo Zumaque

M.Sc. Samuel Giovanny García-Castaño

10.37885/250619545

ABSTRACT

Freshwater accounts for only 2.5% of the world's total, with most of it inaccessible because it is trapped in glaciers or deep aquifers. Groundwater, which makes up 30.1% of freshwater, is crucial for human consumption, agriculture and industry. However, excessive extraction, especially for irrigation in arid and semi-arid regions, has depleted aquifers and caused pollution and subsidence. Agriculture consumes almost 70% of the freshwater extracted, with crops such as rice and wheat standing out for their high demand. Inefficiencies in irrigation and lack of wastewater reuse exacerbate the problem. Climate change alters the water balance, intensifying rainfall variability, floods and droughts, threatening water availability and food security. Although some countries have abundant water resources, they face regional scarcity due to unsustainable practices and weather extremes, while arid nations rely on groundwater and food trade. Research by Dorta-Santos *et al.* (https://doi.org/10.3390/su6106902) suggests that sustainable water management, integrating advanced technologies and global cooperation, is essential to ensure equitable access and mitigate environmental impacts. Reusing wastewater for irrigation is presented as a viable solution to reduce dependence on freshwater and improve soil fertility, contributing to a more balanced hydrological future.

Keywords: climate adaptation; contaminants of emerging concern (CECS); desalination; groundwater management; nutrient recycling; sustainability.

INTRODUCTION

Water is an essential and multi-faceted resource that sustains life and global economic and environmental processes. Its uneven distribution on Earth presents significant challenges to the sustainable management of water resources. According to Gleick (2014), approximately 97.5% of the Earth's water is saline, with only 2.5% being freshwater. Most of this freshwater is inaccessible in glaciers and polar ice caps or stored as groundwater, leaving an infinitesimal amount available in rivers and lakes. According to the Usgs (2024), the Earth holds about 1.2 quintillion m^3 (1,234,000,000,000,000,000,000) of water. Global water distribution of all these water sources (Table 1).

Table 1. Where is the World's water?

	Available water (m^3)	Percentage
Ocean	120,000,000,000,000,000,000,000	97.20%
Glaciers	2,591,491,686,000,000,000,000	2.10%
Groundwater	802,128,379,000,000,000,000	0.66%
Lakes	20,978,742,220,000,000,000	0.02%
Soil Moisture	6,170,218,300,000,000,000	0.01%
Streams, wetlands, and swamps	1,234,043,660,000,000,000	0.05%
Total	123,404,366,000,000,000,000,000	100%

How much water is on Earth? The distribution of water on the Earth's surface is highly uneven. Earth is unique because it has so much water as compared to other known planets. It's in the ground, forms oceans, and it's in our atmosphere. Your body is made up mostly of water. Water covers 71% of Earth's surface and almost all of it. Have different kinds of saltwater but mostly has sodium chloride, the same salt we add to our food. Just 3.5% of the water on Earth is freshwater we can drink, and most of that freshwater, 68%, is trapped in ice and glaciers. A third of the freshwater is groundwater. The last 2% of freshwater is in the rivers, lakes, and streams. A minimal amount is in our atmosphere, where it exists as water vapor, which is what clouds are composed of. If you took all the water on Earth and put it together, it would be a ball 1480 km wide. The National Aeronautics and Space Administration produces a straightforward video to educate us and educate your children. This video is free to download at https://spaceplace.nasa.gov/water/en/.

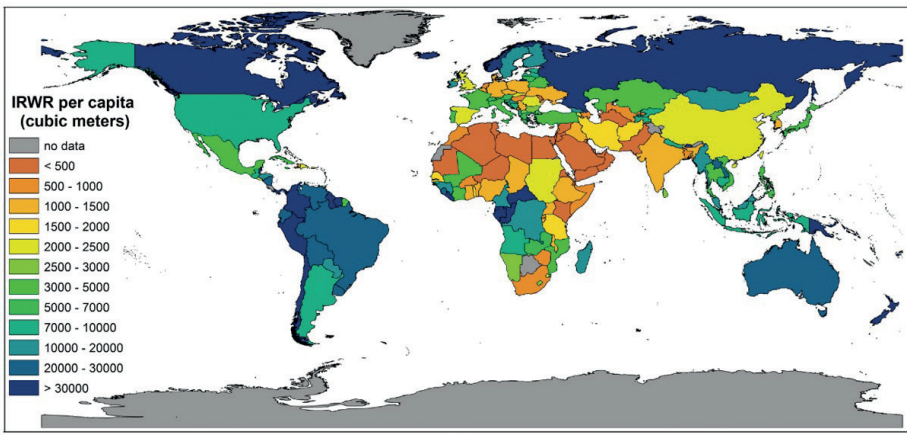
There is a significant variation in rainfall between countries, with more concentrated rainfall in countries with greater vegetative coverage. Many remote areas of the

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

world, such as coastal desert areas in the Middle East and some in the Mediterranean, suffer from a severe drinking water shortage. Figure 1 shows the volume of precipitated water consumed per capita (IRWR) worldwide in 2012. We found that rainfall in areas with hot colors was equal to or less than 500 mm^3 per year. Thus, the largest deposits of freshwater are concentrated in the Americas and Australia. Moreover, many remote areas of the world, such as desert coastal areas in the Middle East and some areas of the Mediterranean, suffer from a severe drinking water shortage.

An increasing water scarcity and drought are expected to occur in the context of tough competition between agribusiness and other sectors of the economy. Since water availability and accessibility are the most significant constraint factors for agricultural production, one alternative to meet the challenge would be the desalination of the oceans. However, this technique is still too expensive to be used on a large scale. An alternative that is increasingly gaining worldwide attention is wastewater reuse in irrigating crops (see sections 4 and 5).

Figure 1. World map of internal renewable water resources (IRWR) per country in 2012 (Mancosu *et al.*, 2015).



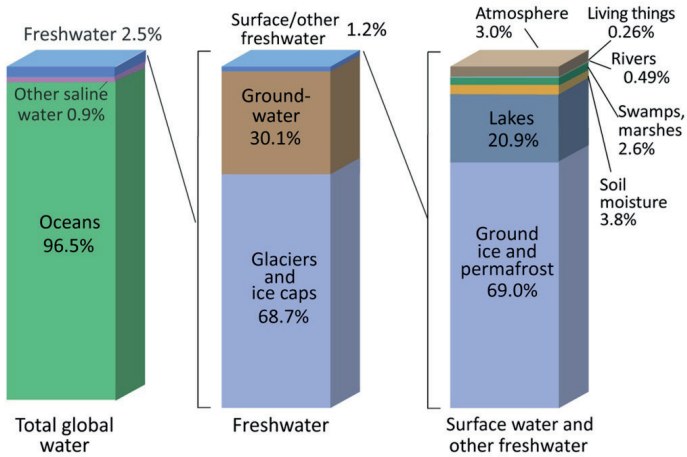
WHERE IS EARTH'S WATER?

Water scarcity is a growing issue affecting over two billion individuals globally, with alarming forecasts for the coming decades due to population growth and climate change (Un-Water, 2018). Water pollution, often resulting from industrial and agricultural discharges, further compromises the availability of potable water. Emerging technologies, such as desalination and advanced wastewater treatment systems, are considered promising solutions to mitigate water scarcity, although they

present considerable challenges in terms of cost and energy efficiency (Elimelech; Phillip, 2011). Figure 2 offers a detailed representation of Earth's water resources distribution, emphasizing the stark disparity between saline and freshwater availability. The ocean reservoir is inaccessible for direct human consumption or agricultural use without expensive desalination processes. Freshwater constitutes a mere 2.5% of global water, highlighting the critical scarcity of this essential resource. Within this small fraction, the distribution is uneven and heavily skewed toward less accessible forms. Glaciers and ice caps account for 68.7% of all freshwaters in polar regions such as Antarctica and Greenland. While these ice reserves represent a significant portion of global freshwater, their inaccessibility limits their direct utility. Additionally, melting glaciers due to climate change pose a dual challenge: contributing to rising sea levels and reducing long-term freshwater reserves.

Groundwater makes up 30.1% of the total freshwater resources, serving as a critical source for human consumption, agriculture, and industry. Groundwater reserves are often more accessible than glacial or ice cap water, particularly in arid and semiarid regions where surface water resources are scarce. However, over-extraction of groundwater has led to declining water tables in many areas, posing long-term sustainability challenges.

Figure 2. Where is Earth's water? (Shiklomanov, 1993).



THE GROUNDWATER, AGRICULTURE, AND SEMIARID REGIONS

Groundwater is the water stored beneath the Earth's surface in aquifers, which are permeable rock or sediment layers that hold and transmit water. Groundwater

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

constitutes approximately 30.1% of the planet's freshwater resources, serving as a vital source for drinking water, irrigation, and industrial processes (Cooke *et al.*, 2024). Stored within aquifers beneath the Earth's surface, groundwater is generally more accessible than glacial water. However, the over-extraction of groundwater, often exceeding natural recharge rates, has led to significant declines in water tables in various regions. This unsustainable withdrawal threatens the long-term availability of groundwater and results in adverse environmental consequences such as land subsidence and reduced surface water flows.

It is a vital resource, providing nearly half of the world's drinking water and supporting agriculture and industry. Significant groundwater reserves include the Great Artesian Basin in Australia (estimated volume – 64.900.000.000.000 m^3), the Guarani Aquifer in South America (estimated volume – 37.000.000.000.000 m^3), the Ogallala Aquifer in the United States (estimated volume – 3.700.000.000.000 m^3), and the North China Plain Aquifer (estimated volume – 1.000.000.000.000 m^3) (Habermehl, 2006). These aquifers are critical for regional water security but face threats from over-extraction, pollution, and climate change. Sustainable management is essential to preserve these resources for future generations (Connor, 2022). Groundwater is a critical resource for socioeconomic development, particularly in arid and semiarid regions, where it supports agriculture, industry, and domestic needs. However, its overexploitation has led to an aquifer overdraft, posing significant socio-environmental challenges. Hoogesteger (2022) emphasizes the complexities of regulating agricultural groundwater use, highlighting the difficulties in organizing users and establishing social control over extraction. The "invisible" nature of groundwater complicates monitoring, and prolonged pumping often results in aquifer declines. Similarly, Wang *et al.* (2021) discuss the overexploitation of groundwater in China's arid regions, where fossil water reserves are being depleted due to climate change and human activities. Both studies underscore the urgent need for sustainable management strategies to balance socioeconomic benefits with long-term aquifer sustainability.

Effective groundwater governance requires a combination of state regulation, co-management, and community involvement. Hoogesteger (2022) argues that co-management arrangements like aquifer management councils can foster shared objectives and strategies among users. However, divergent interests, power imbalances, and the lack of trust among stakeholders often hinder these efforts. Wang *et al.* (2021) argue that this perspective advocates for a comprehensive approach that integrates natural and anthropogenic factors in

groundwater management. They emphasize the importance of understanding spatiotemporal groundwater distribution to devise effective strategies. Both authors agree that sustainable groundwater use demands collaborative efforts between policymakers, scientists, and local communities.

Climate change further exacerbates groundwater challenges, particularly in regions like the Tasuj Plain, Iran, where declining groundwater recharge (GWR) threatens water availability. The use of the Hydrologic Evaluation of Landfill Performance (HELP) model to simulate GWR under various climate scenarios, revealing significant declines under high-emission scenarios like RCP8.5 (Andaryani *et al.*, 2022). While models like HELP provide valuable insights, critics argue that they may oversimplify complex groundwater systems. Despite these limitations, Andaryani *et al.* (2022) maintain that such models are essential for predicting climate change impacts and guiding adaptive water management strategies. This finding aligns with innovative governance models to address groundwater sustainability proposed by Hoogesteger (2022).

Salinity in groundwater presents another critical challenge, particularly in arid and semiarid regions, where it affects agriculture, water quality, and public health. Balaji *et al.* (2021) identify primary, secondary, and tertiary salinity as major issues arising from natural processes and human activities like irrigation. They propose management strategies such as salt-tolerant crops, improved irrigation practices, and soil reclamation techniques. However, critics argue that these traditional methods may not be sustainable in the long term, advocating instead for advanced desalination technologies and integrated water resource management. Despite these differing views, Balaji *et al.* (2021) imput the importance of combining traditional and innovative approaches to address salinity effectively.

The integration of diverse perspectives highlights the need for interdisciplinary research and adaptive management practices. Some scholars (Hoogesteger, 2022; Wang *et al.*, 2021) emphasize the importance of collaboration between stakeholders to implement effective groundwater management strategies. Similarly, Andaryani *et al.* (2022), and Balaji *et al.* (2021) advocate for policies that prioritize groundwater recharge and sustainable use, ensuring water security for future generations. These studies collectively underscore the necessity of addressing groundwater challenges through a combination of scientific innovation, community engagement, and policy reform.

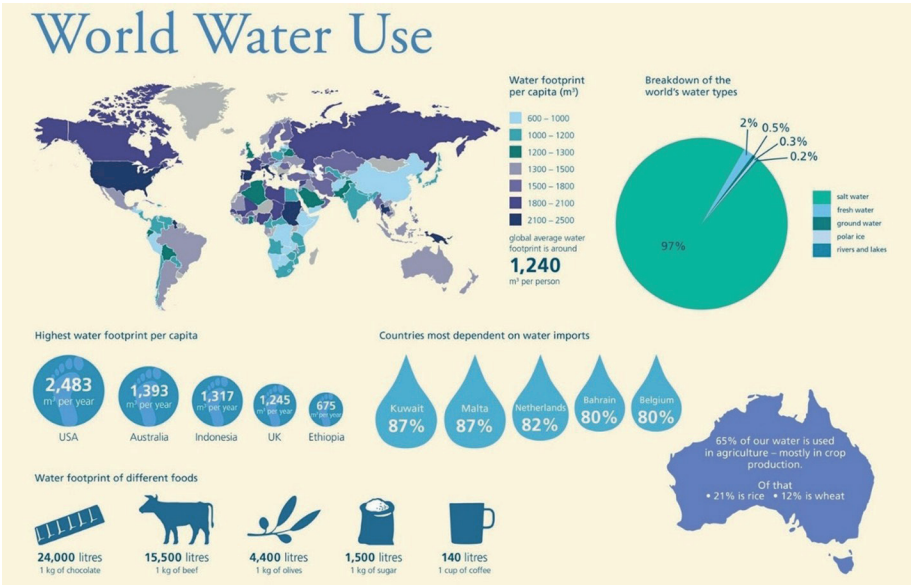
Then, groundwater management in arid and semiarid regions requires a multi-faceted approach that considers socioeconomic, environmental, and climatic

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

factors. Many studies (Andaryani *et al.*, 2022; Balaji *et al.*, 2021; Hoogesteger, 2022; Wang *et al.*, 2021) highlight the complexities of groundwater governance, the impacts of climate change, and the challenges posed by salinity. By integrating traditional and innovative strategies, fostering collaboration among stakeholders, and prioritizing sustainable practices, it is possible to address these challenges and ensure the long-term availability of this vital resource. Future research should focus on developing adaptive management models and interdisciplinary solutions to enhance groundwater sustainability globally.

Figure 3 provides an illustrative image of insights into global water use, distribution, and dependence, shedding light on the disparities and challenges in water resource management. The map highlights variations in water footprints per capita worldwide, with some nations consuming significantly more water per person than others, reflecting socioeconomic, agricultural, and industrial differences. The global average water footprint is 1240 m^3 per person annually, yet this figure masks the extreme variations across continents.

Figure 3. The world uses water and underground water. Source: Museum (2024).



For instance, the United States exhibits one of the highest water footprints per capita at 2483 m^3 per year, indicating high domestic, agricultural, and industrial water use. In contrast, countries like Ethiopia demonstrate much lower footprints, such as 675 m^3 annually, reflecting limited access to water resources

and a predominance of subsistence agriculture. Australia's water footprint per capita, at 1393 m^3 per year, underscores its heavy reliance on water-intensive practices despite being one of the driest inhabited continents. The graphic also underscores the stark realities of water availability and reliance. Countries like Kuwait and Malta are among the most dependent on water imports, with dependency rates as high as 87%. Figure 3 reveals the vulnerabilities of nations with limited freshwater resources and the increasing necessity of international water trade, desalination, and innovative technologies to meet their population's needs.

The composition of the Earth's water is another significant highlight in the figure, with 97% of the planet's water being saline. This insightful breakdown of water consumption in food production reveals the vast quantities required for various commodities. For example, producing 1 kilogram of chocolate necessitates an astonishing 24 m^3 of water, while making 1 kilogram of beef requires 15.5 m^3. Still, 1 kg of orange, potato, cucumber, cabbage, or tomatoes need only 0.46, 0.25, 0.24, 0.20, and 0.18 m^3 of water, respectively (Hoekstra, 2008) (Table 2). Such data underscore the environmental costs of high-water footprints associated with luxury and high-demand foods, sparking debates on sustainable consumption practices.

Agriculture emerges as a dominant water consumer, with 65% of total water usage attributed to this sector, mainly for crop production (Huang *et al.*, 2019). Within this domain, rice and wheat are significant contributors, with 21% and 12% of agricultural water usage dedicated to their cultivation, respectively (Hoekstra, 2008). These figures reflect the intricate balance between ensuring food security and conserving water resources. The disproportionate water usage in agriculture also indicates the pressing need for efficient irrigation techniques, crop diversification, and climate-resilient farming practices to mitigate water stress and secure global food supply chains.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Table 2. Typical values for the volume of water required to produce common foodstuffs.

Food item	Unit	Global average water footprint (m^3)
Chocolate	1 kg	24.00
Beef	1 kg	15.50
Cheese	1 kg	5.00
Pork	1 kg	4.80
Olives	1 kg	4.40
Chicken	1 kg	3.90
Rice	1 kg	3.40
Groundnut (in shell)	1 kg	3.10
Dates	1 kg	3.00
Mango	1 kg	1.60
Sugar (from sugarcane)	1 kg	1.50
Bread (from wheat)	1 kg	1.30
Peach or nectarine	1 kg	1.20
Maize	1 kg	0.90
Banana	1 kg	0.86
Apple of pear	1 kg	0.70
Orange	1 kg	0.46
Milk	1 glass of 250 mL	0.25
Potato	1 kg	0.25
Cucumber or pumpkin	1 kg	0.24
Cabbage	1 kg	0.20
Tomato	1 kg	0.18
Coffee	1 cup of 125 mL	0.14
Lettuce	1 kg	0.13
Wine	1 glass of 125 mL	0.12
Beer (from Barley)	1 glass of 250 mL	0.08
Tea	1 cup of 250 mL	0.03

Source: Hoekstra (2008).

THE FRESHWATER ON THE EARTH'S SURFACE

The remaining 1.2% of freshwater is classified as surface and other freshwater, encompassing rivers, lakes, swamps, soil moisture, atmospheric water, and water contained within living organisms. A closer examination reveals that rivers, although vital for ecosystems and human activities, represent only 0.49% of this category. Lakes, which are more substantial in volume, account for 20.9%, while swamps and marshes add a further 2.6% (Carpenter; Stanley; Zanden, 2024). Ground ice and permafrost contribute significantly, with 69% of surface

freshwater stored in these forms, albeit with limited accessibility. Soil moisture and atmospheric water, comprising 3.8% and 3.0% of surface and other freshwater, respectively, support terrestrial ecosystems and the hydrological cycle. The minuscule 0.26% of water in living organisms underscores the interconnectedness of water systems and life on Earth (Mishra; Dubey, 2015).

The stark disparity between saline and freshwater availability demonstrates the precarious nature of water resources. With most freshwater locked in inaccessible forms, humanity relies heavily on a small fraction of easily accessible water sources. This reliance underscores the importance of sustainable water management, efficient usage, and technological advancements to secure future water needs. Moreover, the distribution of accessible freshwater varies significantly across regions, further complicating water management strategies. Some areas, such as the polar regions, hold vast reserves of glacial freshwater, while others depend heavily on rivers and lakes for their water supply. Groundwater extraction is a lifeline for regions experiencing prolonged droughts or insufficient surface water availability. However, the unsustainable exploitation of groundwater poses significant environmental risks, including aquifer depletion and land subsidence (Scanlon *et al.*, 2023).

Of critical importance are understanding and managing Earth's water resources effectively. While freshwater represents only a tiny fraction of global water, its uneven distribution and accessibility pose challenges that demand coordinated global action. Sustainable practices, advanced water-saving technologies, and international cooperation are essential to address the growing water crisis and ensure equitable access to this vital resource for all.

Strategies to address these challenges must encompass sustainable water use policies, investments in water-saving technologies, and international cooperation to manage transboundary water resources. For instance, enhancing water-use efficiency in agriculture through drip irrigation, adopting crop varieties with lower water demands, and recycling wastewater for irrigation can significantly reduce farming's water footprint. Urban areas, too, must focus on reducing water losses through improved infrastructure, promoting water conservation awareness, and implementing policies that encourage sustainable consumption.

The critical importance of virtual water trade, a concept where water is an integral part of the production of goods traded internationally, is that it allows water-scarce regions to meet their needs indirectly by importing products from water-abundant areas. While virtual water trade can alleviate local water stress,

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

it also raises questions about global equity, environmental costs, and the sustainability of water-intensive exports from donor countries. Figures 2 and 3 offer a compelling visualization of the worldwide water landscape, emphasizing the urgent need for balanced and equitable water resource management. It underscores the interconnectedness of water, food, and energy systems and the critical role of technology, governance, and public awareness in addressing the looming water crises. As global water demand rises, the imperative to transition towards sustainable practices has never been clearer. Addressing these challenges requires a multi-faceted approach that balances environmental conservation, economic development, and social equity to ensure water security for future generations.

CLIMATE CHANGE AND WATER AVAILABILITY

Climate change poses multi-faceted threats to agricultural water availability, altering precipitation patterns, increasing evaporation rates, and shifting temperature regimes. Rising CO_2 levels present a paradox; while higher concentrations can enhance crop water-use efficiency through reduced transpiration, the overarching impacts of temperature stress and reduced water availability tend to negate these benefits (Mancosu *et al.*, 2015). Regions like southern Asia, the Middle East, and North Africa are projected to face the most significant increases in irrigation demands as they grapple with these climatic changes. The global water footprint, a metric quantifying the water volume used in producing goods and services, reveals that agricultural products account for 92% of this footprint, with cereals, meat, and dairy products being the most significant contributors (Dorta-Santos *et al.*, 2014). Virtual water trade—importing water-intensive crops to conserve domestic resources—offers a potential solution but introduces complexities in food security and economic dependencies, particularly for low-income countries reliant on external food sources (Salem; Pudza; Yihdego, 2022).

These frozen reserves in polar regions such as Antarctica and Greenland are crucial in regulating global sea levels and climate patterns. Moreover, ongoing climatic changes induce accelerated melting of these ice masses, contributing to rising sea levels and posing substantial risks to coastal communities worldwide (Xu *et al.*, 2023). Surface water, encompassing rivers, lakes, and swamps, accounts for a mere 1.2% of Earth's freshwater. Within this category, rivers hold only about 0.49%, yet they are indispensable for ecosystems and human societies, providing water for consumption, agriculture, transportation, and energy

generation. Lakes, containing approximately 20.9% of surface freshwater, serve as significant reservoirs for potable water and support diverse biological habitats. Swamps and marshes, comprising around 2.6%, play critical roles in water purification, flood control, and as habitats for various species.

Climate change profoundly influences the global water cycle, affecting patterns of precipitation and evaporation and increasing extreme weather events. Projections of changes in temperature and precipitation regimes suggest an increase in droughts and floods, directly impacting the availability and quality of water resources (Ipcc, 2014). Adaptations to these changes require integrated and coordinated responses at local, regional, and global levels. Climate change introduces additional complexities to water resource management. Altered precipitation patterns, increased frequency of extreme weather events, and accelerated melting of ice reserves contribute to the unpredictability of water availability. Regions historically relying on consistent water sources may face new challenges, necessitating adaptive strategies to ensure water security (Arfasa; Owusu-Sekyere; Doke, 2024; Rivera, 2024).

The impact of climate change on the Vea catchment in Ghana, focusing on changes in temperature, precipitation, and streamflow (Arfasa; Owusu-Sekyere; Doke, 2024). The study uses data from the Ghana Meteorological Agency, satellite sources, and global climate models under SSP 4.5 and SSP 8.5 emission scenarios to predict significant temperature increases and decreases in precipitation and streamflow. The research highlights the importance of effective water resource management to mitigate climate change impacts. Incorporating CHIRPS data and CMhyd software for bias correction and downscaling enhances the accuracy of climate projections, providing a comprehensive understanding of future hydroclimatic conditions in the region.

The study uses the CMIP6 dataset to project changes in Guatemala's climate, predicting significant temperature increases and precipitation decreases throughout the 21st century under various scenarios (Rivera, 2024). By 2100, annual mean temperatures could rise by 1.8°C to 5.4°C, particularly affecting northern regions. Precipitation is expected to decrease by 5% to 22%, with notable reductions in May, June, and July, impacting the north and central areas. Based on WorldClim data and local station averages, the projections highlight the need for climate adaptation and mitigation strategies in Guatemala, providing crucial baseline information for future planning.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Addressing these multi-faceted challenges requires a comprehensive approach integrating scientific understanding, technological innovation, and policy interventions. Implementing efficient water use practices, investing in water storage and distribution infrastructure, and protecting natural ecosystems that regulate the water cycle are critical components of sustainable water management. International cooperation is also paramount, as many water systems transcend political boundaries, necessitating collaborative efforts to manage shared resources effectively.

While Earth's abundant water endows the planet with its distinctive blue appearance, the accessible freshwater resources are limited and unevenly distributed (Edwards, 2024). The intricate interplay between natural processes and human activities dictates the availability and quality of this vital resource. Ensuring the sustainable management of freshwater is imperative to support life, promote economic development, and maintain ecological balance in the face of growing global challenges. Moreover, in the hot areas on the map, the estimated population is about 4 billion, including the African continent (1,460 billion), China (1,409 billion), and India (1,458 billion). This sum equals 12.9-fold and 30.1-fold the United States and Russian populations, respectively. Thus, the challenge of using wastewater in agriculture worldwide is becoming increasingly urgent. However, only some countries have included this technique as a viable alternative for the irrigation of their plantations (Dorta-Santos *et al.*, 2014; Mancosu *et al.*, 2015). From this perspective, the world needs to rethink the indiscriminate use of fresh water to avoid catastrophic predictions such as those expected for 2050. These areas are expected to become as arid as deserts, further limiting the food supply. In this role, Brazil deserves special attention since it is expected for 2021 to be a record export of agricultural products such as soybeans, corn, cellulose, and coffee, which should exceed 37.7 billion tons. Thus, if we could replace 10% of freshwater with wastewater in agriculture in Brazil, a few billion m^3 of freshwater would be spared, making Brazil the focus of attention, not for its ecological disasters, but for the gradual replacement of its freshwater.

WATER SCARCITY AND FUTURE CHALLENGES FOR FOOD PRODUCTION

Water scarcity has emerged as one of the most pressing global challenges of the 21st century, exacerbated by climate change, population growth, and unsustainable water management practices. Agriculture, which accounts for

approximately 70% of global freshwater withdrawals, is particularly vulnerable to water shortages, as irrigation systems are essential for food security in many regions (Mancosu *et al.*, 2015). The dual nature of water resources—renewable (*e.g.*, rivers and surface aquifers) and non-renewable (*e.g.*, deep aquifers)—further complicates the issue, with disparities in water availability amplified at the country level. For instance, nations like Kuwait face extreme water scarcity, with renewable resources as low as 10 m^3 per inhabitant yearly (Shiklomanov, 1993). The Total Renewable Water Resources (TRWR) index, which combines internal and external water resources, underscores the geopolitical vulnerabilities of cross-border water-sharing agreements, particularly in arid regions like the Middle East and North Africa (Dolan *et al.*, 2021).

Biofuels represent an additional strain on water resources, as expanding crops for bioethanol and biodiesel often involves land-grabbing practices that reduce water availability for local populations. Flex crops, which serve multiple purposes (*e.g.*, food, fuel, and industrial materials), exacerbate these challenges, highlighting the trade-offs inherent in resource allocation (Varady *et al.*, 2022). Sustainable water management practices, such as precise irrigation scheduling, adoption of water-saving technologies, and optimization of crop selection based on local conditions, are critical for mitigating water scarcity in agriculture. Techniques like soil monitoring, lysimetry, and advanced irrigation systems (*e.g.*, drip and sprinkler irrigation) offer significant potential for reducing wastage and improving yield efficiency (Grison *et al.*, 2023).

Future projections paint a stark picture, with population growth and urbanization potentially pushing two-thirds of the global population into water-stressed conditions by 2050. Integrating approaches that balance agricultural, industrial, and domestic water demands are imperative to address these challenges. Policies encouraging the adoption of virtual water trade, coupled with investments in infrastructure and technology, can help mitigate regional disparities. However, these efforts require robust international cooperation, particularly in transboundary water management (Koop *et al.*, 2021). The interdependence of water resources and food security calls for a paradigm shift toward sustainable water use driven by technological innovation, policy reform, and global collaboration. Addressing water scarcity is a technical challenge and a social and political imperative, with implications for global stability and human well-being (Yang; Yang; Xia, 2021).

Policies encouraging community participation and integrated watershed management can significantly improve resilience to water challenges.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Nature-based solutions, which mimic natural processes to improve water quality and availability, represent a promising development area. Research in the field has focused on digital innovations that improve water resource management, emphasizing the need for a holistic and adaptive framework capable of coping with the dynamic challenges of climate change and urbanization (Partida, 2023). These advances underline the critical role of interdisciplinary collaboration in fostering resilience and ensuring equitable access to water resources worldwide. Managing water in the modern world is a complex challenge that requires coordination between governments, scientists, and civil society. While technology offers potential solutions, long-term sustainability will depend on effective policies, robust governance, and public awareness (Grison *et al.*, 2023).

The reuse of wastewater in agriculture is emerging as a viable solution to alleviate water scarcity while recycling valuable nutrients. Studies have demonstrated that recycled urban wastewater (RWW) can provide essential nutrients like phosphorus and potassium, which enhance crop productivity and reduce the need for synthetic fertilizers (Bueno *et al.*, 2022). For example, irrigation with RWW has significantly improved crop yields in arid regions such as the Canary Islands. However, concerns about salinity, boron toxicity, and pathogen contamination necessitate rigorous monitoring and advanced treatment processes to ensure sustainability and safety (Dorta-Santos *et al.*, 2014). The future of agriculture will likely see widespread implementation of such technologies to transform wastewater into a reliable input for crop production. This transition will necessitate rigorous regulatory frameworks to ensure the safety of reclaimed water, particularly in preventing contamination from heavy metals, pathogens, and harmful chemicals (Salem; Pudza; Yihdego, 2022).

The accelerated melting of these ice reserves due to rising global temperatures contributes significantly to sea-level rise, threatening coastal communities and ecosystems (Usgs, 2024). NASA reports indicate that melting ice also disrupts freshwater availability by increasing the salinity of nearby water sources, compounding challenges for both agriculture and human consumption (Museum, 2024). Global cooperation and stringent emissions reductions are essential to slow the melting process and mitigate associated risks. The elevation of sea levels is intrinsically tied to climate-induced disasters such as floods and hurricanes, which are becoming increasingly frequent and severe. These events not only displace populations but also contaminate freshwater supplies with saline water, further exacerbating water scarcity (Mancosu *et al.*, 2015).

Urbanization adds complexity to the water crisis as cities demand extensive water supplies while generating significant wastewater. Integrated urban water management systems that emphasize circular economy principles are essential. Recycling wastewater for landscaping, industrial processes, and irrigation can reduce freshwater dependency, as evidenced by successful applications in various cities globally (Dorta-Santos *et al.*, 2014). Public awareness campaigns and policy incentives for water conservation further bolster these efforts. Effective governance and policies are essential for the sustainable management of water resources. Promoting conservation practices, efficient use, and transboundary cooperation are crucial elements in ensuring water security. Initiatives such as the United Nations' Sustainable Development Goals (SDGs) underline the importance of universal and sustainable access to clean water and sanitation (Un, 2015).

Desalination technologies represent a promising solution for addressing water scarcity in arid regions. While historically energy-intensive and costly, recent advancements in membrane technology and renewable energy integration have improved the feasibility of desalination processes (Shiklomanov, 1993). These technologies enable the conversion of abundant saline water into usable freshwater, offering a lifeline for regions facing chronic shortages. However, the environmental impacts of brine disposal and high energy requirements must be carefully managed to ensure sustainability (Usgs, 2024). The interconnectedness of water, food, and energy systems underscores the need for integrated approaches to resource management. For instance, using wastewater to irrigate biofuel crops not only conserves freshwater but also supports renewable energy goals (Dorta-Santos *et al.*, 2014).

The future of agriculture will be defined by its ability to adapt to the challenges of water scarcity, climate change, and sustainability. One of the most promising solutions is the utilization of reclaimed water, a resource that has the potential to revolutionize farming practices while conserving dwindling freshwater reserves. In an era where population growth and urbanization exert unprecedented pressure on natural resources, integrating reclaimed water into agricultural systems offers both a practical and innovative path forward. Reclaimed water, derived from treated municipal, industrial, or agricultural wastewater, serves as an alternative water source that can reduce dependency on freshwater. Advanced treatment technologies, such as membrane filtration, biological processes, and ultraviolet disinfection, have significantly improved the safety and quality of reclaimed water, making it suitable for irrigation purposes (Bueno *et al.*, 2022).

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

The advantages of using reclaimed water in agriculture extend beyond water conservation. Nutrient recycling is a key benefit, as reclaimed water often contains essential nutrients like nitrogen and phosphorus that can enhance soil fertility and reduce reliance on synthetic fertilizers. This dual function not only supports sustainable crop production but also mitigates the environmental impacts associated with chemical fertilizer runoff, such as the eutrophication of water bodies. In the future, precision agriculture technologies, including automated irrigation systems and real-time soil monitoring, will optimize the application of reclaimed water, ensuring that crops receive the right balance of water and nutrients (Salem; Pudza; Yihdego, 2022). In arid and semiarid regions, where water scarcity poses a significant threat to food security, reclaimed water can enable year-round cultivation of high-value crops. For instance, hydroponic and vertical farming systems, which rely on controlled water and nutrient cycles, are ideally suited for incorporating reclaimed water. These systems maximize water-use efficiency and are less vulnerable to the limitations of traditional soil-based farming (Dorta-Santos *et al.*, 2014).

However, integrating reclaimed water into agriculture requires addressing several challenges. Public perception and acceptance remain significant barriers, as many consumers express concerns about the safety of food grown with treated wastewater. To overcome these concerns, transparent communication and education campaigns highlighting the rigorous treatment processes and safety measures involved will be essential. Additionally, investments in infrastructure and research will be critical to scaling up reclaimed water usage, particularly in regions with limited access to advanced treatment technologies. The future of agriculture will also demand a paradigm shift in water governance. Policies encouraging wastewater reuse, coupled with incentives for adopting sustainable practices, will play a crucial role in driving this transformation. International collaboration will be equally important, as sharing technological advancements and best practices can accelerate global progress in water reuse (Grison *et al.*, 2023).

Brazil, despite having vast freshwater resources, is currently grappling with a severe water crisis exacerbated by climate change and deforestation. The country's reliance on hydropower and agriculture, which heavily depend on water availability, has been significantly affected by prolonged droughts. These droughts are intensified by deforestation, mainly in the Amazon Forest, which disrupts moisture transfer, which is crucial for the hydroclimate of Brazil's economically vital south-central region. The consequences of water scarcity

are far-reaching, impacting food production, energy generation, and leading to socioeconomic challenges such as migration and increased poverty in urban areas (Getirana; Libonati; Cataldi, 2021). The article emphasizes the need for a coordinated nationwide Drought Mitigation Plan to address these challenges. To ensure sustainable water management, this plan should involve diverse stakeholders, including researchers, policymakers, and civil society. The authors highlight the importance of diversifying energy sources, such as increasing wind and solar capacity, to reduce dependence on hydropower. Furthermore, improving groundwater and soil moisture monitoring systems is crucial for better understanding and managing water resources (Getirana; Libonati; Cataldi, 2021).

Varady *et al.* (2022) examines the evolution and impact of Global Water Initiatives (GWIs) over recent decades, highlighting their role in shaping global water governance. The authors discuss the diverse nature of GWIs, which include professional societies, special events, organizations, designated periods, and networks, each contributing uniquely to water resource management and policy development. The article emphasizes the importance of these initiatives in fostering international cooperation, knowledge sharing, and awareness-raising about critical water-related issues. By analyzing the historical context and current landscape of GWIs, the authors provide insights into how these initiatives have influenced water governance and management practices on a global scale (Varady *et al.*, 2022). In exploring the contributions of GWIs, the authors underscore the significance of networks and declarations as vital components that have proliferated substantially in recent years. These elements have played a crucial role in advancing collective action and policy dialogue among diverse stakeholders, including governments, NGOs, and academic institutions. The authors highlight the dynamic and interconnected nature of GWIs, which operate within an ever-changing institutional environment to address emerging challenges such as climate change, water scarcity, and sustainable development (Varady *et al.*, 2022).

Salem; Pudza e Yihdego (2022) explore the intricate relationship between water and food security, emphasizing the challenges and opportunities faced by various regions worldwide. It highlights the critical role of the Water-Food (WF) Nexus in addressing the growing demands on water resources driven by factors such as population growth, urbanization, and climate change. The authors discuss the importance of implementing integrated strategies that are considered cross-sectoral, environmental, social, political, and geopolitical dimensions

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

to achieve sustainable development. By examining case studies from different regions, the article provides insights into how the WF Nexus can help create effective programs and synergies to manage water resources efficiently and ensure food security (Salem; Pudza; Yihdego, 2022). The Water-Energy-Food (WEF) Nexus expands on the WF Nexus by incorporating the energy subsystem, underscoring the interdependence and interactions among water, energy, and food subsystems, highlighting the need for a holistic approach to resource management. The authors present the Five W Questions (FWQ) as a framework to encourage interdisciplinary dialogue and produce actionable knowledge (Salem; Pudza; Yihdego, 2022).

The complexities of the hydrological cycle and water resources management in the context of global climate change highlights the intricate interactions between the atmosphere, lithosphere, biosphere, and anthroposphere, emphasizing the profound impact of human activities and socioeconomic development on the global water cycle. Yang; Yang e Xia (2021) explore the challenges posed by climate change and land use changes, which lead to significant spatial and temporal variability in the water cycle, resulting in numerous water-related issues that threaten human water security. The study underscores the need to understand the hydrological cycle and water resources and to effectively address these environmental challenges (Yang; Yang; Xia, 2021). The article further examines the role of water as a fundamental natural resource, crucial for maintaining and promoting sustainable socioeconomic development. It discusses the need for an ideal water resources management system that considers the uncertainties of hydrologic and economic systems and the adaptation mechanisms and behavioral characteristics of water users (Yang; Yang; Xia, 2021). In another way, Bueno *et al.* (2022) investigate the uptake and accumulation of contaminants of emerging concern (CECs) in tomato plants irrigated with reclaimed water. It highlights the growing use of treated wastewater for agricultural purposes as a strategy to combat water scarcity. The study focuses on understanding how different CECs, including pharmaceuticals and pesticides, are absorbed and distributed within the plant tissues and the soil. The authors demonstrate that hydrophilic compounds tend to translocate from roots to leaves and fruits, while hydrophobic contaminants are more likely to remain in the roots and soil. The study presents detailed data on the concentrations of various CECs detected in different parts of the tomato plant and the surrounding soil.

RECYCLED URBAN WASTEWATER FOR IRRIGATION – CRITICAL REVIEW OF DORTA-SANTOS *et al.* (Dorta-Santos *et al.*, 2014)

In the global context, recycled urban wastewater (RWW) in agriculture will increase in water-scarce countries soon, particularly in major cities. Irrigation with RWW has been used as a complementary treatment method for wastewater, use of marginal water as an available water source for agriculture, and as a nutrient source associated with mineral fertilizer. The benefits of RWW use include the conservation of freshwater reserves, less discharge of pollutants into water bodies, the recycling of both water and nutrients, and the supply of nutrients to crops due to the organic matter and nutrients present in this wastewater.

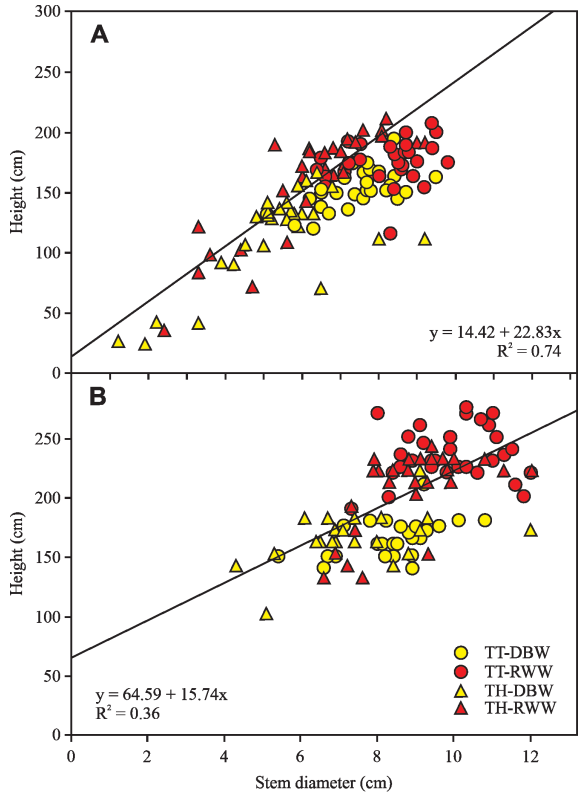
The potential of using non-conventional water resources, such as recycled urban wastewater (RWW), for cultivating *Jatropha curcas* (JCL), a promising biodiesel crop, is excellent. Conducted on the arid island of Fuerteventura, Canary Islands, Spain, this research evaluates the impact of RWW compared to desalinated brackish water (DBW) on JCL growth, seed productivity, and soil conditions. The study holds significant implications for addressing water scarcity and desertification while promoting renewable energy sources.

J. curcas has garnered attention as an energy crop due to its adaptability to degraded soils and arid conditions (Campos *et al.*, 2012; Dorta-Santos *et al.*, 2014; Pompelli *et al.*, 2010). However, its productivity remains dependent on adequate water supply, challenging its use in water-scarce regions. This research tests the hypothesis that RWW, rich in nutrients like phosphorus (P) and potassium (K), can enhance plant growth and yield while reducing dependency on conventional freshwater sources.

The experimental design involved cultivating JCL in two soil types: sandy loam and clay loam. Both were irrigated with RWW and DBW, with growth parameters, leaf mineral composition, and seed production monitored over 39 months. RWW demonstrated significant advantages in supporting plant growth, with plants irrigated with RWW achieving better height and stem diameter than DBW-irrigated plants. Seed productivity under RWW irrigation was approximately 12-fold higher than with DBW, with cumulative yields of ~2258 kg ha^{-1} and ~197 kg ha^{-1}, respectively. This productivity is attributed to the higher nutrient content of RWW, notably P and K, and lower boron (B) toxicity compared to DBW (Fig. 4).

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 4. Stem diameter (A) and height (B) of *Jatropha curcas* at two different stages of cultivation (A, plants 19 months old and B, plants 35 months old) and under different treatments. Figure adapted from Dorta-Santos *et al.* (2014).



According Dorta-Santos *et al.* (2014) sandy-loam soils (TT) provided better aeration and root development, improving plant growth and productivity than clay-loam soils (TH). The interaction between soil type and irrigation water further underscores the need for site-specific management strategies.

While RWW exhibited clear benefits, its use poses risks of soil salinity and boron accumulation. The study notes that prolonged irrigation with RWW increased soil salinity and altered soil pH, particularly in the upper layers. These changes could limit long-term sustainability, requiring careful soil and water management practices such as leaching fractions and calcium amendments to mitigate salinity and sodicity risks. Leaf mineral composition analysis revealed higher boron and sodium levels in DBW-irrigated plants, correlating with reduced growth and productivity. In contrast, RWW-irrigated plants demonstrated balanced nutrient uptake, enhancing their overall performance. The study

emphasizes that excessive boron levels in DBW-irrigated soils and leaves were a critical factor limiting JCL productivity. These findings align with prior research identifying boron toxicity thresholds for JCL and other crops.

Despite its promising results, the study recognizes the challenges of scaling up JCL cultivation with RWW irrigation. Soil degradation risks, particularly salinity and boron accumulation, necessitate improvements in RWW treatment to ensure long-term sustainability. Moreover, while RWW irrigation reduced competition for freshwater resources, its economic feasibility requires further evaluation, particularly in areas reliant on expensive desalination processes. RWW can enhance JCL productivity and support renewable energy goals by providing essential nutrients and mitigating water scarcity. However, the sustainability of this approach hinges on addressing soil degradation risks and optimizing water management practices. The findings contribute valuable knowledge for integrating bioenergy crop production with wastewater reuse strategies, advancing efforts to combat desertification and promoting sustainable agriculture.

From a broader perspective, using RWW to cultivate non-edible plants like *J. curcas* represents a promising strategy for addressing water scarcity and promoting renewable energy sources. The study underscores the importance of site-specific management strategies, as soil type significantly influences plant growth and productivity. The study also raises important questions about the broader application of RWW in agriculture, particularly for edible crops, where health and safety considerations would be paramount. In conclusion, while RWW irrigation enhances *J. curcas* productivity and supports renewable energy goals, its sustainability hinges on optimizing water and soil management practices to mitigate environmental risks. Then, using RWW for growing *J. curcas* offers substantial benefits in terms of enhanced productivity and resource conservation but requires careful management to reduce risks of soil salinity, boron accumulation, and long-term environmental degradation.

CONCLUDING REMARKS

The manuscript underscores the critical importance of water as a finite and unevenly distributed resource, emphasizing its role in sustaining life, agriculture, and global economic systems. It highlights the growing challenges posed by water scarcity, exacerbated by climate change, population growth, and unsustainable management practices. The study explores the potential of innovative

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

solutions such as virtual water trade, recycled urban wastewater (RWW), and advanced desalination technologies to address these challenges. However, it also raises concerns about the environmental and health risks associated with wastewater reuse, particularly in agriculture, where contaminants of emerging concern (CECs) and salinity pose significant threats to soil and crop quality. The interconnectedness of water, food, and energy systems is a recurring theme, emphasizing the need for integrated strategies that balance environmental conservation, economic development, and social equity. The manuscript calls for a paradigm shift in water governance, advocating for policies that promote sustainable practices, technological innovation, and international cooperation to ensure water security for future generations.

Looking ahead, the future of water resource management will depend on the widespread adoption of sustainable practices and advanced technologies. The integration of reclaimed water into agricultural systems offers a promising solution to water scarcity, particularly in arid and semiarid regions. However, this transition will require rigorous regulatory frameworks, public education campaigns, and investments in infrastructure to address concerns about safety and acceptability. Precision agriculture technologies, such as automated irrigation systems and real-time soil monitoring, will play a crucial role in optimizing water and nutrient use. Additionally, interdisciplinary research and collaboration will be essential to develop adaptive management models that can cope with the dynamic challenges of climate change and urbanization. The global community must prioritize equitable access to water resources, fostering international cooperation and knowledge-sharing to address transboundary water issues. By embracing a holistic approach that combines scientific innovation, policy reform, and community engagement, humanity can navigate the complexities of water resource management and ensure a sustainable future for all.

REFERENCES

ANDARYANI, S.; NOURANI, V.; PRADHAN, B.; JALALI ANSARUDI, T. *et al.* Spatiotemporal evaluation of future groundwater recharge in arid and semi-arid regions under climate change scenarios. **Hydrol Sci J**, 67, n. 6, p. 979-995, 2022.

ARFASA, G. F.; OWUSU-SEKYERE, E.; DOKE, D. A. Climate change projections and impacts on future temperature, precipitation, and stream flow in the vea catchment, Ghana. **Environ Chal**, 14, p. 100813, 2024.

BALAJI, E.; ADIMALLA, N.; MADHAV, S.; SOMAGOUNI, S. G. *et al.* Salinity problems in groundwater and management strategies in arid and semi-arid regions. *In*: MADHAV, S. e SINGH, P. (Ed.). **Groundwater Geochemistry: Pollution and Remediation Methods**. Hoboken, NJ, USA: John Wiley & Sons Ltd, 2021.

BUENO, M. J. M.; VALVERDE, M. G.; GÓMEZ-RAMOS, M. M.; SALINAS ANDÚJAR, J. A. *et al.* Fate, modeling, and human health risk of organic contaminants present in tomato plants irrigated with reclaimed water under real-world field conditions. **Sci Total Environ**, 806, p. 150909, 2022.

CAMPOS, M. L. O.; HSIE, B. S.; GRANJA, J. A. A.; CORREIA, R. M. *et al.* Photosynthesis and antioxidant activity in Jatropha curcas L. under salt stress. **Braz J Plant Physiol**, 24, n. 1, p. 55-67, 2012.

CARPENTER, S. R.; STANLEY, E. H.; ZANDEN, J. V. State of the world's freshwater ecosystems: physical, chemical, and biological changes. **Annu Rev Environ Resour**, 36, n. 1, p. 75-99, 2024.

CONNOR, R. **Executive Summary. United Nations World Water Development Report 2022: Groundwater: Making the invisible visible**. Paris: UNESCO, 2022.

COOKE, S. J.; LYNCH, A. J.; TICKNER, D.; ABELL, R. *et al.* Can the planetary health concept save freshwater biodiversity and ecosystems? **Lancet Planet Health**, 8, n. 1, p. e2-e3, 2024.

DOLAN, F. C.; LAMONTAGNE, J.; LINK, R.; HEJAZI, M. *et al.* Evaluating the economic impact of water scarcity in a changing world. **Nature Comm**, 12, p. 1915, 2021.

DORTA-SANTOS, M. A.; TEJEDOR, M.; JIMÉNEZ, C.; HERNÁNDEZ-MORENO, J. M. *et al.* Recycled urban wastewater for irrigation of *Jatropha curcas* L. in abandoned agricultural arid land. **Sustainability**, 6, p. 6902-6924, 2014.

EDWARDS, W. M. Freshwater resources. **National Geographic Education**, v.Available on https://education.nationalgeographic.org/resource/freshwater-resources/. Accessed on 04 Jan 2025.

ELIMELECH, M.; PHILLIP, W. A. The future of seawater desalination: energy, technology, and the environment. **Science**, 333, n. 6043, p. 712-717, 2011.

GETIRANA, A.; LIBONATI, R.; CATALDI, M. Brazil is in water crisis – it needs a drought plan. **Nature**, 600, p. 218-220, 2021.

GLEICK, P. H. Water resources. *In*: CLEVELAND, C. J. (Ed.). **Encyclopedia of energy**. New York: Elsevier, 2014. p. 497-509.

GRISON, C.; KOOP, S. H. A.; EISENREICH, S. J.; HOFMAN, J. *et al.* Integrated water resources management in cities in the world: Global challenges. **Water Resour Manag**, 37, p. 2787–2803, 2023.

HABERMEHL, M. A. The Great Artesian Basin, Australia. *In*: FOSTER, S. e LOUCKS, P. D. (Ed.). **Non-renewable Groundwater Resources: A guidebook on socially-sustainable management for water-policy markers**. Paris: United Nations Educational, Scientific and Cultural Organization, 2006. cap. 5, p. 82-88.

HOEKSTRA, A. Y. The water footprint of food. **Water for food**, v.Available on https://encurtador.com.br/6Vn6A. Accessed on 04 Jan 2025, p. 49-61.

HOOGESTEGER, J. Regulating agricultural groundwater use in arid and semi-arid regions of the Global South: Challenges and socio-environmental impacts. **Curr Opin Env Sci Hl**, 27, p. 100341, 2022.

HUANG, Z.; HEJAZI, M.; TANG, Q.; VERNON, C. R. *et al.* Global agricultural green and blue water consumption under future climate and land use changes. **J Hydrol**, 574, n. 3, p. 242-256, 2019.

IPCC. **Climate change 2014: impacts, adaptation, and vulnerability**. Cambridge: Cambridge University Press, 2014.

KOOP, S. H. A.; GRISON, C.; EISENREICH, S. J.; HOFMAN, J. *et al.* Integrated water resources management in cities in the world: Global solutions. **Sustain Cities Soc**, 86, p. 104137, 2021.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

MANCOSU, N.; SNYDER, R. L.; KYRIAKAKIS, G.; SPANO, D. Water scarcity and future challenges for food production. **Water**, 7, n. 3, p. 975-992, 2015.

MISHRA, R. K.; DUBEY, S. C. Fresh water availability and it's global challenge. **Int J Eng Sci Invention Res Dev**, 2, n. 6, p. 351-407, 2015.

MUSEUM, A. Water around the world. v.Available on https://australian.museum/get-involved/citizen-science/streamwatch/water-catchment/streamwatch-water-around-the-world/. Accessed on 03 Dec 2024.

PARTIDA, J. P. O. We Reviewed More Than 150 Papers on Water Management. Here's What We Learned. **Union of Concerned Scientist**.

POMPELLI, M. F.; BARATA-LUÍS, R. M.; VITORINO, H. S.; GONÇALVES, E. R. *et al.* Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery. **Biomass Bioenerg**, 34, n. 8, p. 1207-1215, 2010.

RIVERA, P. Climate change projections in Guatemala: temperature and precipitation changes according to CMIP6 models. **Model Earth Syst Environ**, 10, p. 2031–2049, 2024.

SALEM, H. S.; PUDZA, M. Y.; YIHDEGO, Y. Water strategies and water–food Nexus: challenges and opportunities towards sustainable development in various regions of the World. **Sustain Water Resour Manag**, 8, p. 114, 2022.

SCANLON, B. R.; FAKHREDDINE, S.; RATEB, A.; GRAAF, I. *et al.* Global water resources and the role of groundwater in a resilient water future. **Nat Rev Earth Environ**, 4, n. 2, p. 87-101, 2023.

SHIKLOMANOV, I. World fresh water resources. *In*: GLEICK, P. H. (Ed.). **Water in Crisis: A guide to the World's Fresh Water Resources 1st edition**. London: Oxford University Press, 1993. p. 504.

UN-WATER. **World Water Development Report 2018: Nature-based Solutions for Water**. Paris: UNESCO, 2018.

UN. **United Nations General Assembly. Transforming our world: the 2030 Agenda for Sustainable Development**. New York: United Nations, 2015.

USGS. How much of the Earth's water is stored in glaciers? **U.S. Geography Survey** v.Available on https://www.usgs.gov/faqs/how-much-earths-water-stored-glaciers. Accessed at 04 Dec 2024.

VARADY, R. G.; ALBRECHT, T. R.; GERLAK, A. K.; HAVERLAND, A. C. Global Water Initiatives Redux: A Fresh Look at the World of Water. **Water**, 14, p. 3093, 2022.

WANG, W.; ZHANG, Z.; YIN, L.; DUAN, L. *et al.* Groundwater recharge and discharge in arid and semi-arid areas of China. **Hydrogeol J**, 29, p. 521–524, 2021.

XU, X.; ZHANG, H.; SUN, Y.; XU, B. *et al.* The effects of climatic change and inter-annual variability on glacier retreat from ~1850s AD moraines in the Kuoqionggangri peak region, southern Tibetan Plateau. **Clim Dyn**, 62, p. 2941–2951, 2023.

YANG, Y.; YANG, D.; XIA, J. Hydrological cycle and water resources in a changing world: A review. **Geogr Sustain**, 2, p. 115–122, 2021.

CHAPTER V

FOLIAR-APPLICATION OF PROLINE MITIGATES SALINITY STRESS IN MAIZE (*Zea maize* L.): A COMPARATIVE STUDY ON GROWTH, PHYSIOLOGY, AND ION COMPOSITION

Prof. Dr. Luis Eliécer Oviedo Zumaque

M.Sc. Andrés José Betin Ruiz

M.Sc. Samuel Giovanny García-Castaño

10.37885/250619546

ABSTRACT

Salinity is a significant constraint for crop production globally, alongside other stresses such as drought. Salinity reduces the growth and yield of maize. A wide range of literature covers that proline, when exogenously applied, helps plants to mitigate the unfavorable impacts of abiotic stresses. While the role of exogenously applied proline in enhancing plant defense has been studied, its specific effects on maize under salinity stress require further exploration. A hydroponic study was conducted using two maize genotypes Monsanto-6525 and Pioneer-33H25, exposing to four treatments including control, salinity (75 mM NaCl), proline (30 mM), and proline (30 mM) + salinity (75 mM NaCl). Foliar application of proline (control, 30 mM) was done at 15 mL per plant after 20 days of transplantation. Proline showed positive results by influencing growth (shoot and root dry weights), physiological (total chlorophyll contents, relative water contents, membrane stability index) responses, proline and ionic compositions i.e. shoot and root sodium (Na) and potassium (K). The Na was reduced, and K was improved in both maize genotypes by foliar-applied proline, however, this improvement was more perceived in Monsanto-6525 cultivar when compared with Pioneer-33H25. Overall, the study suggests that foliar application of proline can be an effective strategy for improving maize tolerance to salinity stress, with cultivar-specific responses indicating the potential for targeted application.

Keywords: abiotic stress; NaCl; crop productivity; plant memory; genetic variability.

INTRODUCTION

In irrigated crop production, salinity is a significant environmental stress that restricts plant growth and productivity [1], and this factor is more evident in arid and semiarid regions. Increased intensity and frequency of extreme weather events, including warming and drought events, have been recorded worldwide. Forecasts indicate an increase in these events, leading to severe implications for crop growth and productivity [2-4]. Changing climatic conditions seriously challenge marginal agricultural ecosystems, which are already facing single or multiple stresses [5,6]. Many scholars have predicted the rapid expansion of semiarid agroclimatic zones due to climate change [7,8]. In accordance with Smanov *et al.* [9], in a Kazakhstan arable layer cornfield soil, the percentage of saline soils is around 25.5% in spring; it increases to 71% in Summer, and in Autumn, the saline soils decrease to 51.7%; data that is corroborated in Morocco irrigation water [10]. Globally, maize is the 3rd important cereal crop, after wheat and rice [11,12]. Most of the crops differ in tolerance against high salt stress, but the maize crop is reported to be moderately salt-sensitive [10].

Salinity has a serious impact on plant growth and productivity and can lead to death, depending on the intensity and duration of exposure [13]. Plants strongly expressed salinity stress in physiological aspects, adopting or mitigating it [14,15]. The first indication of plants that are subjected to salinity stress is chlorophyll photobleaching [16-21], gas exchange [18,22-26], leaf size [16,17,27], and aerial biomass [28,29]. Generally, the high salinity in soil solution imposes an imbalance in osmotic potential, ionic equilibrium, and ion uptake [18,30,31]. Osmotic potential decreases under salt stress that generates the water deficit situation in soil [32,33] and increased concentration of toxic ions (Na^+ and Cl^-) [34]. These toxic ions accumulate within the plant cause ionic imbalance and deficiency of other nutrients [35]. The uptake of nutrients from soil solution is altered by a high concentration of Na^+ [36]. The K^+ efflux also occurs under salinity stress due to an increase in the level of reactive oxygen species (ROS). This rise in ROS concentration depresses the defense mechanism, which results in oxidative stress [37].

The complex nature of salt tolerance in crops is the most essential factor that makes complex breeding programs and improves salt tolerance genotypes [38]. Breeding efforts are hampered due to a lack of understanding of the salt tolerance mechanism and a lack of field and laboratory screening tests [39].

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Genetic manipulation techniques involve differences in plant genetic resources that allow the development of salt-tolerant genotypes of desirable traits that benefit farmers and breeders [40].

To adapt to salt stress, plants increase their concentrations of various cytoplasmic substances (such as glycinebetaine and proline), which promote osmotic balance inside and outside the cell to maintain cell turgor [41-43]. So, exogenous proline application, either as seed priming or foliar spray, is potentially a cost-effective strategy to improve stress tolerance under unfavorable environmental conditions [44,45]. Salt-tolerant plants in common proline steadily increase under salt stress [43,46]. Proline accumulation is essential for selecting drought-tolerant crops [47,48], including *Z. mays* [16,38,49-56]. Salt stress-induced proline production in plants reduces damage caused by salt stress and improves crop productivity. Therefore, the contribution of exogenous proline in alleviating the salinity stress in maize crops at the vegetative stage was investigated in the present hydroponic study.

The main goal of this work was to study the mitigating role of proline foliar application in relieving salt stress in two maize genotypes. Furthermore, this study describes the role of proline in the expression of secondary memory in corn plants grown under salt stress.

MATERIALS AND METHODS

Experimental layout

A hydroponic study was conducted at the Saline Agriculture Research Centre, University of Agriculture, Faisalabad, Pakistan (31º43'10" N; 73º06'95" E; 184 m.a.s.l.) to evaluate the effects of applied proline on maize under salinity stress. Seeds of two maize genotypes (Monsanto-6525 and Pioneer-33H25) were collected from registered seed companies. Seeds of both genotypes were sown in iron trays with washed sand. Adequate moisture level for seed emergence and the seedling foundation was maintained with deionized water, and ten days old uniform-size seedlings were shifted to 6-liter pots fixed with polythene sheets. Seedlings were transplanted to froth-connected gaps to polystyrene sheets sliding over Hoagland's solution [57] at ½ full strength. Pots were kept aerated utilizing air circulation pumps at 0.2 L s^{-1}. The Hoagland solution's pH was sustained daily at 6.5 + 0.5 by using 1M HCl or NaOH. A completely randomized design (CRD) with factorial arrangements was used, each with four replicates.

Treatments

After transplantation, the plants were kept in Hoagland solution in the absence of exogenous treatments to acclimatize. Afterward, 30-days-corn plants were arranged in four treatments: i) Control, ii) 75 mM NaCl, iii) Control + 30 mM proline, and iv) 75 mM NaCl + 30 mM proline. The NaCl was applied directly in the Hoagland solution to be absorbed by the plants roots. In contrast, the proline was applied by a foliar application using a handed 500 mL button spray dispensing 30 mL plant-1 day-1, promoting trichome foliar absorption. NaCl (Sigma-Aldrich Chemical Co, Darmstadt, Germany, part number S9888) provided the salt concentration, while proline (Sigma-Aldrich, part number P3350000) was purchased for experimentation. To ensure maximum absorption of proline, surfactant (Ecosurf SA-4, Sigma-Aldrich, part number STS0017) at 0.1% was added.

Analytical Procedures

Gas exchange and chlorophyll a fluorescence

Gas exchange was measured on the flag leaf using a portable open-flow infrared gas analyzer (LI-6400XT; LI-COR Inc., Lincoln, NE, USA) with integrated fluorescence chamber heads (LI-6400-40; LI-COR Inc.). The net photosynthesis (*A*N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*g*s, mmol H_2O m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), internal-to-ambient CO_2 concentration (Ci/Ca ratio), electron transport rate (ETR) were measured as previously described in Iqbal *et al.* [21]. In the same flag leaf used to gas exchange measurements, the chlorophyll a fluorescence parameters were measured as previously reported in cowpea [16].

Relative Water Content Measurement (RWC)

The +3 attached fully expanded were used to measure the RWC in accordance with Ashraf *et al.* [58] as $RWC\ (\%) = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{oven dry weight})} \times 100$

Determination of Membrane Stability Index

The +3 attached fully expanded leaf from the apex were harvested and processed in accordance with Ashraf *et al.* [58] to measure the membrane stability index (MSI) as (MSI) as $MSI\ (\%) = (\frac{EC1 - EC2}{EC2}) \times 100$.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Biochemical measurements

The same leaf used to estimate the parameters described in topic 2.3.1 was fast frozen in liquid nitrogen and stored at -80ºC until use.

Chlorophyll measurements

Approximately 50 mg of leaf sample was used for the chlorophyll (Chl) and carotenoids (Car) extraction using microtubes containing 2 mL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, part number 472301) as described in Pompelli *et al.* [16].

Determination of K^+ and Na^+

Dried leaf samples were used to determine Na^+ and K^+ after drying in an air forced oven (65º ± 5º C) for 72 h. These samples were processed as previously described in Alnusairi*, et al.* [59] and Amirjani [60].

Proline determination

Free proline contents were determined following Bates *et al.* [61]. Thus, frozen leaf sample (500 mg) was homogenized in 10 mL of 3% sulpho-salicylic acid. The homogenate was centrifuged for 10 min at 3000 ×g, the mixture was heated at 100º C for 1 hour in a water bath after adding glacial acetic acid, Ninhydrin. The supernatant was mixed with toluene and read at 520 nm using a microspectrophotometer. Proline was expressed in mmol kg^{-1} DW.

Antioxidant enzymes

Key antioxidant enzymes, including ascorbate peroxidase (APX) and catalase (CAT), were estimated by measuring the rate of decomposition of H_2O_2 at 290 nm and 240 nm, respectively. Superoxide dismutase (SOD) was estimated by measuring its ability to inhibit the photochemical reduction of *p*-nitro-blue--tetrazolium chloride at 560 nm. Further details have been documented [62].

qRT-PCR

For the qRT-PCR analysis, 1 g leaf samples were collected from five replicates of each treatment after 20 days of germination and 12 hours of stress exposure. These samples were immediately ground to a fine powder in liquid n and then stored at -80°C until further use.

Total RNA extraction was performed using the NucleoSpin RNA Plant kit (Macherey Nagel, Düren, Germany), following the manufacturer's instructions. RNA integrity and DNA contamination were evaluated using a 1% agarose gel. Additionally, RNA concentration and quality were determined using a NanoDrop™ One/OneC spectrophotometer (Thermo Scientific).

Subsequently, the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used to synthesize first-strand cDNA from a total RNA concentration of 1000 mg mL^{-1}, following the manufacturer's instruction. The resulting cDNA was diluted in a 1:10 ratio and stored at -40°C for subsequent use. In the gene expression analysis, a total of 10 genes related to abiotic stress were studied using the pre-established protocol by Chen*, et al.* [63].

Biomass determination

In the final experiments, both shoot and root dry weights were computed. For this, fresh weights were put in an oven at 70ºC for 72 hours. After that, the dry weights were computed using an analytical balance (Sartorius Analytical Balance mod. ENTRIS224-1S, Bradford, MA, USA; accurate to 0.1 mg).

Statistical Analysis

The present study was arranged in a completely randomized design with factorial arrangements. All analyses were replicated four times. Two-way ANOVA analyzed all the data. All means were compared using a SNK test ($p < 0.05$) by Statistic version 14.0 (StatSoft, Tulsa, OK, USA). Principal component analysis (PCA) was performed using Minitab® version 17.0 software (Minitab Ltd. Coventry, UK). Heatmaps were used to compare the meaning of each treatment, using both control treatments as a reference. After log2 transformation, the false color method was used, including a color scale. The heatmaps were constructed using Microsoft® Office 360 (Microsoft Corporation, Redmond, WA, USA) and CorelDRAW Graphics Suite X8 (Corel Corporation, Ottawa, ON, Canada).

RESULTS

Gas exchange and chlorophyll fluorescence analysis

6-week maize plants showed a strong reduction in A_N, whereas salt--stressed-maize plants showed a mean A_N reduction of 76.8% regardless of

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

maize genotype (Fig. 1A). In contrast, plants that received only 30 mM proline, the A_N was increased by 29% and 85.2%, respectively in Monsanto-6525 and Pioneer-33H25 genotype. However, the best performance was shown in plants treated with 75 mM NaCl plus 30 mM proline. In Monsanto-6525, the AN was increased by 691% in comparison to salt-stressed plants. Also, in Monsanto-6525 plants the A_N was 33.2% and 72.4% higher than plants treated only with 30 mM proline or control, respectively.

The *g*s (Fig. 1B) presented the same tendency as A_N; however, the decrease in gs of Pioneer-33H25 was higher than that shown in A_N. Thus, the WUEi presented a similar pattern to previously described features, but the salt-stressed maize plants showed a strong decrease in WUEi of 64% and 69%, respectively, in the Monsanto-6525 and Pioneer-33H25 genotypes.

The internal-to-ambient CO_2 concentration was differentially expressed in accordance with treatments, where salt-stressed plants showed a decrease in Ci:Ca ratio of 39% and 42%, respectively, in Monsanto-6525 and Pioneer-33H25 genotypes. This fall was returned with a slight reduction in the Monsanto-6525 genotype and a non-significative value in the Pioneer-33H25 genotypes (Fig. 1D).

The health of light capture and photochemical reactions involved in photosynthesis is easily accessed by chlorophyll fluorescence analysis. The Fv:Fm ratio shows that in Monsanto-6525, any treatment was able to reduce the Fv:Fm ratio value to a physiological interested fall since that some medians were statistically reduced (Fig. 2A). In Pioneer-33H25, the Fv:Fm ratio was lowest in control (52%) and in salt-stressed (61%) when compared to proline-plants. Since 0.72 ± 0.01 (control mean) is lower than expected, this fall has a physiological effect on plants. While in the salt-stressed Pioneer-33H25 genotype, the fall in the Fv:Fm ratio was 61%, showing physiological effects in the light capture system. Regardless of genotype, ETR was non-significant between control-, salinity-, and proline plants, and with salinity + proline plants. In another way, salinity + Proline Monsanto-6225 plants showed an ETR of 104% and 92% higher than control and salt-stressed plants. Salinity + Proline Pioneer-33H25 plants showed an ETR of 69% and 78% higher than those measured in control or salt-stressed maize plants (Fig. 2B).

Figure 1. Effect of salinity, proline, and salinity + proline on net photosynthesis (A), stomatal conductance (B), intrinsic water use efficiency (C), and internal-to-ambient CO_2 concentration (D) measured in 6-week Monsanto-6525 (dark green) and Pioneer-33H25 (light green) maize plants. Means followed by small letter case denote significant difference between treatments in each genotype and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.

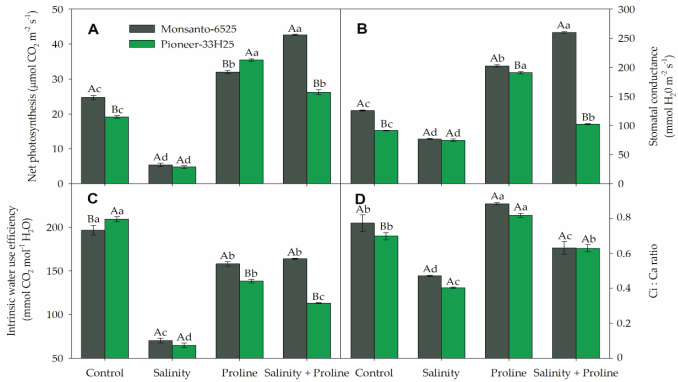
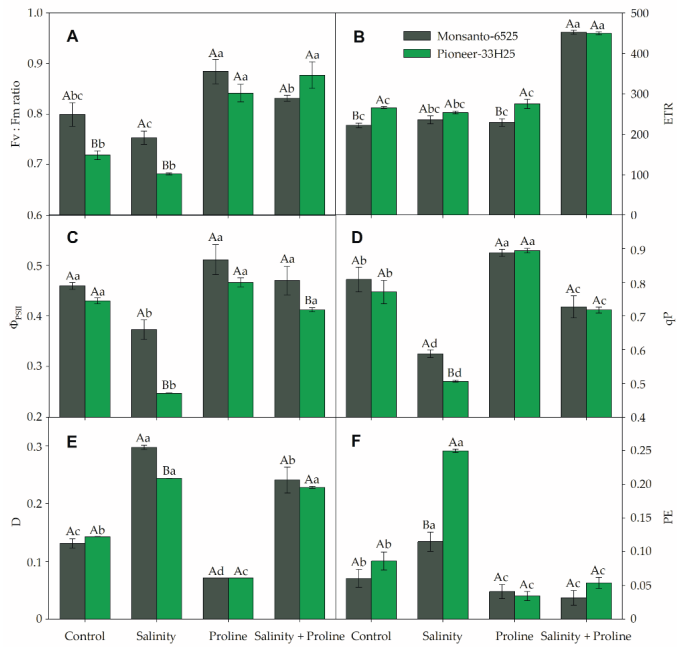


Figure 2. Effect of salinity, proline and salinity + proline on Fv : Fm (A), electron transport rate (ETR; B), quantum yield of photosystem II (Φ_{PSII}; C), photochemical quenching (qP; D), fraction of PAR thermally dissipated (D), and fraction of PAR neither dissipated in photochemical nor thermally (PE) measured in 6-week maize plants of genotypes Monsanto-6525 (dark green) and Pioneer-33H25 (light green). Means followed by small letter case denote significant difference between treatments in each genotype and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.



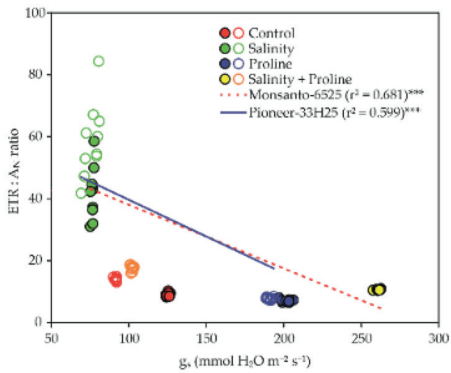
ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

FPSII of the salt-stressed Monsanto-6225 plants show a lesser reduction of 19%, while salt-stressed Pioneer-33H25 plants presented a FPSII strongly reduced in 43% (Fig. 2C).

qP of salt-stressed plants were reduced in about 31% with lesser, but non-significative less qP in Pioneer-33H25 plants (Fig. 2D). Inversely, D (Fig. 2E) and PE (Fig. 2F) increased by 97% and 149%, respectively in salt-stressed plants. In plants treated with 75 mM NaCl + 30 mM proline, the plants were able to thermally dissipate the fraction of absorbed light (Fig. 2E) but don't modulate the PE quenching (Fig. 2F).

Figure 3 shows that, regardless of treatment, the Pioneer-33H25 has lesser g_s for the same ETR:A_N ratio. Here, we highlight the salt stress that decrease g_s with a light increase in ETR:A_N due to low available carbon to reduce aligned with a lesser efficiency of electron use (higher ratio). The plants under salt stress combined with proline also deserve mention because in Pioneer-33H25, the plants showed the same electron use efficiency with 63% less g_s, with water use efficiency overwhelmed electron use efficiency.

Figure 3. Regression between the ratio of electron transport rate (ETR) and net photosynthesis (*A*N) versus stomatal conductance (g_s) measured in 6-week maize plants of genotypes Monsanto-6525 (full symbols) and Pioneer-33H25 (open symbols). The plants were subjected to 75 mM NaCl (orange symbols), 30 mM proline (blue symbols), combination of two first salts (yellow symbols), plus control (red symbols). The regression coefficient (r^2) is shown. *** Significant at $p < 0.001$.



Relative water content in leaves

The change in relative water content (RWC) was low, ranging from 86% to 96%. In Monsanto-6525, the salt stress shows a 4.1% decrease in RWC compared to the control and a reduction of 6.4% when the base of comparison is plants under proline treatment (Fig. 4A). In Pioneer-33H25, the salt stress decreases the RWC by 6.6% and 9.0%, respectively, in comparison to control and proline plants.

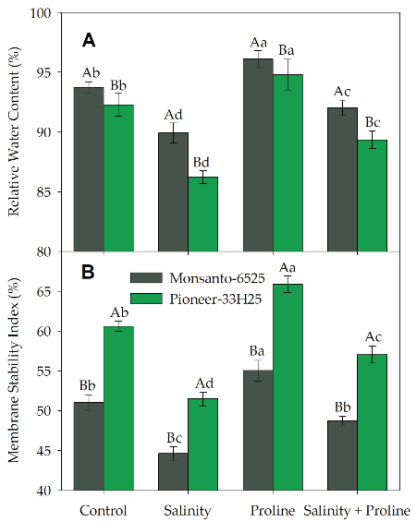
Membrane stability index (MSI)

Similarly, the MSI shows lesser values in salt-stressed plants, followed by control and proline plants, a pattern that is independent of genotype. However, Pioneer-33H25 plants showed an MSI 15.4%, 17%, 18.7%, and 19.7% higher than the MSI registered in Monsanto-6525 (Fig. 4B).

Photosynthetic pigments

The total chlorophyll content of both genotypes was significantly decreased under salt stress as compared to control (Fig. 5A). There was a 58.6% and 48.2% reduction in total chlorophyll content of Monsanto-6525 and Pioneer-33H25 respectively in salt stress when compared to control. It was observed that the Pioneer-33H25 genotype had higher salt tolerance than Monsanto-6525 due to a lesser chlorophyll decrease in salt-stressed plants compared to the control. Both genotypes showed significant improvement in total chlorophyll content in response to proline application under non-saline and saline conditions. In plants subjected to proline, the total chlorophyll was increased by 39.8% and 93.3% in Monsanto-6525 and Pioneer-33H25, when control was the base of mensuration (Fig. 5A).

Figure 4. The effect of salinity, proline and salinity + proline on relative water content (A) and membrane stability index (B) measured in 6-week maize plants of genotypes Monsanto-6525 (dark green) and Pioneer-33H25 (light green). Means followed by small letter case denote significant difference between treatments in each genotype and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.

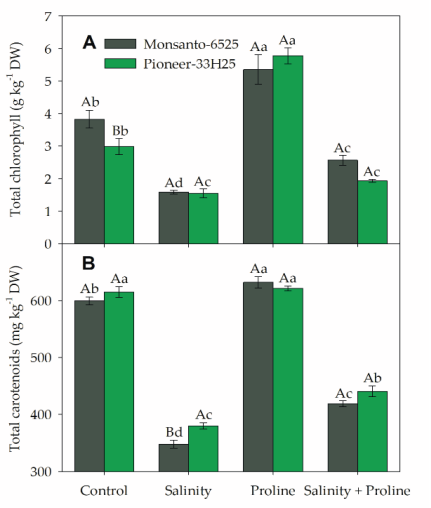


ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Total carotenoids show the same pattern previously described for chlorophyll (Fig. 5B). The main difference results in Pioneer-33H25, which shows more carotenoids than Monsanto-6525 since this higher concentration is not statistically significant in control and saline + proline.

In salt-stressed plants, ETR did not change with a change in chlorophyll concentration (Fig. 6A). However, the response of Φ_{PSII} to changes in chlorophyll concentration is completely different from that described in ETR. Except for salt-stressed plants, all other treatments increase Φ_{PSII} as total chlorophyll increases (Fig. 6B). However, only in Monsanto-6525 is this relationship significant (r^2 = 0.762), and in Pioneer-33H25 the correlation between chlorophyll and FPSII results in a *r*2 of 0.135, due to the high variance measured in Proline plants, since this variation also occurred in Monsanto-6525.

Figure 5. The effect of salinity, proline and salinity + proline on total chlorophyll (A) and carotenoids (B) measured in 6-week maize plants of genotypes Monsanto-6525 (dark green) and Pioneer-33H25 (light green). Means followed by small letter case denote significant difference between treatments in each genotype and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.



Root and Shoot Ionic Composition

The shoot and root K^+ concentrations of both genotypes were significantly decreased under salinity. In salt-stressed plants, K^+ concentration in shoots was decreased in 25.6% and 24.4% as compared to its controls (Fig. 7A). This

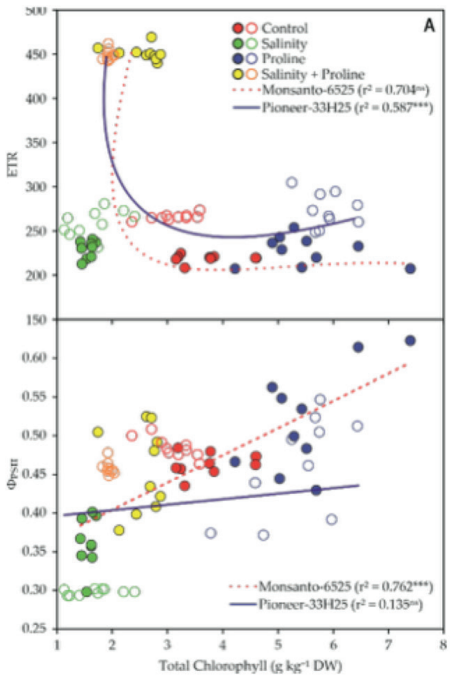
decrease was slight reverted in salt-stressed plus proline plants in 20% and 19.1%, respectively in Monsanto-6525 and Pioneer-33H25. Regardless of roots, the K^+ concentration was 44.1% and 45.2% lesser in salt-stressed plants as compared to its controls (Fig. 7B). However, salt-stressed plants plus proline were 41.3% and 34% higher in favor to salt-stressed plus proline plants, both in Monsanto-6525 and Pioneer-33H25, respectively.

Similarly, shoot and root Na^+ concentrations of both genotypes were significantly increased under salinity stress as compared to control (Fig. 7C,D). In shoot, there was a 74.1% and 50.3% increase in Na^+ concentration respectively in Monsanto-6525 and Pioneer-33H25 genotype, when comparig salt-stressed plants with its controls. In other way, salt-stressed plus proline plants there are a significant decrease of 11.8% and 10.4% compared to salt-stressed Monsanto-6525 and Pioneer-33H25 plants, respectively. In maize roots plant, there was a 38% and 32.2% increase in Na^+ concentration, respectively in Monsanto-6525 and Pioneer-33H25 genotype (Fig. 7D). When compared salt-sressed plus proline plants, proline application decreased Na^+ concentration of 10.4% and 11.7%, respectively in salt-stressed Monsanto-6525 and Pioneer-33H25.

More than analyzing the K^+ and Na^+ in isolated form, it's very important to show the ratio between K^+ and Na^+ due to both ions are highly competitive in some biological reactions. So, the K^+:Na^+ ratio measured in shoot (Fig. 7E) and root (Fig. 7F) of salt stressed plants was 0.30 ± 0.01 (shoot) and 0.16 ± 0.1 (root) in Monsanto genotype and 0.30 ± 0.01 (shoot) and 0.26 ± 0.01 (root) in Pioneer-33H25 salt-stressed plants. This relation is very important, thus we describe that the salt-stressed Monsanto-6525 plants showed a decrease in 57.7% (shoot; Fig. 7E) and 59.5% (root; Fig. 7F) in K^+ : Na^+ ratio. When comparing salt stressed plants with salt-stressed plus proline plants, we describe that the last shows an increase in K^+:Na^+ ratio of 198% and 202% in Monsanto-6525 plants and in 161% and 197% in Pioneer-33H25 plants.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Fig. 6. Relationship between total chlorophyll and electron transport rate (ETR; A) and total chlorophyll and quantum yield of PSII (Φ_{PSII}; B) in 6-week maize plants of genotypes Monsanto-6525 (full symbols) and Pioneer-33H25 (open symbols). The plants were subjected to 75 mM NaCl (orange symbols), 30 mM proline (blue symbols), combination of two first salts (yellow symbols), plus control (red symbols). Each symbols denote one repetition. The regression r^2 are shown. ns, non-significative, *** significative at $p < 0.001$.



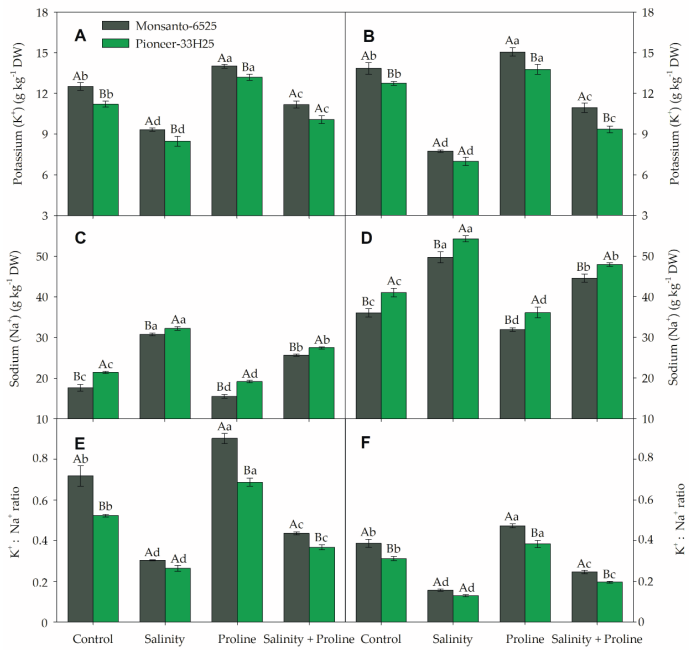
In *totun*, it was observed that the Monsanto-6525 had higher salt tolerance as compared to Pioneer-33H25 due to more K^+ and less Na^+ concentration both shoot (Fig. 7A,C) and root (Fig. 7B,D).

Antioxidative defense

In salt-stressed plants, the superoxide dismutase (SOD) activity was increased by 47.7% and 40.1%, respectively in Monsanto-6525 and Pioneer-33H25 (Fig. 8A). Moreover, in salt-stressed plus proline plants, the SOD was weekly decreased in 4.6% in Monsanto-6525 and moderately in 18.5% in Pioneer-33H25 plants; both means statistically different from control or salt-stressed without proline plants. Catalase (CAT) activity shows a similar pattern to those described to SOD, however with higher intensity. Monsanto-6525 and Pioneer-33H25 salt-stressed shows an increase of 149.1% and 110.1%, respectively when compared to its controls (Fig. 8B);

however, salt-stressed plus proline plants shows a significant decrease in 13.5% and 24.9% in CAT activity when compared to salt stressed plants. When analyzed the role of genotype in SOD and CAT activity, we showed that Pioneer-33H25 modulated more than Monsanto-6525, mainly in salt-stressed plants. This pattern was inverted when we analyzed the ascorbate peroxidase (APX) activity. In all cases, Pioneer-33H25 shows lesser values of APX than Monsanto-6525. Likewise other antioxidative enzymes, APX activity was increased in 45.5% and 89.3% respectively in Monsanto-6525 and Pioneer-33H25 salt-stressed plants (Fig. 8C). A peculiar pattern was shown in Pioneer-33H25 salt-stressed plus proline plants which shows a decrease in APX activity by 56% while an increase of 2.4% (ns) was observed in Monsanto-6525 salt-stressed plus proline plants.

Figure 7. The effect of salinity, proline and salinity + proline on potassium (K^+, A-B), sodium (Na^+, C-D), and K^+:Na^+ ratio (E-F) measured in shoot (A, C, E) and root (B, D, F) of 6-week maize plants of genotypes Monsanto-6525 (dark green) and Pioneer-33H25 (light green). Means followed by small letter case denote significant differences between treatments in each genotype, and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.



The successive increase in proline level was observed in the order of control, salinity, proline, and salinity plus proline plants (Fig. 8D). In all cases, Pioneer-33H25 plants showed higher value when compared to Monsanto-6525

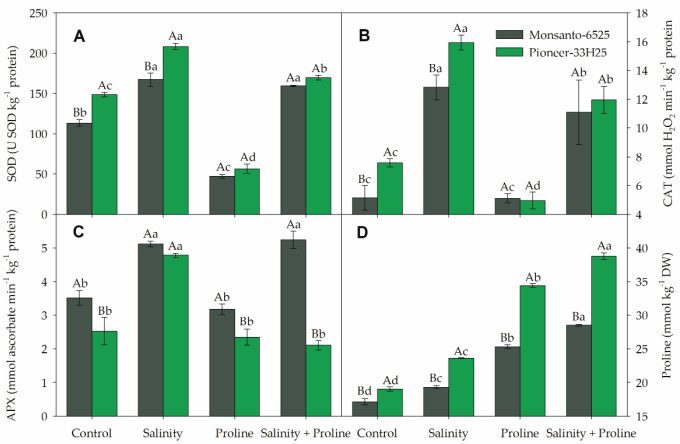
ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

plants. Furthermore, the salt-stressed plants show an increase in proline in 12.7% (Monsanto-6525) and 24.2% (Pioneer-33H25). When comparing salt-stressed plus proline plants with salt-stressed plants, the first shows an increase of 47.8% in Monsanto-6525 and 64.1% in Pioneer-33H25 plants. Moreover, a comparison between salt-stressed plus proline plants and their controls first shows an increase of 66.5% and 103.9%, respectively, in Monsanto-6525 and Pioneer-33H25 plants.

qRT-PCR

All amplicons resulted in the expected lengths, for the 10 genes under the four evaluated conditions (control, salinity (75 mM), proline (30 mM), and proline (30 mM) + salinity (75 mM)) in the two genotypes (Table 1).

Figure 7. The effect of salinity, proline and salinity + proline on superoxide dismutase (SOD, A) catalase (CAT, B), ascorbate peroxidase (APX) and proline (D) measured in 6-week maize plants of genotypes Monsanto-6525 (dark green) and Pioneer-33H25 (light green). Means followed by small letter case denote significant difference between treatments in each genotype and capital letter case denote the differences between genotypes in the same treatment. All bars denote mean (± SE). n =5.



The measured sets of values for the different genes in the plants subjected to salinity and proline treatments were generally higher. Differences between treatments were detected with an increase in the expression of some genes (Figure 8). All evaluated genes showed a more pronounced difference in the Monsanto 6525 genotype, as shown in Figure 8. However, the proline (30 mM) + salinity (75 mM) treatment was very similar to the control. Gene expression results did not show significant differences between the two genotypes

evaluated; however, the Monsanto 6525 genotype exhibited higher expression of the evaluated genes.

The combined expression and physiological analysis revealed that the proline treatment had a more evident mitigating effect on the Pioneer-33H25 genotype. Therefore, we speculate that despite showing tolerance to salinity stress, this material is less tolerant than the Monsanto 6525 genotype.

Biomass measurements

The shoot and root dry weights of both genotypes were significantly decreased in salt-stressed plants in contrast to their respective control (Table 1). There was 39% and 49.8% less shoot dry weight, respectively, in Monsanto-6525 and Pioneer-33H25 salt-stressed plants. In salt-stresses plus proline plants, there was an increase in shoot dry weight in Monsanto-6525 (32.8%) and Pioneer-33H25 (13.8%) when compared to their respective control.

Similarly, the root dry weight decreases by 12.4% and 60%, respectively, in Monsanto-6525 and Pioneer-33H25 salt-stressed plants (Table 2). Both genotypes showed significant improvement in shoot and root dry weight due to the application of proline in both non-saline and saline conditions.

Multifactorial analysis

Even though most of the analyses showed a positive effect of the genotype, sometimes with significant interaction with the treatments, the output multifactorial data shows us that the effect of treatments is overwhelmed by genotype effects. The analysis of component 1 (PC1) corresponds to the largest change in the parameter (72.9%), while component 2 (PC2) reflects the maximum parameter change in the same plane (16.2%), on which the location of the point with the coordinate of PC1/PC2 showed the state of net photosynthesis and the effects of salt stress against it. The PC1 and PC2 reflect total 89.1% of changes (Fig. 9A). In PC1, both qP and RWC contribute with 24.8% of variations in *A*N, followed by Ci:Ca ratio (24.7%), potassium in root (24.4%), potassium in shoot (23.8), and chlorophyll (23.5%). The content of sodium in shoot (25%), CAT (24.6%), SOD (24.1%), sodium in root (24%), and D (22.8%) were the factors that contribute to reduction of A_N. As other PCs contributes less to increase or decrease in A_N, we do not describe this effect.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Table 1. Primer sequences used for qRT-PCR analysis in this article.

GeneID	Description	Forward primer	Reverse primer
100284352	seven-transmembrane-domain protein 1	AGTTGCCATTCATCGCTCTTC	TTCTTTGACACGACACGCAC
100383492	putative alpha-amylase family protein	TACTGCGTCTGGGAGAAAGC	CGTTATGCAGTGTCGTGCTG
100501464	Sulfate transporter 1.2	GCCTTGGAGGAGTTGCTGAA	TCTATGTTAGCGGGGTATCGC
103641785	POU domain, class 3, transcription factor 3	CACCGGTACCATCAGTTCGG	GCCACACCAAACGTTATCCA
103643513	glycine-rich cell wall structural protein 2-like	AAGATGGCGACCAAACACCT	ATAACCCAGTTTGCGGTTTGC
100281135	Protein P21	CCCACCGACTACTCCAAGTT	GAGCTCATGGGCAGAAGACG
103631966	probable carboxylesterase 15	GCAATGTGCCGACTGTAACG	ACACGTGGTACATCCATCGC
103643618	potassium transporter 21-like	GGGGAGAAGATGCTGTCGATT	CGGAAACATGTCCAGATCCCA
100280929	DNA binding protein	GATCCACCGCGACTCTACG	ATCTGAAGAGCACCGGGAAC
100279614	putative O-Glycosyl hydrolase superfamily protein	AGGCTTACTGCGTACTGGTAG	GACGACAGTGCTGAACAGAGA

Figure 8. Comparative results of gene expressions for all treatments, based on qRT-PCR of ten genes related to ion transport, signal transduction, secondary metabolism, and elimination of reactive oxygen species.

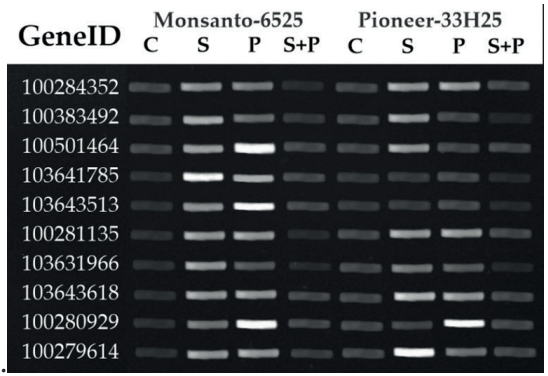


Table 2. The effect of salinity, proline, and salinity + proline on shoot and root dry weight in Monsanto-6525 and Pioneer-33H25 maize genotypes. Upper-case letters denote significant differences between genotypes in each feature, and small-case letters denote significant differences between treatments in each genotype. All data represent the means (± SE), n = 5.

	Shoot dry weight (g per plant)			
	Control	**Salinity**	**Proline**	**Salinity + Proline**
Monsanto-6525	2.58 ± 0.09 Ab	1.57 ± 0.07 Ad	2.89 ± 0.02 Aa	2.09 ± 0.09 Ac
Pioneer-33H25	1.19 ± 0.02 Bb	0.60 ± 0.07 Bc	1.79 ± 0.08 Ba	0.68 ± 0.05 Bc
	Root dry weight (g per plant)			
Monsanto-6525	0.79 ± 0.01 Ab	0.69 ± 0.02 Ac	0.85 ± 0.03 Aa	0.74 ± 0.02 Ab
Pioneer-33H25	0.53 ± 0.02 Bb	0.21 ± 0.02 Bd	0.60 ± 0.02 Ba	0.29 ± 0.01 Bc

Multifactorial analysis

Even though most of the analyses showed a positive effect of the genotype, sometimes with significant interaction with the treatments, the output multifactorial data shows us that the effect of treatments is overwhelmed by genotype effects. The analysis of component 1 (PC1) corresponds to the largest change in the parameter (72.9%). In comparison, component 2 (PC2) reflects the maximum parameter change in the same plane (16.2%), on which the location of the point with the coordinate of PC1/PC2 showed the state of net photosynthesis and the effects of salt stress against it. The PC1 and PC2 reflect a total of 89.1% of changes (Fig. 9A). In PC1, both qP and RWC contribute with 24.8% of variations in A_N, followed by Ci:Ca ratio (24.7%), potassium in root (24.4%), potassium in shoot (23.8), and chlorophyll (23.5%). The content of sodium in shoot (25%), CAT (24.6%), SOD (24.1%), sodium in root (24%), and D (22.8%) were the factors that contribute to reduction of A_N. As other PCs contribute less to the increase or decrease in A_N, we do not describe this effect.

After critical evaluation of the multifactorial analysis, we should describe four distinct groups, composed exactly by treatments. However, there are a positive interaction of proline and control plants, which should be joined in a single cluster if we consider 81.7% of similarity, in distinct form when the cluster is analyzed in 47.5% of similarity (Fig. 9B-C).

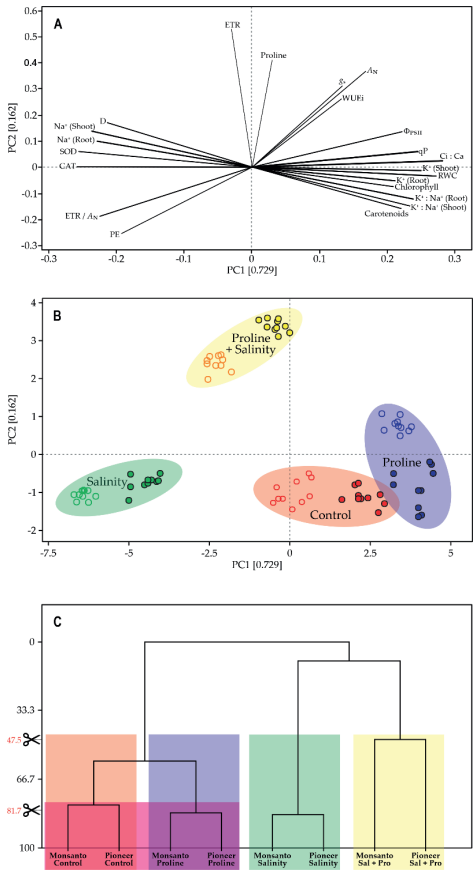
DISCUSSION

Distinctive methodologies are used to mitigate the harmful impact of salt stress. Among them, foliar spray of osmoprotectants like proline is more efficient for enhancing crop salt resistance [64]. The present research demonstrated the defensive role of exogenous proline on maize genotypes under salt stress. Both genotypes responded differently to proline under saline conditions. However, foliar-applied proline was found to be more effective in reducing the adverse effects of salinity in both genotypes, Monsanto-6525 and Pioneer-33H25. Moreover, extreme changes were observed in Monsanto-6525. Proline influenced plant characteristics like shoot and root dry weights, total chlorophyll content, proline content, MSI, RWCs, Na^+, and K^+ content in both root and shoot. Significant differences were observed between the shoot and root dry weight of both genotypes; however, the most noteworthy increase was observed in Monsanto-6525 regarding shoot and root dry weight under saline conditions. The main reason behind low shoot and root dry weight might be an addition in the

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

osmotic potential that causes an increment in salt concentration, leading to dehydration, ionic imbalance in transpiring leaves, inhibited meristem activity, cell elongation, and subsequently hinders plant growth [16]. Salt accumulation in the root zone caused a reduction in the cell wall elasticity and changes in the metabolic pathway and resulted in decreased membrane stability [58]. The foliar proline application increased crops' root and shoot dry weight by antagonizing salts' harmful effects. Increased root and shoot dry weight due to proline application minimized the harmful impact of salinity on the stem and leaf anatomy [37,59].

Figure 9. Multivariate analysis to assess the morphophysiological characteristics of maize genotypes under salt stress and salt stress plus proline. (A) Spatial distribution of all analyzed features, showing the strength of each influencing the *A*N. In (B) and (C), all treatments are displayed in the PC1 and PC2 to show that both analyzed genotypes there are lesser influence on clustering than treatments. *A*N, net photosynthesis; CAT, catalase; Ci:Ca, internal-to-ambient CO_2 concentration; D, fraction of PAR thermally dissipated; ETR, electron transport rate; FPSII, quantum yield of photosystem II; *g*s, stomatal conductance; PE, fraction of PAR neither dissipated in photochemical nor thermally; qP, photochemical quenching; RWC, relative water content; SOD, superoxide dismutase; WUEi, intrinsic water use efficiency.



In the present study, significant reduction in gaseous exchange parameters (Fig. 1-2) and total chlorophyll contents (Fig. 3) were observed due to salinity stress in comparison to control condition and maximum gaseous exchange reactions and total chlorophyll reading was observed in Monsanto-6525. Reduction in chlorophyll contents can be attributed to ion accumulation and functional disorder observed during stomatal regulation under salinity stress [51,52]. Moreover, a decrease of chlorophyll contents under the saline condition is described as rapid maturation of leaves [53]. The reduction in photosynthetic pigments also impairs the net photosynthesis rate [13], which is also linked to low stomatal conductance, ETR, and misfunctioning of photosystems observed in this study. The increase in PE suggests that the down-regulation of PSII to prevent over--reduction of QA was insufficient to compensate for the decreased demand for electrons through $NADP^+$ consumption. Salt-tolerant species usually remained less affected, with minor changes in chlorophyll content under salinity conditions. Contrarily, a significant reduction in chlorophyll contents of salt-sensitive species suggested it as a biochemical marker of salt tolerance in plants [54,55]. Salinity stress induces chlorophyllase enzyme that is responsible for chloroplast damage, causes reduction in photosynthetic pigment and photosynthesis rate [65]. The proline significantly reduced the antagonistic impact of salt stress on chlorophyll content [56]. Proline application improved gaseous exchange reactions and chlorophyll content, thus improved the photosystem efficiency and photosynthesis rate in maize genotypes (Fig. 1-3). Proline also protects the RuBisCO and ETC under stress conditions, thus improve photosynthesis processes [65]. Our findings confirmed the findings of Wani, *et al.* [66] who reported that exogenous application of proline increased photosynthesis rate, stomatal conductance, and efficiency of Fv:Fm in *Brassica juncea* under salt stress.

Salinity damages the membrane, and it can be assessed indirectly by estimating the MSI [60]. Increasing salinity stress in the present study caused a decrease in MSI of both genotypes compared with control plants. However, proline application improved MSI (Fig. 4). The use of exogenous proline adjusted the stress-mediated damage to the plasma membrane, as was clear from the significant increase in MSI of both genotypes associated with those of the reference controls. The present results are in according with Talat *et al.* [61]. This authors described that the exogenous proline application increased MSI in wheat under salt stress. Leaf water content is a criterion to define the water content of plants [62,63]. The significant differences were observed between

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

leaves RWC of both genotypes however the most noteworthy water content was observed in Monsanto-6525. Huge salt concentration considerably decreased the percent RWCs up to several folds. A decrease in RWC was observed in rice seedlings cv. *Tarom Azmoon* at 200 mM salinity stress [64]. Exogenous application of proline radically diminished the lethal consequences for RWC in pea plants grown under saline conditions (Shahid *et al.* 2014). In the barley seedlings, RWC increased after proline application under saline conditions [65].

The proline content of both genotypes was significantly increased under salinity stress. Still, the exogenous application of proline decreased proline content in the plant (Fig. 6). Accumulation of proline is mostly reported to be prompted by water stress in plants and involved in stress tolerance mechanisms [66,67]. Remarkable increment in proline content was detected in leaves of salt-resistant cultivars compare to salt-sensitive cultivars [68]. The higher buildup of proline might be because of (a) rise in proline biosynthesis (b) decrease in proline consumption (c) and degradation (d) increase in Glutamine synthetase activity [69]. In soybean, the intracellular proline content was increased as salinity level increased [70,71]. Reduced free proline under saline stress was improved by the foliar application of proline in pea plants [72]. A similar application of proline to *Pancratium maritimum* produces a substantial increase in the endogenous level of proline stressed in both stressed and non-stressed plants [73].

Many crop species experience subsequently saline conditions and offer a decrease in development K^+ concentration and an increase in Na^+ concentration. The present study showed that the supply of Na^+ was enhanced under salinity stress in both genotypes whereas foliar-applied proline significantly reduced the root and shoots Na^+ content in both genotypes and improve the growth of plants concerning control (Figs. 7). Proline excludes maximum Na^+ from roots and shoots of both genotypes and maintains the plant's osmotic potential as proline plays a vital role in the buildup of the osmotic adjustment [74]. The foliar application of 25 mM proline caused a substantial decrease in Na^+ and Cl^- content [59]. Potassium efflux was significantly decreased using proline and ionic homeostasis was maintained by enhancing the H^+ ATPase activity [75,76]. Proline application significantly increased the root and shoots K^+ content under salt stress and improved the growth of maize plants. In ionic parameters, the Monsanto-6525 genotype showed a better response to proline application under saline conditions than others. Similar results were found by Dawood *et al.* (2014) [59]. They reported significant increases in K^+, and K^+:Na^+ ratio by foliar proline application

in *Vicia faba*. Foliar proline applications increased the K^+ concentrations, and decreased Na^+ ion concentrations, in salt-affected *Phaseolus vulgaris* L. plants. Thus, the K^+:Na^+ ratio increased [77]. Proline application mitigated the salinity stress and improved the growth of both maize genotypes. Proline spray may raise the uptake of beneficial macro-nutrients to keep the osmotic adjustment by minimizing toxic ion (Na^+ and Cl^-) concentrations to facilitate normal growth and development of crops [26,28]. Our study showed that K^+:Na^+ ratio increased in Monsanto-6525 and Pioneer-33H25 upon exposure to all treatments especially proline (Fig. 7), which indicates the proline induced tolerance and homeostasis in these genotypes. However, Monsanto-6525 tolerates stress conditions better than Pioneer-33H25, which is confirmed from the enhanced expression of genes in former genotype (Fig. 8). The amplification results showed that all genes exhibited the expected lengths in the four evaluated conditions. However, there were notable variations in gene expression levels among treatments. Some genes displayed higher expression levels in response to salinity and proline treatments, indicating their involvement in the plant's response to abiotic stress. Notably, the Monsanto 6525 genotype demonstrated more pronounced differences in gene expression compared to Pioneer-33H25. The study revealed that the Pioneer-33H25 genotype exhibited a more prominent mitigating effect in response to proline treatment. This suggests that this genotype may possess inherent mechanisms for better coping with salinity stress when supplemented with proline. On the other hand, the Monsanto 6525 genotype displayed a higher overall expression of the evaluated genes, indicating its potential for better stress tolerance. Like our findings, following genes (TaPTF1, TaP5CS, TaGSK1, and TaSRG) were expressed in wheat upon exogenous application of proline under salt stress [67]. The study highlights the positive influence of exogenously applied proline on the growth and physiological responses of maize plants under abiotic stress. The differential responses observed between genotypes suggest variations in their inherent stress tolerance mechanisms. Further research is warranted to elucidate the specific molecular pathways and mechanisms underlying the observed responses and to explore the potential application of these findings in enhancing crop productivity under salinity stress conditions.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

CONCLUSION

The present study showed that salinity causes a significant effect on the growth of maize and that can be minimized by the application of proline during the vegetative growth stage. Crop cultivation in saline areas might be profitable with proline applications. Under the saline condition, Pioneer-33H25 showed better results in chlorophyll content, proline content, shoot K, shoot, and root Na concentration while Monsanto-6525 performed better in case of shoot and root dry weight, RWC, MSI and root K concentration. Maize cultivar Monsanto-6525 showed better results compared to Pioneer-33H25 when proline was applied as a foliar spray, which also showed strong gene expression in the former maize genotype under salt stress. Crop plants with better response to proline confer tolerance to salinity. Protective role of proline under salinity in maintaining the ionic balance in plants by increasing K^+, and decreasing Na+ concentration, makes it a more suitable treatment under saline conditions. Based on these findings, the use of proline to enhance plant growth and production in maize cultivar Monsanto-6525 grown under saline conditions can be recommended. However, the present results need to be confirmed in field trials and economic feasibility must be worked out.

DIRECT STUDY

Foster, S.; Pulido-Bosch, A.; Vallejos, Á.; Molina, L.; Llop, A.; MacDonald, A.M. "Impact of irrigated agriculture on groundwater-recharge salinity: a major sustainability concern in semi-arid regions." **Hydrogeol J**, 2018, 26, 2781-2791. DOI: 10.1007/s10040-018-1830-2.

Wen, W.; Timmermans, J.; Chen, Q.; van Bodegom, P.M. "A review of remote sensing challenges for food security with respect to salinity and drought threats." **Remote Sens**, 2021, 13, 6. DOI: 10.3390/rs13010006.

Rehmani, M.I.A.; Ding, C.; Li, G.; Ata-Ul-Karim, S.T.; *et al.* "Vulnerability of rice production to temperature extremes during rice reproductive stage in Yangtze River Valley, China." **J King Saud Univ Sci**, 2021, 33, 101599. DOI: 10.1016/j.jksus.2021.101599.

Rehmani, M.I.A.; Wei, G.; Hussain, N.; *et al.* "Yield and quality responses of two indica rice hybrids to post-anthesis asymmetric day and night open-field warming in lower reaches of Yangtze River delta." **Field Crops Res**, 2014, 156, 231-241. DOI: 10.1016/j.fcr.2013.09.019.

Cheeseman, J. "Chapter 7 - Food Security in the Face of Salinity, Drought, Climate Change, and Population Growth." In **Halophytes for Food Security in Dry Lands**, Academic Press: San Diego, CA, USA, 2016, pp. 111-123.

Research Agreement: This chapter was written based on its original form published in Journal of Plant Growth Regulation. Naz, T.; Iqbal, M.M.;, Ahmad, I.; Sohail, M.A.; Iqbal, S.; Jamal, A.; Rodriguez-Paez, L.A.; Pompelli, M.F. Foliar-application of proline mitigates salinity stress in two mayze (Zea mays L.) genotypes: A comparative study of growth, physiology, chlorophyll fluorescence, and ionic composition. **Journal of Plant Growth Regulation**, 2025. Doi: Under the permission of authors. Springer Nature Licence.

REFERENCES

1. Foster, S.; Pulido-Bosch, A.; Vallejos, Á.; Molina, L.; Llop, A.; MacDonald, A.M. Impact of irrigated agriculture on groundwater-recharge salinity: a major sustainability concern in semi-arid regions. **Hydrogeol J** 2018, 26, 2781-2791, doi:10.1007/s10040-018-1830-2.

2. Wen, W.; Timmermans, J.; Chen, Q.; van Bodegom, P.M. A review of remote sensing challenges for food security with respect to salinity and drought threats. **Remote Sens** 2021, 13, 6, doi:10.3390/rs13010006.

3. Rehmani, M.I.A.; Ding, C.; Li, G.; Ata-Ul-Karim, S.T.; Hadifa, A.; Bashir, M.A.; Hashem, M.; Alamri, S.; Al-Zubair, F.; Ding, Y. Vulnerability of rice production to temperature extremes during rice reproductive stage in Yangtze River Valley, China. **J King Saud Univ Sci** 2021, 33, 101599, doi:10.1016/j.jksus.2021.101599.

4. Rehmani, M.I.A.; Wei, G.; Hussain, N.; Ding, C.; Li, G.; Liu, Z.; Wang, S.; Ding, Y. Yield and quality responses of two indica rice hybrids to post-anthesis asymmetric day and night open-field warming in lower reaches of Yangtze River delta. **Field Crops Res** 2014, *156*, 231-241, doi:10.1016/j.fcr.2013.09.019.

5. Cheeseman, J. Chapter 7 - Food Security in the Face of Salinity, Drought, Climate Change, and Population Growth. In **In Halophytes for Food Security in Dry Lands**, Khan, M.A., Ozturk, M., Gul, B., Ahmed, M.Z., Eds.; Academic Press: San Diego, CA, USA, 2016; pp. 111-123.

6. Corwin, D.L. Climate change impacts on soil salinity in agricultural areas. **Eur J Soil Sci** 2021, 7*2*, 842-862, doi:10.1111/ejss.13010.

7. Mukhopadhyay, R.; Sarkar, B.; Jat, H.S.; Sharma, P.C.; Bolan, N.S. Soil salinity under climate change: Challenges for sustainable agriculture and food security. **J Environ Manage** 2021, 280, 111736, doi:10.1016/j.jenvman.2020.111736.

8. Hopmans, J.W.; Qureshi, A.S.; Kisekka, I.; Munns, R.; Grattan, S.R.; Rengasamy, P.; Ben-Gal, A.; Assouline, S.; Javaux, M.; Minhas, P.S.; *et al.* Chapter One - Critical knowledge gaps and research priorities in global soil salinity. In **In Advances in Agronomy**, Sparks, D.L., Ed.; Academic Press: San Diego, CA,USA, 2021; pp. 1-191.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

9. Smanov, Z.M.; Laiskhanov, S.U.; Poshanov, M.N.; Abikbayev, Y.R.; Duisekov, S.N.; Tulegenov, Y.A. Mapping of cornfield soil salinity in arid and semi-arid regions. **J Ecol Eng** 2023, 24, 146-158, doi:10.12911/22998993/155952.

10. Rafik, F.; Saber, N.; Iben-Halima, O.; Douaik, A. The effects of the quality of irrigation water used on agricultural soils in coastal Chaouia, Morocco. **J Ecol Eng** 2023, 24, 50-60, doi:10.12911/22998993/156691.

11. Iqbal, S.; Hussain, S.; Qayyaum, M.A.; Ashraf, M. The response of maize physiology under salinity stress and its coping strategies. In **Plant Stress Physiology**, Iqbal, S., Hussain, S., Qayyaum, M.A., Ashraf, M., Saifullah, Eds.; IntechOpen: London, UK, 2020; pp. 1-25.

12. Zörb, C.; Geilfus, C.-M.; Dietz, K.-J. Salinity and crop yield. **Plant Biol** 2019, 21, 31-38, doi:10.1111/plb.12884.

13. Askari-Khorasgani, O.; Rehmani, M.I.A.; Wani, S.H.; Kumar, A. Osmotic stress: An outcome of drought and salinity. In **Handbook of Plant and Crop Physiology**, Pessarakli, M., Ed.; CRC Press: Boca Raton, Fl, USA, 2021; pp. 445-464.

14. Kumar, V.; Raghuvanshi, N.; Pandey, A.K.; Kumar, A.; Thoday-Kennedy, E.; Kant, S. Role of halotolerant plant growth-promoting rhizobacteria in mitigating salinity stress: Recent advances and possibilities. **Agriculture** 2023, 13, 168, doi:10.3390/agriculture13010168.

15. Al-Tabbal, J.; Al-Jedaih, M.; Al-Zboon, K.K.; Alrawashdeh, K.A.B. Mitigation of salinity stress effects on kochia (*Bassia scoparia* L.) biomass productivity using biochar application. **Int J Phytoremediation** 2023, 25, 1-7, doi:10.1080/15226514.2022.2164248.

16. Pompelli, M.F.; Arrieta, D.V.; Rodríguez, Y.Y.P.; Ramírez, A.M.J.; Bettin, A.M.V.; Avilez, M.A.Q.; Cárcamo, J.A.A.; Castaño, S.G.G.; González, L.M.M.; Cordero, E.D.F.; *et al.* Can Chlorophyll a fluorescence and photobleaching be a stress signal under abiotic stress in *Vigna unguiculata* L.? **Sustainability** 2022, 14, 15503, doi:10.3390/su142315503.

17. Pompelli, M.F.; Espitia-Romero, C.A.; Jaraba-Navas, J.D.; Rodriguez-Paez, L.A.; Jarma-Orozco, A. *Stevia rebaudiana* under a CO_2 enrichment atmosphere: Can CO_2 enrichment overcome stomatic, mesophilic and biochemical barriers that limit photosynthesis? **Sustainability** 2022, 14, 14269, doi:10.3390/su142114269.

18. Pompelli, M.F.; Jarma-Orozco, A.; Rodrígues-Páez, L.A. Salinity in *Jatropha curcas*: a review of physiological, biochemical, and molecular factors involved. **Agriculture** 2022, 12, 594, doi:10.3390/agriculture12050594.

19. He, Y.; Wang, Y.; Li, X.; Guo, Y.; Ma, L. Breaking the bottleneck of organic light conversion agents: Preparation, performance evaluation and intrinsic mechanism. **Spectrochim Acta A Mol Biomol Spectrosc** 2023, *228*, 122161, doi:10.1016/j.saa.2022.122161.

20. Qin, F.; Bai, J.; Zhu, Y.; He, P.; Wang, X.; Wu, S.; Yu, X.; Ren, L. Searching for the true origin of the red fluorescence of leaf-derived carbon dots. **Phys Chem Chem Phys** 2023, 25, 2762-2769, doi:10.1039/D2CP05130C.

21. Iqbal, W.; Afridi, M.Z.; Jamal, A.; Mihoub, A.; Saeed, M.F.; Székely, Á.; Zia, A.; Khan, M.A.; Jarma-Orozco, A.; Pompelli, M.F. Canola seed priming and its effect on gas exchange, chlorophyll photobleaching, and enzymatic activities in response to salt stress. **Sustainabiity** 2022, 14, 9377, doi:10.3390/su14159377.

22. Mukhtar, E.; Siddiqi, E.H.; Bhatti, K.H. Gas exchange attributes can be valuable selection criteria for salinity tolerance in canola cultivars (*Brassica napus* L.). **Pak J Bot** 2013, 45, 35-40.

23. Praxedes, S.C.; Lacerda, C.F.; DaMatta, F.M.; Prisco, J.T.; Gomes-Filho, E. Salt tolerance is associated with differences in ion accumulation, biomass allocation and photosynthesis in cowpea cultivars. **J Agron Crop Sci** 2010, 196, 193-204, doi:doi: 10.1111/j.1439-037x.2009.00412.x.

24. Dorta-Santos, M.A.; Barriola, I.; Wassner, D.F.; Ploschuk, E.L. Photosynthesis, fluorescence and mesophyll conductance responses to increasing salinity levels in *Jatropha curcas* at early vegetative stages. **J Agron Crop Sci** 2020, 206, 52-63, doi:10.1111/jac.12373.

25. Pan, T.; Liu, M.; Kreslavski, V.D.; Zharmukhamedov, S.K.; Nie, C.; Yu, M.; Kuznetsov, V.V.; Allakhverdiev, S.I.; Shabala, S. Non-stomatal limitation of photosynthesis by soil salinity. **Crit Rev Env Sci Tec** 2020, *51*, doi:10.1080/10643389.2020.1735231.

26. Wungrampha, S.; Joshi, R.; Singla-Pareek, S.L.; Pareek, A. Photosynthesis and salinity: are these mutually exclusive? **Photosynthetica** 2018, 56, 366-381, doi:10.1007/s11099-017-0763-7.

27. Castillejo-Morales, A.; Jarma-Orozco, A.; Pompelli, M.F. Physiological and morphological features denote that salt stress in Stevia rebaudiana is based on nonstomatic instead of stomatic limitation. **Rev Colombiana Cien Hortíc** 2021, 15, e12928, doi:10.17584/rcch.2021v15i3.12928

28. Shahid, M.A.; Sarkhosh, A.; Khan, N.; Balal, R.M.; Ali, S.; Rossi, L.; Gómez, C.; Mattson, N.; Nasim, W.; Garcia-Sanchez, F. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. **Agronomy** 2020, 10, 938, doi:10.3390/agronomy10070938.

29. Yasir, T.A.; Khan, A.; Skalicky, M.; Wasaya, A.; Rehmani, M.I.A.; Sarwar, N.; Mubeen, K.; Aziz, M.; Hassan, M.M.; Hassan, F.A.S.; *et al.* Exogenous sodium nitroprusside mitigates salt stress in lentil (*Lens culinaris* Medik.) by affecting the growth, yield, and biochemical properties. **Molecules** 2021, *26*, 2576, doi:10.3390/molecules26092576.

30. Nazir, M.F.; Sarfraz, Z.; Mangi, N.; Shah, M.K.N.; Mahmood, T.; Mahmood, T.; Iqbal, M.S.; Rehmani, M.I.A.; El-Sharnouby, M.; Shabaan, M.K.A.; *et al.* Post-anthesis mobilization of stem assimilates in wheat under induced stress. **Sustainability** 2021, *13*, 5940, doi:10.3390/su13115940.

31. Munns, R.; Passioura, J.; Colmer, T.; Byrt, C. Osmotic adjustment and energy limitations to plant growth in saline soil. **New Phytol** 2020, 225, 1091-1096, doi:10.1111/nph.15862.

32. Osthoff, A.; Donà dalle Rose, P.; Baldauf, J.A.; Piepho, H.-P.; Hochholdinger, F. Transcriptomic reprogramming of barley seminal roots by combined water deficit and salt stress. **BMC Genomics** 2019, 20, 325, doi:10.1186/s12864-019-5634-0.

33. Li, P.-C.; Yang, X.-Y.; Wang, H.-M.; Pan, T.; Yang, J.-Y.; Wang, Y.-Y.; Xu, Y.; Yang, Z.-F.; Xu, C.-W. Metabolic responses to combined water deficit and salt stress in maize primary roots. **J Integr Agric** 2021, *20*, 109-119, doi:10.1016/S2095-3119(20)63242-7.

34. Mel, V.C.; Bado, V.B.; Ndiaye, S.; Djaman, K.; Aissata Bama Nati, D.; Manneh, B.; Futakuchi, K. Predicting rice yield under salinity stress using K/Na ratio variable in plant tissue. **Comm Soil Sci Plant Anal** 2019, 50, 1321-1329, doi:10.1080/00103624.2019.1614605.

35. Bidalia, A.; Vikram, K.; Yamal, G.; Rao, K.S. Effect of salinity on soil nutrients and plant health. In **In Salt Stress, Microbes, and Plant Interactions: Causes and Solution**: Volume 1, Akhtar, M.S., Ed.; Springer Singapore: Singapore, 2019; pp. 273-297.

36. Hurtado, A.C.; Chiconato, D.A.; Prado, R.M.; Sousa Junior, G.S.; Felisberto, G. Silicon attenuates sodium toxicity by improving nutritional efficiency in sorghum and sunflower plants. **Plant Physiol Bioch** 2019, 142, 224-233, doi:10.1016/j.plaphy.2019.07.010.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

37. Acosta-Motos, J.R.; Ortuño, M.F.; Bernal-Vicente, A.; Diaz-Vivancos, P.; Sanchez-Blanco, M.J.; Hernandez, J.A. Plant responses to salt stress: adaptive mechanisms. **Agronomy** 2017, *7*, 18, doi:10.3390/agronomy7010018.

38. Majeed, A.; Siyyar, S. Salinity stress management in field crops: An overview of the agronomic approaches. In **Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives II: Mechanisms of Adaptation and Stress Amelioration**, Hasanuzzaman, M., Ed.; Springer Singapore: Singapore, 2020; pp. 1-6.

39. Hanin, M.; Ebel, C.; Ngom, M.; Laplaze, L.; Masmoudi, K. New insights on plant salt tolerance mechanisms and their potential use for breeding. **Front Plant Sci** 2016, *7*, 1787, doi:10.3389/fpls.2016.01787.

40. Saradadevi, G.P.; Das, D.; Mangrauthia, S.K.; Mohapatra, S.; Chikkaputtaiah, C.; Roorkiwal, M.; Solanki, M.; Sundaram, R.M.; Chirravuri, N.N.; Sakhare, A.S.; *et al.* Genetic, epigenetic, genomic and microbial approaches to enhance salt tolerance of plants: A comprehensive review. **Biology** 2021, 10, 1255, doi:10.3390/biology10121255.

41. Cao, Y.; Zhou, Z.; Song, H.; Zhang, M.; Jiang, C. Advances in deciphering salt tolerance mechanism in maize. **Crop J** 2023, *in press*, doi:10.1016/j.cj.2022.12.004.

42. Oh, G.G.K.; O'Leary, B.M.; Signorelli, S.; Millar, A.H. Alternative oxidase (AOX) 1a and 1d limit proline-induced oxidative stress and aid salinity recovery in Arabidopsis. **Plant Phvsiol** 2022, 188, 1521-1536, doi:10.1093/plphys/kiab578.

43. Shafi, A.; Zahoor, I.; Mushtaq, U. Proline accumulation and oxidative stress: diverse roles and mechanism of tolerance and adaptation under salinity stress. In **Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches**: Volume 2, Akhtar, M.S., Ed.; Springer Singapore: Singapore, 2019; pp. 269-300.

44. Singh, M.; Kumar, J.; Singh, S.; Singh, V.P.; Prasad, S.M. Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. **Rev Environ Sci Biotechnol** 2015, 14, 407-426, doi:10.1007/s11157-015-9372-8.

45. Subramanyam, K.; Du Laing, G.; Van Damme, E.J.M. Sodium selenate treatment using a combination of seed priming and foliar spray alleviates salinity stress in rice. **Front Plant Sci** 2019, *10*, 116, doi:10.3389/fpls.2019.00116.

46. Meena, M.; Divyanshu, K.; Kumar, S.; Swapnil, P.; Zehra, A.; Shukla, V.; Yadav, M.; Upadhyay, R.S. Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. **Heliyon** 2019, 5, e02952, doi:10.1016/j.heliyon.2019.e02952.

47. Dodd, I.C. Root-to-shoot signalling: Assessing the roles of 'Up' in the up and down world of long-distance signalling in planta. **Plant Soil** 2005, 274, 251-270, doi:10.1007/s11104-004-0966-0.

48. Le Gall, H.; Philippe, F.; Domon, J.-M.; Gillet, F.; Pelloux, J.; Rayon, C. Cell wall metabolism in response to abiotic atress. **Plants** 2015, 4, 112-166, doi:10.3390/plants4010112.

49. Cha-Um, S.; Kirdmanee, C. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. **Pak J Bot** 2009, 41, 87-98.

50. Claussen, W. Growth, water use efficiency, and proline content of hydroponically grown tomato plants as affected by nitrogen source and nutrient concentration. **Plant Soil** 2002, 247, 199-209, doi:10.1023/A:1021453432329.

51. Tobiasz-Salach, R.; Mazurek, M.; Jacek, B. Physiological, biochemical, and epigenetic reaction of maize (*Zea mays* L.) to cultivation in conditions of varying soil salinity and foliar application of silicon. /**nt J Mol Sci** 2023, 24, 1141, doi:10.3390/ijms24021141.

52. Kishor, P.B.K.; Sreenivasulu, N. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? **Plant Cell Environ** 2014, *37*, 300-311, doi:10.1111/pce.12157.

53. Nguyen, H.T.T.; Das Bhowmik, S.; Long, H.; Cheng, Y.; Mundree, S.; Hoang, L.T.M. Rapid accumulation of proline enhances salinity tolerance in Australian wild rice *Oryza australiensis* Domin. **Plants** 2021, 10, 2044, doi:10.3390/plants10102044.

54. Berteli, F.; Corrales, E.; Guerrero, C.; Ariza, M.J.; Pliego, F.; Valpuesta, V. Salt stress increases ferredoxin-dependent glutamate synthase activity and protein level in the leaves of tomato. **Physiol Plant** 1995, 93, 259-264, doi:10.1111/j.1399-3054.1995.tb02226.x.

55. Kumar, R.G.; Shah, K.; Dubey, R.S. Salinity induced behavioural changes in malate dehydrogenase and glutamate dehydrogenase activities in rice seedlings of differing salt tolerance. **Plant Sci** 2000, *156*, 23-34, doi:10.1016/s0168-9452(00)00224-7.

56. Feng, Y.-X.; Li, C.-Z.; Yang, L.; Yu, X.-Z. Spatial-temporal variations of proline and related amino acids reveal distinct nitrogenous utilization strategies in rice during detoxification of exogenous cyanide. **Chem Biol Interact** 2023, *369*, 110267, doi:10.1016/j.cbi.2022.110267.

57. Epstein, E.; Bloom, A.J. **Mineral Nutrition of Plants: Principles and Perspectives**; Sinauer: New York, 2004; p. 380.

58. Ashraf, M.; Shahzad, S.M.; Imtiaz, M.; Rizwan, M.S.; Iqbal, M.M. Ameliorative effects of potassium nutrition on yield and fiber quality characteristics of cotton (*Gossypium hirsutum* L.) under NaCl stress. **Soil Environ** 2017, *36*, 51-58, doi:10.25252/SE/17/31054.

59. Alnusairi, G.S.H.; Mazrou, Y.S.A.; Qari, S.H.; Elkelish, A.A.; Soliman, M.H.; Eweis, M.; Abdelaal, K.; El-Samad, G.A.; Ibrahim, M.F.M.; ElNahhas, N. Exogenous nitric oxide reinforces photosynthetic efficiency, osmolyte, mineral uptake, antioxidant, expression of stress-responsive genes and ameliorates the effects of salinity stress in wheat. **Plants** 2021, *10*, 1693, doi:10.3390/plants10081693

60. Amirjani, M. Effect of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. **Am J Plant Physiol** 2010, *5*, 350-360, doi:10.3923/ajpp.2010.350.360.

61. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. **Plant Soil** 1973, 39, 205-207, doi:10.1007/BF00018060.

62. Pompelli, M.F.; Martins, S.C.V.; Antunes, W.C.; Chaves, A.R.M.; DaMatta, F.M. Photosynthesis and photoprotection in coffee leaves is affected by nitrogen and light availabilities in winter conditions. **J Plant Physiol** 2010, 167, 1052-1060, doi:10.1016/j.jplph.2010.03.001.

63. Chen, F.; Fang, P.; Zeng, W.; Ding, Y.; Zhuang, Z.; Peng, Y. Comparing transcriptome expression profiles to reveal the mechanisms of salt tolerance and exogenous glycine betaine mitigation in maize seedlings. **Plos One** 2020, 15, e0233616, doi:10.1371/journal.pone.0233616.

64. Kubala, S.; Wojtyla, t.; Quinet, M.; Lechowska, K.; Lutts, S.; Garnczarska, M. Enhanced expression of the proline synthesis gene P5CSA in relation to seed osmopriming improvement of *Brassica napus* germination under salinity stress. **J Plant Physiol** 2015, 183, 1-12, doi:10.1016/j.jplph.2015.04.009.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

65. El Moukhtari, A.; Cabassa-Hourton, C.; Farissi, M.; Savoure, A. How does proline treatment promote salt stress tolerance during crop plant development? **Front Plant Sci** 2020, 11, 1127, doi:10.3389/fpls.2020.01127

66. Wani, A.S.; Ahmad, A.; Hayat, S.; Tahir, I. Is foliar spray of proline sufficient for mitigation of salt stress in *Brassica juncea* cultivars? **Environ Sci Pollut Res** 2016, 23, 13413–13423, doi:10.1007/ s11356-016-6533-4.

67. Aycan, M.; Baslam, M.; Mitsui, T.; Yildiz, M. The TaGSK1, TaSRG, TaPTF1, and TaP5CS gene transcripts confirm salinity tolerance by increasing proline production in wheat (*Triticum aestivum* L.). **Plants** 2022, 11, 3401, doi:10.3390/ plants11233401.

CHAPTER VI

HOW 2,6-DICHLOROPHENOLINDOPHENOL (DCPIP) DAMAGE CHLOROPHYLL CHAIN ELECTRON TRANSPORT, BLOCKING THE PHOTOSYNTHESIS AND PLANT DEAD

M.Sc. Samuel Giovanny García-Castaño

M.Sc. Marvin José Perneth-Montaño

M.Sc. Andrés José Betin Ruiz

10.37885/250619547

ABSTRACT

This classroom protocol presents a detailed investigation into the dynamics of photosynthesis through practical experiments that explore the effects of light intensity and herbicidal inhibition on the electron transport chain. Using DCPIP (2,6-dichlorophenolindophenol) as a Hill reagent, the reduction kinetics of photosystem II were monitored via changes in absorbance at 600 nm under varying light conditions. The experiments demonstrated a direct relationship between irradiance and the electron transport rate, affirming the centrality of photon energy in driving photosynthesis. Additionally, the herbicide Diuron was employed to disrupt the quinone pool, effectively halting the reduction of DCPIP and illustrating its inhibitory impact on photosynthetic processes. The findings, visualized through detailed figures and graphs, highlight the robustness of photosynthetic machinery and its susceptibility to external stressors. These experiments serve as a practical bridge between theoretical concepts and real-world applications. They offer valuable insights into photosynthetic efficiency, the biochemical basis of herbicide action, and their broader ecological implications. By combining spectrophotometric data with analytical observations, the study underscores the importance of photosynthesis as a cornerstone of plant physiology and its pivotal role in sustaining life on Earth.

Keywords: electron transport chain; herbicide inhibition; hill reaction; photochemical efficiency; photosystem II.

INTRODUCTION

Until the 1930s, it was widely believed that the O_2 released in the photosynthetic process originated from the cleavage of the CO_2 molecule. This assumption stemmed from the prevailing, yet unverified, hypothesis that carbon dioxide was directly split during photosynthesis. However, two critical discoveries during this period fundamentally overturned this perspective. The first breakthrough came with the observation that certain photosynthetic bacteria, such as *Rhodospirillum rubrum*, could reduce CO_2 in the presence of light but without producing O_2. This indicated that oxygen production was not inherently tied to the reduction of CO_2, suggesting that a different electron donor might be involved in these processes. Water (H_2O) was subsequently identified as this electron donor for plants and algae. When oxidized through the light-dependent Hill reaction, H_2O yields molecular oxygen (O_2). This discovery was pivotal in redefining the mechanisms of oxygenic photosynthesis and disproved the earlier notion of a direct link between O_2 evolution and CO_2 reduction [1,2].

Robert Hill's work in the mid-1940s further elucidated the mechanisms underlying photosynthetic oxygen evolution. Hill isolated chloroplasts and observed that these organelles could not release O_2 even in the presence of light, a result that initially puzzled him. He hypothesized that the isolation of chloroplasts from plant tissue disrupted some critical components necessary for oxygen release. However, when potassium ferricyanide, an artificial electron acceptor, was added to the isolated chloroplast preparation, O_2 was released, and ferricyanide was reduced. This experimental result conclusively demonstrated that isolated chloroplasts retained the capacity for O_2 evolution when provided with an external electron acceptor, now referred to as a Hill reagent. Since then, a variety of Hill reagents, including benzoquinone and dichlorophenolindophenol, have been identified, all of which play a role in facilitating electron transport in isolated chloroplasts [1,3].

The photochemical stage of photosynthesis, where light energy is captured and converted into chemical energy, involves a sophisticated sequence of electron transfers. In oxygenic photosynthesis, water acts as the primary electron donor, undergoing oxidation to yield O_2 and protons. The electrons liberated from water are transferred through the photosynthetic electron transport chain, culminating in the reduction of $NADP^+$ to NADPH. This process is facilitated by the photosystems (PSII and PSI) and a series of protein complexes and mobile

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

electron carriers, such as plastoquinone and plastocyanin. Although Hill's chloroplast preparations were capable of releasing O_2 in the presence of artificial acceptors, they were unable to reduce CO_2, highlighting the necessity of intact cellular machinery for the integration of light-dependent reactions with the Calvin cycle. This discovery provided critical insights into the compartmentalization and specialization of photosynthetic processes within plant cells [3,4].

The Hill reaction specifically illuminated the role of photolysis in the evolution of oxygen. In this process, light energy absorbed by chlorophyll in photosystem II drives the splitting of water molecules into protons, electrons, and molecular oxygen. This reaction, which occurs at the oxygen-evolving complex of PSII, is a hallmark of oxygenic photosynthesis and is absent in anoxygenic photosynthetic organisms. Hill's findings underscored the significance of water as an electron source, a concept that distinguished oxygenic photosynthesis in plants, algae, and cyanobacteria from the mechanisms observed in photosynthetic bacteria, which utilize other electron donors such as hydrogen sulfide [5]. Moreover, Hill's experiments paved the way for a deeper understanding of the Z-scheme model of electron transport in photosynthesis. This model describes the energy changes of electrons as they move through the photosynthetic apparatus, driven by photon absorption in photosystem II and photosystem I. Hill's findings demonstrated that artificial acceptors could substitute for $NADP^+$ as electron sinks, thus decoupling the light-dependent reactions from the subsequent biochemical reduction of CO_2 in the Calvin cycle. This decoupling highlighted the modular nature of photosynthetic processes and provided a framework for studying the mechanistic underpinnings of electron transport and energy conversion in isolated systems [6].

In addition to its historical significance, Hill's work has practical implications for modern research. The discovery of Hill reagents has facilitated studies on the efficiency of photosynthetic electron transport and the factors influencing O_2 evolution. These reagents remain invaluable tools for investigating photosynthetic mechanisms and have contributed to advances in artificial photosynthesis research, where the goal is to mimic natural processes to produce renewable energy. Hill's experiments laid the groundwork for understanding the biochemical intricacies of photosynthesis, from the dynamics of the oxygen-evolving complex to the interplay between light absorption, electron transport, and CO_2 fixation [7].

Ultimately, the studies conducted by Hill and his contemporaries reshaped the scientific understanding of photosynthesis, revealing it as a highly coordinated

series of reactions. These reactions not only involve the transfer of electrons from water to $NADP^+$ but also depend on the Calvin cycle for the assimilation of CO_2 into organic molecules. Together, these processes illustrate the remarkable efficiency and complexity of photosynthesis, a fundamental biological pathway that sustains life on Earth by driving global carbon and oxygen cycles [8,9].

Herbicides play a critical role in disrupting the chloroplast electron transport chain, effectively halting photosynthesis and leading to plant death. These compounds, often targeting specific protein complexes such as photosystem II (PSII), bind to key sites within the photosynthetic apparatus, such as the D1 protein, preventing the transfer of electrons from plastoquinone. This blockage interrupts the generation of ATP and NADPH, essential for the Calvin cycle and other metabolic processes. As a result, the plant experiences a cascade of effects, including oxidative stress caused by the accumulation of reactive oxygen species (ROS), membrane damage, and the depletion of energy reserves. Over time, this disruption leads to the cessation of growth and ultimately the death of the plant. Common herbicides such as atrazine and diuron are widely used for this purpose, highlighting the importance of understanding their molecular mechanisms to manage weed resistance and minimize environmental impact [10]. Since photosynthesis is basically a transfer of electrons from the water molecule to $NADP^+$, which reacts with organic molecules in the Calvin cycle to reduce atmospheric CO_2 to triose phosphates, any molecule capable of receiving electrons from water could be a Hill reductant. In artificial laboratory situations, this reductant is DCPIP (2,6-dichlorophenolindophenol), a chemical compound that, when oxidized, has a bluish coloration. This coloration disappears upon reduction, resulting in a decrease in absorbance at 600 nm (Fig. 1). However, Diuron [3-(3,4-Dichlorophenyl)-1,1-dimethylurea], a herbicide widely used in agriculture, is capable of blocking the transfer of electrons between the quinone pool, located between photosystems II and I, blocking the electron transport chain and photosynthesis. In this specific case, DCPIP will remain oxidized, and the color should not change, showing how a herbicide can lead to the death of the plant by inhibiting photosynthesis. (Fig. 2).

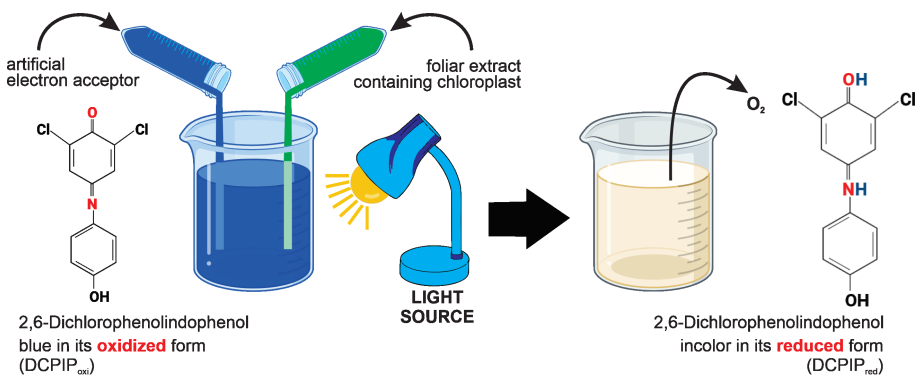
MATERIALS

- Tube centrifuge
- Glass cuvettes for spectrophotometer

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

- Spectrophotometer
- Spinach leaves (20 g)
- 200 W light sources
- Medical gauze (wad)
- Deionized H_2O
- Blender
- Aluminum foil
- Parafilm
- Glass volumetric pipettes or calibrated micropipettes
- 0.2 mM DCPIP solution
- 0.10 mM Diuron solution (2 mL)
- 0.1 μM Diuron solution (2 mL)
- 0.5 M Sucrose Solution, kept in an ice bath (100 mL)
- 0.10 M Phosphate Buffer (pH 6.5), kept at room temperature (~10 mL)
- 0.10 M Phosphate Buffer (pH 6.5), kept in an ice bath (~240 mL)
- Polypropylene tubes, "Falcon" type

Figure 1. How DCPIP is reduced by light, inhibiting the electron transference between photosystem II and photosystem I.

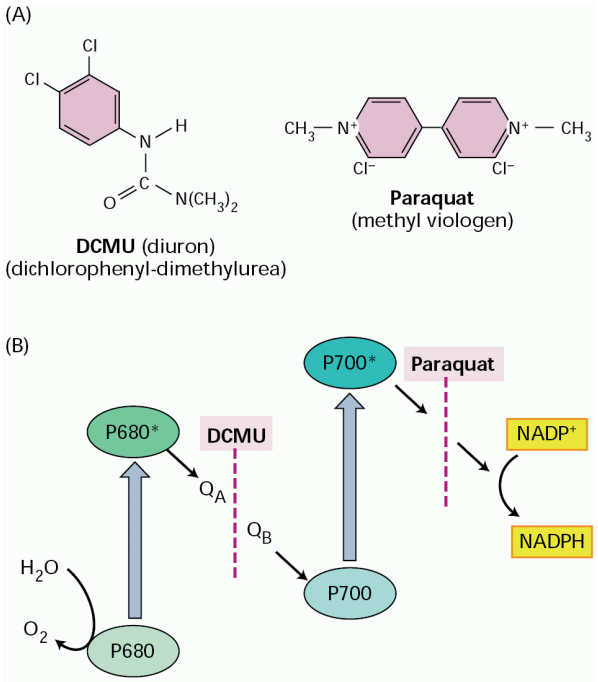


$$2\,H_2O + 2A \rightarrow 2\,O_2 + 2\,AH_2$$

where A is electron aceptor, on plants $NADPH^+$; AH_2 is NADPH, here DCPIP

IMPORTANT NOTE: ALL CHLOROPLAST SOLUTIONS MUST BE MAINTAINED IN DARKNESS TO AVOID REDUCTION. THE SUCROSE SOLUTION MUST BE LEAVE IN REFRIGERATOR 2-3ºC OR SMOOTH FROZEN

Figure 2. Schematics showing the chemical structure of the main blockers (A) of the electron transport chain (B) within the photochemical stage of photosynthesis. Adapted from Blankenship (2010).



METHOD

Preparation of chloroplast suspension

Separate the spinach leaf blades, which should be thoroughly cleaned in running water. Then, reserve 20 grams of leaf blades and grind them very well with a blender. To homogenize, use 100 mL of sucrose solution (0.5 M), always keeping this solution cooled in an ice bath.

After homogenization, the chloroplast solution should be filtered with two layers of gauze, and the filtrate should be recovered in small centrifuge tubes. Centrifuge the tubes for 10 minutes at 3000 rpm, discarding the supernatant. Resuspend the pellet in Potassium Phosphate Buffer (TFK) 0.10 M (pH 6.5) kept in an ice bath until use.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

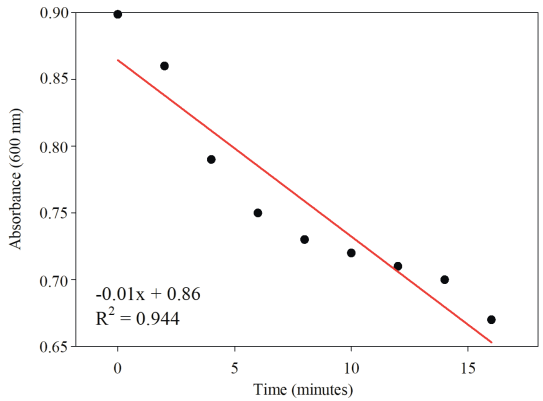
Kinetics of DCPIP reduction

There are several ways to determine the rate of reduction of DCPIP. One of them is to continuously monitor the disappearance of the color of DCPIP, which is blue when oxidized but becomes colorless when exposed to light. This is technically very difficult to visualize, especially if the chloroplast solution is exposed to light. So, we will use only two points, two absorbances – the initial and the final – and calculate a delta (Δ) from this, which will be proportional to the time between the first and last reading.

So, we will prepare a glass cuvette containing 0.7 mL of DCPIP (0.2 mM), 0.7 mL of TFK 0.10 M (pH 6.5), 1.4 mL of deionized water, and 0.7 mL of the freshly prepared chloroplast suspension. Mix the mixture well and read the absorbance at 600 nm. The absorbance values should be between 0.75 and 0.90. Then, position the cuvette 10 cm from the light source and read the absorbance for 14 minutes with 2-minute intervals between each reading. Note: Whenever transporting the cuvette between the light source and the spectrophotometer, use gloves and aluminum foil to wrap the cuvette.

Construct a graph with the readings obtained from the spectrophotometer (Fig. 3).

Figure 3. Diagram showing the reduction of DCPIP over time of exposure to light.

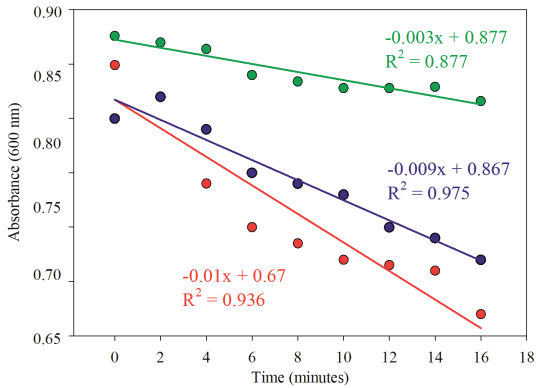


Effect of light intensity on the Hill reaction

To evaluate the effect of irradiance on photosynthesis, we will repeat the experiment above at two other distances from the light source. We will use distances of 10 cm, 20 cm, and 50 cm. If possible, quantify the light intensity at

each of these points. Then, all the data will be combined in a graph showing the effect of light intensity on the photoreduction of DCPIP (Fig. 4).

Figure 4. Scheme showing the reduction of DCPIP over time of exposure to light as a function of exposure to different light intensities, promoted by a light source located 10 cm (red), 20 cm (blue), and 50 cm (green) from the samples.



Effect of herbicides on the activity of Hill reactions

Now that we have evaluated the effect of light on the photoreduction of DCPIP, we will determine how adding a herbicide interferes with photosynthesis by inhibiting the photoreduction of DCPIP. To do so, we will use the following concentrations of the herbicide Diuron (0.10 mM and 0.10 μM).

Omitting the chloroplasts, the following dilutions are prepared:

- 0.7 mL TFK + 0.7 mL DCPIP + 1.4 mL water – Tube 1
- 0.7 mL TFK + 0.7 mL DCPIP + 1.4 mL 0.10 mM Diuron – Tube 2
- 0.7 mL TFK + 0.7 mL DCPIP + 1.4 mL 0.01 mM Diuron – Tube 3

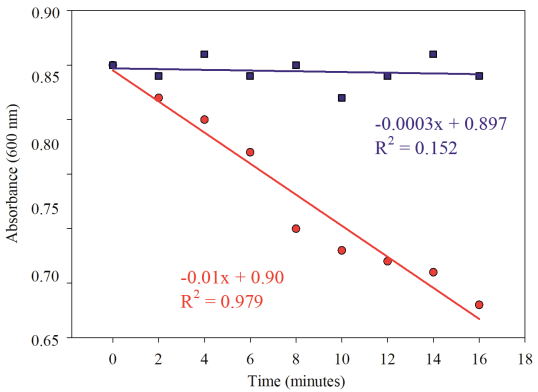
Next, add 0.7 mL of the chloroplast suspension to tube 1 only. After mixing everything, read the absorbance at 600 nm. Take the same reading in the other tubes that did not receive the chloroplast suspension and set aside the values.

Position the cuvettes at the 10 cm mark from the light source and keep the tubes in this position until you verify a color change in tube 1, which should change to blue or green and occur after about 10 minutes. Once this is done, transfer all the cuvettes to the dark and record the time the tubes remained in the light.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Now, determine the final absorbance of each cuvette. Then, calculate the reduction rate of DCPIP by the change in absorbance at 600 nm per minute – this is the kinetics of the Hill reaction. Construct the graph, like Fig. 5.

Figure 5. Diagram showing the reduction of DCPIP over time of exposure to light as a function of the absence (red) and presence of Diuron (blue) as an inhibitor of the electron transport chain within the quinone pool.



Although this practice may seem complicated at first glance, it provides tremendous information, and dozens of essential information can be extracted from it. There is no doubt that photosynthesis represents one of the most complex mechanisms in Plant Physiology. Still, it is through practices of this nature that one realizes that photosynthesis is limited to a complex set of oxidation-reduction reactions that add up to the conversion of CO_2 into carbohydrates. With this class, the student must verify in practice how light interferes with photosynthesis and how increased irradiance affects photosynthetic activity. Furthermore, the student will be guided to understand how photosynthesis is inhibited and how herbicides cause the death of plants. Therefore, this practice has a huge practical value for biologists, agronomists, and other students of plant biology.

DISCUSSION

Photosynthesis is the cornerstone of terrestrial life. It converts light energy into chemical energy, which is stored in organic compounds. The photochemical phase, involving light absorption and electron transport, is integral to this process, with water serving as the electron donor. The experiments involving DCPIP (2,6-dichlorophenolindophenol) and Diuron, detailed in this classroom protocol, offer critical insights into the electron transport chain's mechanisms

and vulnerabilities. These studies highlight both the robustness of photosynthetic machinery and its susceptibility to chemical disruptions [11].

The Hill reaction, first described by Robert Hill in 1937, demonstrated that isolated chloroplasts could evolve oxygen in the presence of light and an artificial electron acceptor, such as DCPIP. This reagent, which changes from blue to colorless upon reduction, allows researchers to quantify electron flow through photosystem II (PSII). The experimental setup elegantly reveals the relationship between light intensity and the rate of electron transport, as DCPIP reduction increases proportionally with irradiance, emphasizing the role of photon energy in driving the Z-scheme of photosynthesis [1,3]. This model highlights the sequential energy boosts provided by PSII and PSI to electrons as they move towards $NADP^+$ reduction [6].

Diuron, a PSII inhibitor, disrupts this electron flow by blocking the plastoquinone-binding site, effectively halting the Hill reaction and demonstrating the herbicide's ability to inhibit photosynthesis. As seen in the figures, DCPIP remains oxidized in the presence of Diuron, visually underscoring the interruption of electron transport. Such studies are pivotal for understanding the biochemical underpinnings of herbicide action and its implications for agricultural and environmental management [10].

From a practical perspective, these experiments bridge theoretical concepts with real-world applications. They elucidate how light intensity affects photosynthetic rates and demonstrate the direct impacts of herbicides on plant physiology. Diuron's ability to induce oxidative stress by halting electron transport has broader ecological consequences, influencing plant growth and resilience [5]. Such findings inform strategies to mitigate herbicide resistance in weeds and minimize environmental impacts through precise application methods [10].

Furthermore, the experiments align with historical milestones in photosynthesis research. Hill's foundational discoveries provided a framework for isolating light-dependent reactions, separating them from the Calvin cycle's CO_2 fixation process. Modern studies, leveraging tools like DCPIP, continue to build on this legacy, offering detailed views of photosynthetic efficiency and its perturbations [2,8]. The modular nature of photosynthesis revealed by these studies aids in designing artificial photosynthetic systems, which seek to replicate natural processes for sustainable energy production [3].

However, experimental limitations should be acknowledged. While DCPIP reduction provides a clear proxy for PSII activity, it does not fully replicate the

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

complexities of intact chloroplast systems. Factors such as feedback regulation within the thylakoid membrane and the influence of stromal metabolites are not accounted for in isolated systems. Additionally, variations in herbicide effects across different plant species and environmental conditions underscore the need for broader ecological studies [5].

CONCLUSIONS

The proposed practical experiments effectively elucidate critical aspects of photosynthesis, particularly the electron transport chain's role in converting light energy into chemical energy. The utilization of DCPIP as an artificial electron acceptor demonstrates the precise functionality of photosystem II, where light-driven reactions facilitate electron transfer. The reduction of DCPIP, evidenced by changes in absorbance at 600 nm, underscores the dependency of photosynthetic efficiency on light intensity. By increasing irradiance, the rate of electron transport and, consequently, the rate of photosynthetic activity are enhanced. These findings align with the Z-scheme electron flow model in photosynthesis, showcasing the dual energy-boosting mechanisms of photosystems II and I. Additionally, the experiments underscore the susceptibility of this finely tuned system to disruptions, mainly through the inhibitory effects of Diuron on the quinone pool. The observed persistence of DCPIP in its oxidized state in the presence of this herbicide highlights its effectiveness in halting photosynthesis and causing physiological stress in plants.

The practical exercises, supported by detailed experimental figures, bridge theoretical knowledge with empirical observation, allowing students and researchers to analyze photosynthetic dynamics under various conditions. This approach reinforces understanding of fundamental principles such as light-driven reactions and offers insights into real-world applications, including herbicide action and environmental impacts on plant systems. By integrating visual data from absorbance readings and graphical trends, the experiments provide a comprehensive understanding of the complexities and vulnerabilities of photosynthetic processes, fostering critical thinking and analytical skills in plant biology.

DIRECT STUDY

How is the Hill reaction used to demonstrate photosynthesis and electron transport, and how is this evaluated using DCPIP and varying light intensities?

Expected Answer: The Hill reaction demonstrates photosynthesis by showcasing electron transport in chloroplasts, using DCPIP as an electron acceptor. When reduced, DCPIP loses its blue color, indicating active electron transport. Light intensity affects the rate of DCPIP reduction, with higher irradiance enhancing photosynthesis. This relationship is assessed by measuring DCPIP absorbance at time intervals under different distances from the light source.

How do herbicides like Diuron interfere with photosynthesis, and how can this be experimentally observed using DCPIP?

Expected Answer: Diuron disrupts photosynthesis by blocking electron transport between photosystems II and I, halting ATP and NADPH production. Experimentally, the presence of Diuron prevents the reduction of DCPIP, which remains blue, indicating the disruption of electron transport. This effect is observed by measuring DCPIP absorbance in the presence and absence of the herbicide after light exposure.

REFERENCES

1. Hill, R. Oxygen evolved by isolated chloroplasts. **Nature** 1937, 139, 881-882, doi:10.1038/139881a0.

2. Blankenship, R.E.; Hartman, H. The origin and evolution of oxygenic photosynthesis. **Trends Biochem Sci** 1995, 20, 93–98, doi:10.1016/S0968-0004(00)88920-0.

3. Shevela, D.; Messinger, J. Studying the oxidation of water to molecular oxygen in photosynthesis: From classical chemistry to artificial photosynthesis. **Front Plant Sci** 2013, 4, 473, doi:10.3389/fpls.2013.00473.

4. Govindjee, G. A role for a light-harvesting antenna complex of photosystem II in photoprotection. **Plant Cell** 2002, 14, 1663-1668, doi:10.1105/tpc.140810.

5. Foyer, C.H.; Neukermans, j.; Queval, G.; Noctor, G.; Harbinson, J. Photosynthetic control of electron transport and the regulation of gene expression. **J Exp Bot** 2012, 63, 1637–1661, doi:10.1093/jxb/ers013.

6. Govindjee, G. Photosystem II: The water/plastoquinone oxidoreductase in oxygenic photosynthesis. **Photosynth Res** 2010, 87, 331-335, doi:10.1007/s11120-006-9035-2.

7. Heinz, S.; Liauw, P.; Nickelsen, J.; Nowaczyk, M. Analysis of photosystem II biogenesis in cyanobacteria. **Biochim Biophys Acta** 2015, 1857, 274-287, doi:10.1016/j.bbabio.2015.11.007.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

8. Blankenship, R.E. Photosynthesis: The light reactions. In **Plant Physiology and Development**, 6th edition, Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., Eds.; Sinauer Associates, Inc.: Sunderland, USA, 2014; pp. 163-197.

9. Langdale, J.A. C4 cycles: past, present, and future research on C4 photosynthesis. **Plant Cell** 2011, 23, 3879-3892, doi:10.1105/tpc.111.092098.

10. Rutherford, A.W.; Krieger-Liszkay, A. Herbicide-induced oxidative stress in photosystem II. **Trends Biochem Sci** 2001, 26, 648-653, doi:10.1016/s0968-0004(01)01953-3.

11. Gondim, E.; Rondon, J.N. The crucial role of photosynthesis in sustaining plant life and its impact on the global ecosystem, climate, and human survival. **Aust Herb Insight** 2020, 3, 1-5, doi:10.25163/ahi.319925.

SECTION III

POST HARVEST AND SHORT PLANT PHYSIOLOGY CLASSES

The journey into the postharvest and physiological lives of fruits takes a vibrant turn in this third section of the book, where science, tradition, and innovation come together to reveal the hidden potential of plant materials and the intricacies of water dynamics. This section explores native species with immense industrial value, uncovers the delicate biochemical changes that occur after harvest, and dives deep into the fundamental processes that sustain plant life — all through the lens of practical, insightful research.

Chapter Seven highlights the technological possibilities and nutritional richness of pequi (*Caryocar* spp.), a native Brazilian fruit with growing economic importance. The chapter provides a comprehensive overview of drying methods and how they affect the final quality of pequi pulp, revealing how drying—especially freeze-drying and vacuum drying—can preserve bioactive compounds such as carotenoids and vitamin C. With detailed insights into various drying techniques, from convective to osmotic dehydration and fluidized bed drying, this chapter demonstrates how processing can extend the fruit's shelf life, reduce waste, and unlock new applications in the food, pharmaceutical, and cosmetic industries. The discussion is framed around optimizing sensory and nutritional properties, highlighting pequi's value as both a cultural staple and a high-potential industrial ingredient.

In Chapter Eight, the focus shifts to practical approaches for assessing and preserving the quality of fruits and vegetables after harvest. With a startling reminder that up to 40% of produce is lost before it reaches consumers, the chapter underscores the urgency of postharvest technologies. Topics range from respiration control and ethylene management to refrigeration, modified atmospheres, and edible coatings, all aimed at extending shelf life and minimizing waste. The second half of the chapter dives into classroom practices for determining soluble solids content (Brix), linking biochemical ripeness to harvest timing, flavor, and processing yield. By integrating theoretical knowledge with hands-on measurement techniques, this chapter equips readers with tools to evaluate fruit quality, optimize harvest strategies, and ensure profitability in the supply chain.

Chapter Nine takes a more experimental route, guiding the reader through

practical demonstrations of osmotic potential and xylem water transport. Using hydrogel beads and white roses in dyed water, the chapter brings abstract physiological concepts to life, such as water potential gradients, osmotic flow, and transpiration. Through these visual and accessible experiments, students and researchers alike can observe how water movement responds to solute concentrations and environmental stimuli. The lessons link water balance to real-world agricultural concerns like irrigation efficiency and drought tolerance, while also revealing the elegant coordination between transpiration, photosynthesis, and xylem function. These classroom protocols not only reinforce theoretical learning but also emphasize the ecological and agronomic importance of water regulation in plants.

Altogether, this section blends food science, postharvest physiology, and plant water relations into a coherent narrative that connects practical methods with deeper biological meaning. Whether optimizing a fruit's shelf life or modeling osmotic flow in a lab, each chapter invites readers to see plants not just as crops, but as dynamic systems constantly interacting with their environment. The section demonstrates how scientific understanding can translate into innovation, sustainability, and greater food security.

Now, take a deep breath and get ready to dive into the magic behind every fruit basket! Section III is a delicious mix of tropical aromas, science experiments, and stories from the field and the lab. You'll learn how a humble fruit like pequi becomes a superstar through drying, how sugar levels can shape juice yields, and how flowers drink through invisible rivers of water. So grab your lab coat — or your blender — and join us for this flavorful, enlightening adventure into the secrets that make our fruits and vegetables so incredible. Let's get growing!

The authors

CHAPTER VII

DRYING OF PEQUI (*Caryocar* spp.): COMPOSITION, DRYING METHOD, AND FINAL PRODUCT QUALITY

Franciscleudo Bezerra da Costa
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Rodolfo Rodrigo de Almeida Lacerda
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Marilia Hortência Batista Silva Rodrigues
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Toshik Iarley da Silva
Center for Agricultural, Biological, and Environmental Sciences, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Brazil;

Rayane Alves Pereira
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Geraldavane Lacerda Lopes
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Ivislanne de Sousa Queiroga Lacerda
Paraíba State Department of Education, Pombal, Brazil

Giuliana Nayara Barros Sales
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Fernandes Antônio de Almeida
Agrifood Science and Technology Center, Program of Postgraduate in Tropical Horticulture, Universidade Federal de Campina Grande, Pombal, Brazil

Wellington Souto Ribeiro
Department of Agronomy, Program of Postgraduate in Plant Sciences, Universidade Federal de Viçosa, Viçosa, Brazil.

10.37885/250619548

ABSTRACT

Pequi (*Caryocar brasiliense*) is a fruit species native to the Brazilian Cerrado, with recognized nutritional, cultural, and socioeconomic importance. Its pulp is rich in bioactive compounds such as carotenoids, phenolic compounds, vitamin C, fibers, and unsaturated lipids, characteristics that confer high nutritional and functional value to the fruit. However, pequi has high perishability due to its elevated moisture content. In this context, drying the pequi pulp emerges as a viable alternative to extend the product's shelf life, facilitate transportation and storage, and expand its potential use in food, cosmetic, and pharmaceutical formulations. The adoption of appropriate drying technologies enables the preservation of the fruit's physicochemical and sensory properties, maintaining its nutritional value and adding value to the final product. Therefore, this chapter aims to provide an overview of the technological use of pequi, with emphasis on pulp drying and its impacts on the final product's quality. The main methods applied to pequi pulp include convective drying (hot air), vacuum drying, osmotic dehydration, lyophilization, spouted bed drying, and microwave drying. The choice of method directly influences the quality of the final product, especially regarding the retention of carotenoids and vitamin C, which are highly sensitive to heat and oxidation. Convective drying, although widely used due to its economic accessibility, may result in significant losses of bioactive compounds when performed at high temperatures and for prolonged periods. In contrast, lyophilization and microwave drying have stood out for their greater ability to preserve the nutritional and sensory properties of the pulp, although they involve higher operational costs. Osmotic and vacuum drying also present themselves as viable alternatives, particularly when combined with other techniques. In addition to technical aspects, the use of powdered pequi represents a strategy to strengthen local production chains, allowing the fruit to enter new markets, such as those of functional and nutraceutical foods. Thus, drying the pequi pulp is presented as an efficient tool to mitigate post-harvest losses, expanding its application across different industrial sectors. The adoption of drying methods that preserve bioactive compounds is essential to maintain the nutritional and functional value of the product. However, further studies are still needed to deepen

the understanding of the effects of different drying processes, their economic and technical feasibility, and the stability of the final product during storage.

Keywords: pequi powder; convective drying; drying techniques; bioactive compounds; post-harvest technology; fruit processing.

INTRODUCTION

Brazil harbors one of the greatest biodiversities of natural resources in the world, including numerous fruit species with high nutritional, functional, and economic potential. Among the native species, the pequi tree (*Caryocar* spp.) stands out for its significant ecological and socioeconomic importance within the Cerrado and Caatinga biomes (Costa; Pinto; Soares, 2017; Garcia *et al.*, 2024). Within this genus, *Caryocar brasiliense*, widely distributed across the Cerrado, and *C. coriaceum*, endemic to the Caatinga, are particularly notable (Nascimento-Silva; Naves, 2020; Garcia *et al.*, 2024). Both species play key roles in food security, income generation, and the preservation of traditional knowledge and cultural practices among local communities in these regions.

The edible portion of the fruit, corresponding to the inner mesocarp and popularly known as pequi, has been associated with several health benefits, including antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective, and even potential anticancer properties (Nascimento-Silva; Naves, 2020). Due to its high concentration of bioactive compounds, the pulp has been widely used by the agro-industry in the production of products such as oil, ice cream, and liqueur, in addition to showing promising applications in the pharmaceutical and cosmetic sectors (Deus, 2008). As a result, there has been significant growth in the commercialization and market value of pequi, driven by the increasing number of studies and scientific evidence supporting its nutritional value (Nascimento-Silva; Naves, 2020).

Given the growing appreciation for pequi, the adoption of effective preservation methods becomes essential to enable the full utilization of the fruit and expand its access to diversified markets. In this context, the pulp drying technique and its subsequent transformation into powder emerge as a promising alternative, as it reduces the food's water activity, thereby inhibiting microbial growth and consequently extending its shelf life (Singhal *et al.*, 2020).

This type of processing significantly contributes to product stability and enables its integration into different production chains, adding value to the fruit and enhancing its commercialization potential. Moreover, drying ensures year-round availability of the product by converting fresh produce into dehydrated forms, while maintaining, or even improving, its nutritional and sensory properties. It also reduces the volume and weight of the final product, facilitating both transportation and storage (Calín-Sánchez *et al.*, 2020). Another important

benefit of drying is the expansion of the possible applications of pequi pulp, which can be incorporated into food formulations such as seasonings, sauces, and industrialized products, promoting consumption diversification and increasing the product's added value (Silva *et al.*, 2018).

Another relevant benefit of the drying process is the preservation of the product's sensory characteristics, such as color, aroma, and flavor, especially when the process is carried out under controlled temperature conditions, such as at 105 °C (Silva *et al.*, 2018). In addition, this technique promotes the retention of essential bioactive compounds, including vitamin C and carotenoids, substances of great interest to the functional food industry due to their well-documented health benefits (Sousa *et al.*, 2021).

Despite the significant increase in scientific research on this topic, the available information remains scattered throughout the literature, making it difficult to access systematic data on the composition, drying methods, and the effects of these processes on the quality of pequi powder. In light of this, the objective of this chapter is to compile and synthesize the main existing evidence, providing a comprehensive overview of the technological utilization of pequi, with a focus on pulp drying, its impact on the final product quality, and its potential applications across different industrial sectors.

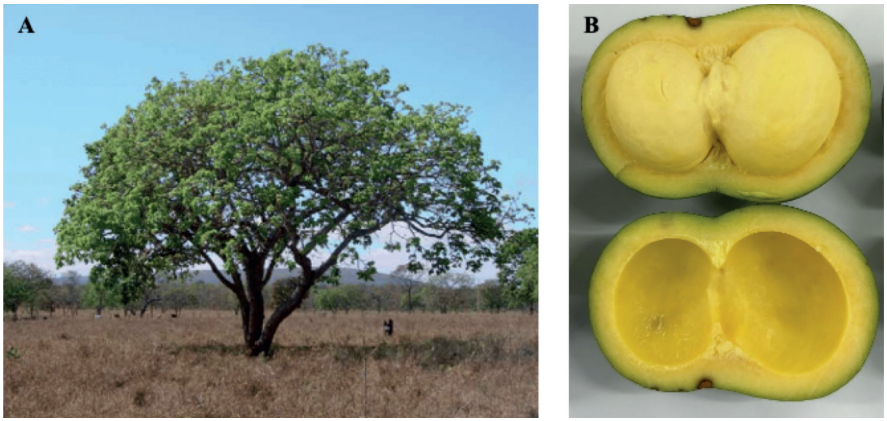
PEQUI: COMPOSITION AND INFLUENCE OF DRYING ON THE QUALITY OF PULP POWDER

Plant composition and fruit properties

The pequi tree, belonging to the family Caryocaraceae and the genus *Caryocar*, has its origins in Indigenous culture, with its name derived from Tupi, meaning "spiny shell", a reference to the characteristic spines of the fruit's endocarp (Amaral *et al.*, 2014) (Figure 1A). Commonly known by various names, such as piqui, piquiá, piqui-do-cerrado, piquiá-bravo, amêndoa espinhosa, castanha-da-índia, suarí, and pequerim, it is a tropical and exotic fruit native to the Brazilian Cerrado, remarkable for its unique morphological structure, with seeds surrounded by rigid spines (Silveira *et al.*, 2024) (Figure 1B).

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 1. A – A Pequi tree (*Caryocar* spp.). Source: Google Images; B – Internal structure of the pequi fruit. Source: personal archive.



The Caryocaraceae family is predominantly distributed across Central and South America, with a higher occurrence in tropical forest regions. In Brazil, the most representative species is *C. brasiliense* (Pinto *et al.*, 2018). The pequi tree is a perennial plant whose fruiting period occurs between November and February, although this may vary depending on the region (Nunes *et al.*, 2021). The pequi fruit is rounded, edible, and known for its strong and distinctive flavor. It is a non-climacteric fruit, botanically classified as a drupe, typically containing two to four seeds per fruit. Its structure consists of a green-colored exocarp (or pericarp), followed by an outer mesocarp and an inner mesocarp with a yellow hue, which constitutes the edible portion of the fruit (Pinto *et al.*, 2018; Batista; Sousa, 2019; Costa *et al.*, 2024).

The economic activity related to pequi is primarily based on sustainable extractivism, playing a crucial role in food security and income generation for numerous families, especially during the harvest season (Batista; Sousa, 2019). In 2022, Brazil's national pequi production reached 47,698 tons, with the state of Minas Gerais standing out as the main contributor to this volume (IBGE, 2024).

The quality of pequi fruits is directly influenced by their stage of maturation (Oliveira *et al.*, 2017), as the pulp undergoes significant changes in its chemical composition and physical structure throughout the ripening process. These changes include variations in moisture content, yield, sugar levels, and bioactive compounds, all factors that directly affect the fruit's post-harvest shelf life (Vieira *et al.*, 2024). The pequi pulp is particularly notable for its high levels of

carotenoids, lipids, fibers, and phenolic compounds, with their concentrations varying according to the fruit's developmental stage (Nascimento-Silva *et al.*, 2020).

According to Silva *et al.* (2025), in their evaluation of the drying kinetics of *Spondias purpurea* fruits, flours obtained from ripe fruits exhibited higher values of yield, moisture content, pH, soluble solids, and reducing sugars. In contrast, unripe fruits showed higher titratable acidity. Regarding bioactive compounds, a decrease in total chlorophyll, ascorbic acid, and phenolic content was observed throughout the ripening process, while total carotenoids increased due to chlorophyll degradation and the activation of carotenoid biosynthesis. Additionally, total anthocyanin and flavonoid levels rose during the final stages of maturation.

Pequi fruits are considered ripe when they naturally detach from the tree and fall to the ground. After falling, if not collected immediately, they begin to deteriorate rapidly, becoming soft within an average period of two to three days (Oliveira *et al.*, 2006). In addition to their high perishability, the fruit's seasonal availability poses another challenge for its preservation and storage. Therefore, the adoption of appropriate preservation methods becomes essential to extend shelf life and enable commercialization throughout the year, including during the off-season (Leite *et al.*, 2020; Nascimento-Silva *et al.*, 2020).

Chemical and nutritional composition

Pequi pulp stands out for its rich and diverse chemical composition, serving as an important source of proteins, lipids, and dietary fibers such as pectin and hemicellulose. Its lipid content ranges from 46 to 51%, with a predominance of unsaturated fatty acids, particularly oleic acid. In addition, the pulp contains high concentrations of carotenoids, phenolic compounds, vitamin C, and tocopherols, substances that contribute to the fruit's antioxidant properties. The fiber content, which may vary from 8 to 15%, also plays a significant role in enhancing the functional and nutritional value of the pulp (Cordeiro *et al.*, 2013; Moreno, 2014; Carneiro *et al.*, 2023).

Among the main bioactive compounds found in pequi, carotenoids and phenolic compounds stand out, both widely recognized for their functional properties. The intense yellow coloration of the pulp is attributed to its high carotenoid contente, natural pigments commonly found in plants that exhibit antioxidant activity and, in many cases, serve as precursors of vitamin A. These compounds contribute to the neutralization of free radicals and reactive oxygen

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

species, playing a key role in preventing oxidative damage to cells (Pinto *et al.*, 2018; Narita *et al.*, 2022).

Phenolic compounds represent one of the main groups of phytochemicals found in plants, playing essential roles from both physiological and structural perspectives. Several studies have reported that these substances, known for their antioxidant activity, are strongly associated with the reduced incidence of chronic and degenerative diseases in humans (Monteiro *et al.*, 2015; Leão *et al.*, 2017; Narita *et al.*, 2022). Evidence suggests that pequi pulp contains high levels of phenolic compounds, surpassing commonly consumed fruits in Brazil such as guava, strawberry, and açaí (Lima *et al.*, 2007). Among these compounds, quercetin and ellagic acid are particularly noteworthy, both of which are linked to a range of health benefits, including antioxidant, anti-inflammatory, and cardioprotective properties. These effects contribute to the mitigation of oxidative stress and further reinforce the functional value of pequi as a promising food for health promotion (Lemes *et al.*, 2017).

A study conducted by Narita (2022), which investigated the in vitro bioaccessibility of carotenoids, phenolic compounds, and antioxidant activity in flours derived from pequi fruit (*C. brasiliense*), demonstrated that flours obtained from the pulp, kernel, and peel contained total levels of α-carotene, β-carotene, and lycopene ranging from 9.34 to 63.81 μg/g. Similar results were reported by Lima *et al.* (2007), who identified high total carotenoid levels in the fresh pulp (72.5 μg/g) and in the kernel (2.95 μg/g). Complementing these findings, Leão *et al.* (2017) observed significant carotenoid concentrations in flours prepared from the combined epicarp and mesocarp (34.99 μg/g), as well as in flours obtained exclusively from the mesocarp (21.16μg/g).

Although the antioxidant activity values reported by Schiassi *et al.* (2018) for flours derived from pequi kernel and pulp are lower than those observed in other native Cerrado fruits, such as buriti, cagaita, and mangaba, this does not diminish the nutritional and functional importance of pequi. The diversity of bioactive compounds present in its various parts, combined with its versatility of use, highlights the fruit's significant role within the context of functional foods and the valorization of native species from the biome. The integral use of the fruit, made possible through methods such as drying and flour production, contributes to expanding its applications, facilitating storage, and preserving its nutritional and functional properties.

Drying methods

Drying is one of the oldest and most widely used preservation techniques, based on the removal of water from the product through evaporation or sublimation under controlled heat conditions (Sousa *et al.*, 2021). This process helps preserve the physiological and physicochemical characteristics of foods, ensuring their stability, microbiological safety, and extending storage time without compromising quality (Santos *et al.*, 2019).

In addition to its preservative function, drying as a processing technique enables the conversion of raw materials into products such as flours, promoting an increase in the concentration of essential nutrients, including bioactive compounds, fibers, and minerals. This method also contributes to the reduction of post-harvest losses (Santos *et al.*, 2022), expands the range of product formats available to the final consumer, and provides the industry with higher technological quality products and greater added value (Castiglioni *et al.*, 2013).

The efficiency of the drying process is directly influenced by variables such as temperature, exposure time, and the intrinsic characteristics of the material being dehydrated. During drying, the heat transferred to the food promotes the movement of internal moisture toward the surface, where it evaporates. Higher temperatures favor vapor formation, facilitating the removal of water from the product (Castiglioni *et al.*, 2013). Various types of dryers and drying methods are commercially employed for the dehydration of fruits and vegetables, depending on their specific requirements (Mousakhani-Ganjeh *et al.*, 2021). The selection of the most appropriate technique depends on factors such as the shape and properties of the material, the physical configuration, the desired characteristics of the final product, as well as operational conditions and associated costs (Sokhansanj; Jayas, 2006).

Currently, hot air drying is the most widely used method in the food industry due to its operational simplicity, low cost, and broad applicability across different types of food products. However, alternative and emerging methods are being explored and optimized with the aim of improving process efficiency, reducing drying time, and, most importantly, preserving the nutritional, sensory, and functional quality of dehydrated products.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Convective drying and vacuum drying

Hot air convective drying stands out as one of the most widely used techniques in the food industry for reducing water content in both solid and liquid products. This is a simple, low-cost method that uses the circulation of heated air to promote the evaporation of moisture present in foods (Stepien *et al.*, 2019). This approach allows for effective control of water activity, inhibiting metabolic processes that lead to spoilage, while also ensuring uniform drying even under varying temperature conditions (Santos *et al.*, 2019; Sahoo *et al.*, 2022).

In this process, heat is transferred to the system through heated air or other gases, such as superheated steam, direct combustion gases, or inert gases, which flow over the surface of the material. Moisture removal occurs by thermal vaporization under controlled airflow, humidity, and temperature conditions, enabling the preservation of the product's physicochemical properties (Mujumdar, 2006; Silva *et al.*, 2022). Studies have shown that using temperatures between 50 and 70 °C can significantly reduce the pulp's moisture content, extending shelf life while maintaining bioactive compounds at satisfactory levels (Souza, 2015). However, strict control of time and temperature is essential to prevent the degradation of heat-sensitive compounds such as carotenoids and vitamin C.

Vacuum drying, in turn, is a dehydration technique performed under reduced pressure, allowing moisture removal at lower temperatures than those used under atmospheric pressure, typically ranging between 35 and 70 °C (Orikasa *et al.*, 2014). Due to the combination of low temperatures and pressures, this method is particularly suitable for products containing heat-sensitive or volatile compounds. The main advantage of vacuum drying lies in minimizing the thermal degradation of sensitive nutrients while preserving the sensory characteristics of the fruit, such as flavor, aroma, and color. Compared to traditional convective drying, vacuum drying can offer significant advantages (ElGamal *et al.*, 2023), particularly in terms of bioactive compound retention and the overall quality of the final product (Dağhan *et al.*, 2018).

Osmotic dehydration

Osmotic dehydration is a technique used for the partial reduction of water activity in foods, based on the osmotic phenomenon characteristic of plant tissues. The process involves immersing the food in a hypertonic solution, which induces the movement of water from inside the plant tissue to the external

medium, while, to a lesser extent, allowing the diffusion of solutes from the solution into the food (Pinto *et al.*, 2022). Since plant tissues exhibit lower permeability to sugars and high molecular weight compounds compared to water, the outward flow of water from the food is significantly higher than the inward flow of osmotic substances into the tissue, thus promoting osmotic dehydration (Lewicki; Lenart, 2006).

Osmotic dehydration offers several advantages, including the possibility of direct consumption of the product without the need for rehydration, the ability to control the amount of osmotic agents that penetrate the plant tissue, allowing adjustments according to technological and nutritional objectives, the modulation of the food's chemical composition to meet specific dietary requirements, and the reduction of raw material mass, which facilitates transportation and storage (Lewicki; Lenart, 2006). In the specific case of pequi, this technique represents a promising alternative for extending shelf life and increasing market availability while preserving sensory and nutritional characteristics similar to those of the fresh fruit (*in natura*). Additionally, Jayaraman and Gupta (2006) highlight other benefits of osmotic dehydration, such as lower degradation of color and flavor due to reduced thermal damage, decreased discoloration caused by oxidative enzymatic browning, better retention of volatile compounds responsible for aroma, and lower energy consumption, since water removal occurs without a phase change.

Specific studies on pequi pulp indicate that osmotic dehydration is effective in significantly reducing the fruit's moisture content, with values decreasing from 54.63 to 31.88% when using a 70% sucrose solution at a 1:2 ratio (mass/mass) over a period of 6 hours. In addition to the reduction in moisture content, the process promotes an increase in acidity, ash, and total sugar levels, while reducing pH and lipid content, resulting in a product with physicochemical characteristics similar to those of the fresh fruit (*in natura*) (Silva *et al.*, 2017). However, it is important to emphasize that osmotic dehydration may lead to the leaching of bioactive compounds, such as ascorbic acid and carotenoids, which can partially compromise the nutritional value of the final product (Mendonça *et al.*, 2017).

Freeze-drying

The technique of moisture reduction through freezing followed by water sublimation is known as freeze-drying. This process demonstrates high efficiency

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

compared to other drying methods, as it preserves the structural integrity of the material by preventing severe shrinkage, minimizes the loss of volatile compounds, reduces protein denaturation, and limits thermal decomposition. As a result, freeze-drying ensures product stability and quality throughout storage (Ordóñez, 2005; Fellows, 2006).

According to the observations of Boss (2004), freeze-drying offers significant advantages over conventional drying methods, particularly due to the reduction of degradation reactions, which is favored by the direct transition from the hydrated to the dehydrated state. In addition, this technique provides greater product stability during storage, allows moisture removal at lower temperatures, and contributes to the preservation of the physical structure of the food, key factors for maintaining the final product quality.

Freeze-drying consists of three distinct stages: freezing, primary drying (sublimation), and secondary drying (or desorption). In the first stage, the water present in the food is converted into ice crystals, while solutes become concentrated, interrupting chemical and biological reactions that could compromise product quality (Ordóñez, 2005). Next, during the primary drying phase, water removal occurs through sublimation under vacuum conditions with controlled heat application. Finally, in the secondary drying stage, the product remains under vacuum for an additional period of 2 to 6 hours, allowing the elimination of residual moisture and ensuring the stability of the final product (Ordóñez, 2005).

The application of freeze-drying for obtaining dried pequi pulp requires a careful evaluation of its chemical composition, physical properties, and antioxidant activity, with the aim of ensuring maximum utilization and high quality of the processed product. The use of this technique offers significant advantages, such as extended shelf life, stability of bioactive compounds, protection against oxidative and enzymatic degradation, and the possibility of making the product available throughout the year (Soares, 2018).

Nascimento *et al.* (2017), in their analysis of the physicochemical characteristics, phenolic and carotenoid contents, as well as the antioxidant capacity of pequi pulp, found that freeze-drying was effective in preserving bioactive compounds, particularly carotenoids and phenolics. The study also demonstrated that the use of opaque, light-resistant packaging materials, such as laminated films, significantly contributed to the preservation of these compounds during storage. From a nutritional perspective, the freeze-dried pulp exhibited remarkable values: 48.87 g/100 g of lipids, 6.17 g/100 g of proteins, 4.20 g/100 g of total

dietary fiber, and an energy value of 556.79 kcal/100 g, highlighting its potential as a functional ingredient for applications in the food industry.

Alves (2007), in a study on powders obtained from freeze-dried pequi pulp, evaluated hygroscopicity, microstructure, and carotenoid retention during storage. The results indicated that the combination of sucrose and ethanol led to lower variations in water activity, the formation of more stable crystalline structures, reduced adhesiveness, and satisfactory carotenoid retention. These findings reinforce the effectiveness of freeze-drying as a drying method for pequi pulp, contributing to the preservation of its bioactive compounds and functional properties over time.

Fluidized bed drying

The fluidized bed drying technique stands out as a promising alternative for the dehydration of fruit pulps and purees. In the literature, this process has been identified as an attractive option for drying pastes and suspensions, promoting the production of high-quality powders while offering low operational costs (Bezerra *et al.*, 2013). In recent decades, in response to the growing demand for higher productivity in the food industry, this technique has gained relevance within the agricultural sector (Almeida; Rocha, 2002). According to Gomes, Figueirêdo, and Queiroz (2004), fluidized bed drying has shown significant advancements, establishing itself as a viable alternative to spray drying in food processing. The use of this method for drying fruit pulps has been progressively expanding, leading to products with satisfactory quality. However, as noted by Pallai *et al.* (2006), despite the powder obtained meeting the minimum requirements for commercial use, issues such as flow instability, particle agglomeration, and material buildup inside the bed may represent limitations to its application.

Several studies have been conducted applying the fluidized bed drying technique for the dehydration of various fruits, including umbu, seriguela, mango, sugar apple, avocado (Medeiros *et al.*, 2001), acerola (Gomes, Figueirêdo, Queiroz, 2004), cajá (Melo *et al.*, 2010), long shelf-life tomato (Larrosa, Muszinski, Pinto, 2011), and green banana (Bezerra *et al.*, 2013), among others. These studies reinforce the feasibility of fluidized bed drying as a relevant technological alternative for the preservation and value addition of horticultural products.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Drying kinetics and other approaches

The analysis of drying kinetics has been widely addressed in the literature due to its relevance for monitoring changes in moisture content over time, playing a key role in understanding the dynamics of the dehydration process. This monitoring allows for the evaluation of the system's energy performance and the efficiency of heat and mass transfer, essential aspects for the development and optimization of equipment used in food drying (Silva *et al.*, 2014). According to Alves *et al.* (2013), different mathematical models can be applied to describe drying kinetics, depending on the operational conditions of the process and the specific characteristics of the product. In the case of pequi pulp, several studies have focused on the characterization of drying kinetics, with an emphasis on moisture loss over time, energy efficiency, and the behavior of heat exchange processes involved (Alves, 2007; Aquino *et al.*, 2009; Batista; Sousa, 2019; Carneiro *et al.*, 2023). These investigations are strategic not only for improving drying methods but also for the development and enhancement of equipment used in the agro-industrial sector.

Among the mathematical models used to simulate the drying of pequi pulp, the model proposed by Midilli has stood out for its high fitting capacity to experimental data. In a study conducted by Sousa *et al.* (2017), which evaluated the drying of pequi pulp at different temperatures (50, 60, 70, and 80 °C) and thicknesses (0.5, 1.0, and 1.5 cm), the Midilli model achieved coefficients of determination (R^2) ranging from 0.9990 to 0.9999, demonstrating its high accuracy in representing the drying process.

In addition to the conventional approach of convective drying, other techniques have been investigated, such as oven drying, solar drying, and microwave drying. Solar drying, for instance, stands out for its low implementation cost and operational simplicity, representing a viable alternative in regions with high solar radiation levels. However, its efficiency is limited by climatic conditions, particularly due to the overlap between the seasonality of agricultural products, such as pequi, and rainy periods, which compromises the optimal use of solar energy (Guimarães, 2023).

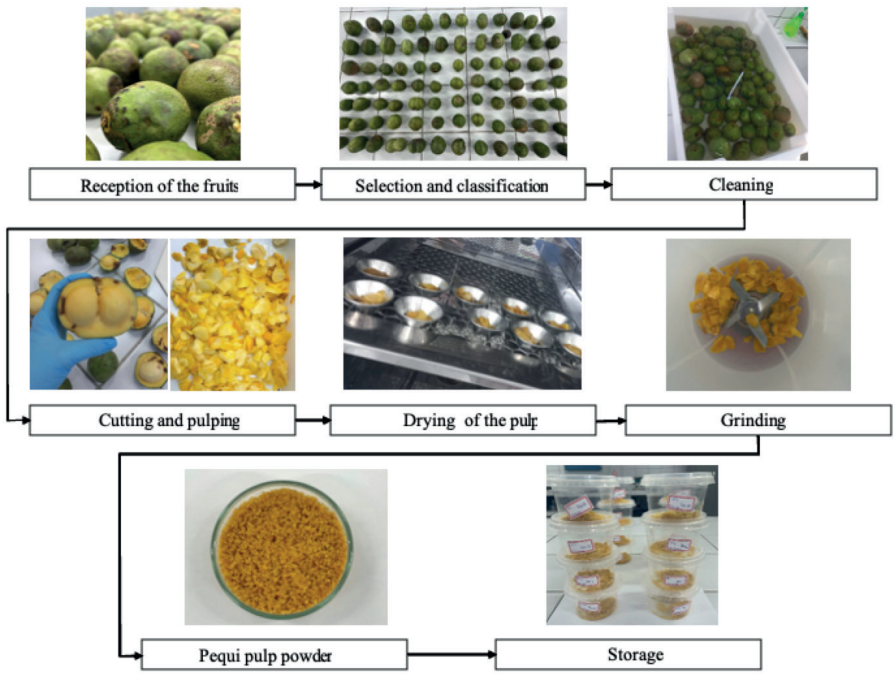
On the other hand, microwave drying, still scarcely explored in the specific case of pequi, has attracted increasing interest due to its rapid processing and characteristic volumetric heating, which favor the preservation of bioactive compounds. However, this technique requires strict control of operational

parameters, particularly to prevent localized overheating, which may compromise the physicochemical and sensory quality of the final product (Lemes *et al.*, 2017).

Impacts on powder quality and properties

Drying is an effective technique for food preservation; however, the conditions applied during the process, particularly temperature and time, can significantly affect the quality of the final product (Figure 2). Inadequate exposure to heat may lead to the degradation of bioactive compounds, such as vitamin C, phenolics, and flavonoids, in addition to compromising the sensory attributes of the food (Silva Junior *et al.*, 2023). On the other hand, when properly controlled, the drying process offers new opportunities for the utilization and preservation of fruit pulp, extending its shelf life and enabling the development of differentiated food products (Machado *et al.*, 2010).

Figure 2. Convective drying process and powder production from pequi pulp. Source: personal archive.According to Fennema, Parkin, and Damodaran (2018), although drying is often identified as a factor that compromises carotenoid stability, due to the sensitivity of these compounds to moisture reduction, there is evidence that a minimal residual moisture content in dehydrated foods may exert a protective effect. This remaining water fraction can hinder oxygen diffusion within the product, contributing to the preservation of carotenoids and, consequently, to the extension of the product's shelf life.



ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Given the high sensitivity of carotenoids, it is essential to adopt precautionary measures throughout all stages of food processing. This includes the careful selection of operational variables, such as the drying method and heat exposure time, which are critical for preserving the integrity of these compounds and maintaining the quality of the final product (Aquino *et al.*, 2009). Factors such as excessive heat, oxygen presence, light exposure, acidic pH, and low relative humidity may induce structural changes in carotenoids, compromising their stability and reducing their biological activity (Rodriguez-Amaya, 1993).

In this context, it becomes essential to carefully adjust process variables such as temperature, drying time, and the physicochemical characteristics of the fruit, including moisture content, thickness, and composition, in order to optimize nutrient retention and ensure the stability of the final product (Silva Junior *et al.*, 2023). Although higher temperatures may accelerate the drying process, they can compromise the integrity of thermosensitive compounds, such as carotenoids and lipids. Studies conducted by Aquino *et al.* (2009) and Souza *et al.* (2017) demonstrated that drying at 60 °C for 16 hours resulted in significant carotenoid degradation, along with an increase in peroxide values in the extracted oil, indicating the occurrence of lipid oxidation. In contrast, the use of lower temperatures, such as 40 °C, favored the preservation of these compounds, although associated with a considerably longer drying time.

Environmental factors also have a significant influence on fruit composition. Sunlight, for example, while stimulating the biosynthesis of bioactive compounds, may also contribute to their photodegradation (Xu *et al.*, 2020). In the case of phenolic compounds, whose synthesis occurs predominantly through the shikimic acid pathway, their production is closely related to the environmental stress experienced by the plant. For this reason, higher concentrations of these compounds have been identified in the powder obtained from pequi peel, which is directly exposed to solar radiation, high temperatures, and physical impacts during the maturation process (Santos-Sánchez *et al.*, 2019).

Santana *et al.* (2014) demonstrated that spray drying of pequi pulp resulted in vitamin C contents ranging from 145.78 to 292.11 mg of ascorbic acid per gram of solids, depending on the operational conditions applied. Similarly, Sousa *et al.* (2021), in their evaluation of pulp drying in a forced-air circulation oven at different temperatures (50 to 80 °C) and thicknesses (0.5 to 1.5 cm), observed a significant reduction in the final moisture content of the product, with values ranging from 1.38% to 4.33%.

The physical properties of the powders obtained were considered satisfactory, showing good solubility and low cohesiveness. Notably, the condition of 60 °C with a thickness of 0.5 cm resulted in a powder with excellent flowability. The product's adsorption isotherm, classified as Type II, was best fitted by the GAB model, with a coefficient of determination (R^2) higher than 0.98, providing relevant information for proper storage and the development of formulations based on pequi powder (Sousa *et al.*, 2021).

The feasibility of producing pequi powder through convective drying of the pulp was evaluated by Silva *et al.* (2018) at different temperatures (50 °C, 60 °C, 90 °C, and 105 °C) and varying drying times. The study found that drying at 105 °C was the most efficient, promoting a moisture loss of 58.88% without compromising the color, aroma, and flavor of the fruit. Although drying at 50 °C resulted in a lower final moisture content (40.45%), it required a longer processing time (12 hours), whereas intermediate temperatures such as 60 °C and 90 °C showed variations influenced by the sample's mass and thickness. Sensory analysis indicated that, even at higher temperatures, the sensory characteristics of pequi were preserved or even enhanced, suggesting that thermal processing can be effective without compromising the fruit's identity.

From a physicochemical perspective, the results indicated that the pH of the pulp decreased from 8.08 (fresh) to 6.95 after the drying process, accompanied by an increase in total acidity from 0.233% to 0.3667% and in the concentration of soluble solids (°Brix), which rose from 1.5 to 3.5. An increase in ash content was also observed, from 0.764% to 2.528%, reflecting the higher concentration of minerals as a result of water removal. After grinding, the powder showed a predominance of particles retained on a sieve with a 0.725 mm opening (46.36%), with a Sauter mean diameter of 0.6059 mm, demonstrating good particle size uniformity and technological potential for application in food formulations.

These results demonstrate that, when properly conducted, the drying process of pequi pulp can yield a stable and functional product with high potential for industrial use, particularly in the formulation of foods with nutritional and sensory appeal. However, this operation requires strict control, especially considering the presence of thermosensitive compounds such as carotenoids and vitamin C, whose preservation depends directly on the processing conditions adopted. Nevertheless, drying stands out as a promising strategy to diversify consumption options for the fruit, facilitate its incorporation into new products, extend its shelf life, and add value to its use in the food industry.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

FINAL CONSIDERATIONS

When properly conducted, the drying process allows for the production of a stable and functional product with high potential for industrial applications. Pequi powder can be incorporated into various formulations, adding nutritional and sensory value while expanding its consumption possibilities. However, the presence of thermosensitive compounds, such as carotenoids and vitamin C, requires strict control of key variables like temperature, drying time, and sample thickness throughout the process. This technique represents a viable alternative for the full utilization of pequi, promoting extended shelf life and enhancing its value within the food industry. In addition to contributing to the conservation of the fruit, drying broadens its applicability, supporting the sustainable use of native species from the Cerrado biome. It is worth noting that certain techniques, such as microwave drying, freeze-drying, and infrared drying, remain underexplored specifically for pequi, representing promising opportunities for future scientific research and technological development.

REFERENCES

Almeida, C.; Rocha, S. C. S. Fluidodinâmica de sementes de brócolis em leito fluidizado e leito de jorro. **Ciência Agrícola**, v. 59, n. 4, p. 645-652, 2002. http://dx.doi.org/10.1590/S0103-90162002000400004

Almeida, R. F; Machado, A. P. O. Secagem de alimentos e seu impacto na pós-colheita. *In*: **Anais do Congresso Brasileiro Interdisciplinar em Ciência e Tecnologia. Anais**...Diamantina(MG) UFVJM, 2021. Disponível em: https//www.even3.com.br/anais/cobicet/390601-SECAGEM-DE-ALIMENTOS-E-SEU-IMPACTO-NA-POS-COLHEITA. Acesso em: 15/04/2025.

Alves, C. C. O. **Pós obtidos da polpa de pequi (*Caryocar brasiliense* Camb.) liofilizada**: Higroscopicidade e microestrutura. 2007, 168 f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) Universidade Federal de Lavras, Minas Gerais, 2007.

ALVES, G. E.; Isquierdo, E. P.; Borém, F. M.; Siqueira, V. C.; Oliveira, P. D.; Andrade, E. T. Cinética de secagem de café natural para diferentes temperaturas e baixa umidade relativa. **Coffee Science**, Lavras, v. 8, n.2, p. 238-247, 2013. http://repositorio.ufla.br/jspui/handle/1/13699

Amaral, L. F. B.; Moriel, P.; Foglio, M. A.; Mazzola, P.G. *Caryocar brasiliense* supercritical co_2 extract possesses antimicrobial and antioxidant properties useful for personal care products. **Complementary and Alternative Medicine**, v.14, n.73, p.1-7, 2014. https://doi.org/10.1186/1472-6882-14-73.

Aquino, L. P.; Ferrua, F. Q.; Borges, S. V.; Antoniassi, R.; Correa, J. L. G.; Cirillo, M. A. Influência da secagem do pequi (*Caryocar brasiliense* Camb.) na qualidade do óleo extraído. **Ciênc. Tecnol. Aliment.**, v.29, n. 2, p. 354-357. 2009. https://doi.org/10.1590/S0101-20612009000200018

Batista, F. O.; Sousa, R. S. Bioactive compounds in fruits pequi (*Caryocar brasiliense* Camb.) e baru (*Dipteryx alatavogel*) and their potential uses: a review. **Brazilian Journal of Development**, v. 5, n. 7, p. 9259-9270, 2019. https://doi.org/10.34117/bjdv5n7-120

Bezerra, C. V.; Amante, E. R.; Oliveira, D. C.; Rodrigues, A. M. C.; Silva, L. H. M. Banana verde (*Musa cavendishii*) farinha obtida em leito de jorro - Efeito da secagem na fisico-características químicas, funcionais e morfológicas do amido. **Culturas e produtos industriais**, v. 41, n. 1, p. 241-249, 2013. http://dx.doi.org/10.1016/j.indcrop.2012.04.035

Boss, E. A. Modelagem e otimização do processo de liofilização: aplicação para leite desnatado e café solúvel. 2004. 107 f. Tese (Doutorado em Engenharia Química) – Universidade Estadual de Campinas, Faculdade de Engenharia Química, Campinas, SP, 2004. Disponível em: https://hdl.handle.net/20.500.12733/1596991. Acesso em: 15 abr. 2025.

Calín-Sánchez, Á.; Lipan, L.; Cano-Lamadrid, M.; Kharaghani, A.; Masztalerz, K.; Carbonell-Barrachina, Á. A.; Figiel, A. Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. **Foods**, v. 9, n. 9, p. 1261, 2020. https://doi.org/10.3390/foods9091261

Carneiro, C. R.; Alhaji, A. M.; Silva, C. A. S.; Sousa, R. C. S.; Monteiro, S.; Coimbra, J. S. R. Potential challenges of the extraction of carotenoids and fatty acids from pequi (*Caryocar brasiliense*) Oil. **Foods**, v. 12, n.1, p.1-21, 2023. https://doi.org/10.3390/foods12091907

Castiglioni, G. L.; Silva, F. A.; Calliari, M.; Junior, M. S. S. Modelagem matemática do processo de secagem da massa fibrosa de mandioca. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.17, n. 9, p.987-994, 2013. https://doi.org/10.1590/S1415-43662013000900012

Cordeiro, M. W. S.; Cavallieri, Â. L. F.; Ferri, P. H.; Naves, M. M. V. Características físicas, composição químico-nutricional e dos óleos essenciais da polpa de *Caryocar brasiliense* nativo do estado de Mato Grosso. **Revista Brasileira de Fruticultura**, v. 35, n. 4, p.1127-1139, 2013. https://doi.org/10.1590/S0100-29452013000400024

Costa, A. P. F.; Pinto, E. G.; Soares, D. S. B. Obtenção de farinha do mesocarpo de pequi. **Agrarian**, v.10, n.38, p. 349-354, 2017. https://doi.org/10.30612/agrarian.v10i38.7051

Costa, C. A. R.; Nascimento, S. V.; Valadares, R. B. S.; Silva, G. M.; Machado, G. G. L.; Costa, I. R. C.; Nahon, S. M. R.; Rodrigues, L. J.; Boas, E. V. B. V. Proteome and metabolome of *Caryocar brasiliense* Camb. fruit and their interaction during development. **Food Research International**, v. 191, n.1, p.1-10, 2024. https://doi.org/10.1016/j.foodres.2024.114687

Damodaran, S. Parkin, K. L. **Química de Alimentos de Fennema**. Londres: CRC Press, 2018. 1120p.

Dağhan, Ş.; Yildirim, A.; Yilmaz, F.M.; Vardin, H.; Karaaslan, M. The effect of temperature and method of drying on Isot (*Urfa pepper*) and its vitamin C degradation kinetics. **Italian Journal of Food Science,** v.30, n.1, p.504–521, 2018. https://doi.org/10.14674/IJFS-1070

Deus, T. N. **Extração e caracterização de óleo do pequi (Caryocar brasiliense Camb.) para o uso sustentável em formulações cosméticas óleo/água (O/A)**. 2008. 132 f. Dissertação (Mestrado em Ciências Ambientais e Saúde) – Pontifícia Universidade Católica de Goiás, Goiânia, 2008.

ElGamal, R.; Song, C.; Rayan, A. M.; Liu, C.; Al-Rejaie, S.; ElMasry, G. Thermal degradation of bioactive compounds during drying process of horticultural and agronomic products: A comprehensive overview. **Agronomy**, v.13, n.6, p.1580, 2023. https://doi.org/10.3390/agronomy13061580

Fellows, P. J. **Tecnologia do processamento de alimentos: princípios e prática**. Porto Alegre: Artmed, 2006. 602p.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Garcia, L.; Cunha, A.; Costa, B.; Oliveira, G.; Mendonça, H.; Reis, G. D.; Silva, V.; Correia, V. T. A. V. Reina, L. D. C. B.; Melo, J. Pequi (*Caryocar brasiliense* e *Caryocar coriaceum*): importância socioeconômica, principais fitoquímicos e propriedades bioativas. *In*: MELO, J. O. F.; DAMIANI, C.; ARAÚJO, R. L. B. De; LUCENA, R. F. P. (org.). *Árvores, plantas e* **frutos do Cerrado: aplicações e possibilidades**. 1. ed. São Carlos: Editora Científica Digital, p. 97-114, 2024.

Gomes, P. M. A; Figueirêdo, R. M. F.; Queiroz, A. J. M Armazenamento da polpa de acerola em pó a temperatura ambiente. **Ciência e Tecnologia de Alimentos**, v. 24, n. 3, p.384-389, 2004. http://dx.doi.org/10.1590/S0101-20612004000300014

Guimarães, A. S. **Obtenção de pó de caju por secagem solar:** estudo de viabilidade e caracterização. 2023. Trabalho de Conclusão de Curso (Graduação em Engenharia de Alimentos) – Universidade Federal do Rio Grande do Norte, Natal, 2023.

IBGE - Instituto Brasileiro de Geografia e Estatística. Diretoria de Pesquisas, Produção Agropecuária. Disponível em: https://www.ibge.gov.br/explica/producao-agropecuaria/pequi/br. Acesso: 03 de abril de 2025.

Jayaraman, K. S.; Gupta, D. K. Drying of fruits and vegetables. *In*: MUJUMDAR, A. S. **Handbook of industrial drying.** New York: Marcel Dekker, 2006. p. 606-631.

Larrosa, A. P. Q.; Muszinski, P. Pinto, L. A. A. Programação linear para formulação de pasta de vegetais e operação de secagem em leito de jorro. **Ciência Rural**, v.41, n.11, p.2032-2038, 2011. https://doi.org/10.1590/S0103-84782011001100030

Leão, D. P.; Franca, A. S.; Oliveira, L. Ls.; Bastos, R.; Coimbra, M. A. Physicochemical characterization, antioxidant capacity, total phenolic and proanthocyanidin content of flours prepared from pequi (*Caryocar brasilense* Camb.) fruit by-products. **Food Chemistry**, v. 225, n.1, p. 146-153, 2017. http://dx.doi.org/10.1016/j.foodchem.2017.01.027

Leite, J. F.; Feitosa, A. C.; Zuniga, A. D. G.; Guida, L. M.; Silva, D. X. Quality of pequi fruit *(Caryocar Brasiliense* Camb.*)* stored under vacuum at different temperatures. **Brazilian Journal of Development**, v. 6, n.4, p.21951-21958, 2020. https://doi.org/10.34117/bjdv6n4-385

Lemes, E. O.; Fernandes, M. M. C.; Rosa, V. P.; Nascimento, A. H. Levantamento da Utilização do Pequi (*Caryocar brasiliense* Camb.*)* como Agente Antioxidante na Prevenção de Doenças Neurodegenerativas. **Uniciências**, v. 21, n.2, p. 110-114, 2017. https://doi.org/10.17921/1415-5141.2017v21n2p110-114

Lewicki, P. P.; Lenart, A. Osmatic dehydration of fruits and vegetables. *In*: Mujumdar, A. S. **Handbook of industrial Drying**. New York: Marcel Dekker, p. 665-681, 2006.

Lima, A.; Silva, A. M. O.; Trindade, R. A.; Torres, R. P.; Filho, J. M. Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (*Caryocar brasiliense* Camb.). **Revista Brasileira de Fruticultura**. v. 29, p. 695-698, 2007. https://doi.org/10.1590/S0100-29452007000300052

Machado, A. V; Oliveira, E. L.; Santos, E. S.; Oliveira, J. A.; Freitas, L. M. Estudo cinético da secagem do pendúculo de caju e um secador convencional. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v.5, n.1, p.36-42, 2010.

Medeiros, M. F. D; Alsina, O. L. S.; Rocha, S. C.; Jerônimo, C. E. M; Mata, A. L. M. L.; Medeiros, U. K. L.; Furtunato, A. A. Escoabilidade de leitos de partículas inertes com polpa de frutas tropicais: efeitos na secagem em leito de jorro. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 5, n. 3, p. 475-480, 2001. http://dx.doi.org/10.1590/S1415-43662001000300018

Melo, K. S.; Nascmento, M. A.; Gomes, W. C.; Cabral, S. B.; Rocha, A. P. T. Fluidodinâmica de leito de jorro com leite de cabra e polpa de cajá. **Revista Verde**, v. 5, n. 4, p. 61-67, 2010. Disponível em: https://www.gvaa.com.br/revista/index.php/RVADS/article/view/392. Acesso em: 16 apr. 2025.

Mendonça, K. S.; Corrêa, J. L.; Junqueira, J. R.; Cirillo, M. A,; Figueira, F. V.; Carvalho, E. E. Influences of convective and vacuum drying on the quality attributes of osmo-dried pequi (*Caryocar brasiliense* Camb.) slices. **Food Chemistry**. 2017 v. 1, n. 224, p.212-218. https://doi.org/10.1016/j.foodchem.2016.12.051

Monteiro, S. S.; Silva, R. R.; Silva, S. C. M.; Barin, J. S.; Rosa, C. S. Phenolic compounds and antioxidant activity of extracts of pequi peel (*Caryocar brasiliense* Camb). **International Food Research Journal**, v. 22, n. 5, p.1985-1992, 2015.

Moreno, L, G. **Efeitos biológicos da associação da ingestão da polpa de pequi (***Caryocar brasiliense)* **ao exercício físico aeróbico regular em ratos**. 2014. 95f. Dissertação (Mestrado em Ciências Fisiológicas) – Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, 2014.

Mousakhani-Ganjeh, A.; Amiri, A.; Nasrollahzadeh, F.; Wiktor, A.; Nilghaz, A.; Pratap-Singh, A.; Khaneghah, A. M. Electro-based technologies in food drying-A comprehensive review. **Lwt**, v.145, n.1, p.111315, 2021. https://doi.org/10.1016/j.lwt.2021.111315

Mujumdar, A. S. **Handbook of industrial drying**. New York: Marcel Dekker, 2006

Narita, I. M. P.; Filbido, G. S.; Ferreira, B. A.; Pinheiro, A. P. O.; Silva, D. C.; Nascimento, E.; Villa, R. D.; Oliveira, A. P. In vitro bioaccessibility of carotenoids and phenolic compounds and antioxidant capacity of pequi fruit *(Caryocar brasiliense* Camb.*)* flours. **Brazilian Journal of Food Technology**, v. 25, n.1, p. e2021068, 2022. https://doi.org/10.1590/1981-6723.06821

Nascimento-Silva, N. R. R.; Mendes, N. S.; Silva, F. A. Nutritional composition and total phenolic compounds content of pequi pulp (*Caryocar brasiliense* Cambs.). **Journal of Bioenergy and Food Science**. v.7, n.1, p. 2812019, 2020. http://dx.doi.org/10.18067/jbfs.v7i2.281

Nascimento, N. R. R.; Alves, A. M.; Silva, M. R.; Naves, M. M. V. Antioxidant capacity of pequi (*Caryocar brasiliense* Camb.) pulp is preserved by freeze-drying and light-resistant packaging. **Revista Brasileira de Fruticultura**, v. 39, n. 1, p.e-150, 2017. https://doi.org/10.1590/0100-29452017150

Nunes, A. C.; Souza, A. L. C.; Santos, T. A.; Silva, T. P. Extraction of essential oils from *Caryocar coriaceum* (pequi) by enzymatic pathways. **Research, Society and Development**, v. 10, n.16, p. 237101623496, 2021. https://doi.org/10.33448/rsd-v10i16.23496

Oliveira, M. N. S.; Gusmão, E.; Lopes, P. S. N.; Simões, M. O. M.; Ribeiro, L. M.; Dias, B. A. Maturity stage of fruits and factors related to nutritive and texture characters of pequi *(Caryocar brasiliense* Camb.*)* pulp. **Revista Brasileira de Fruticultura**, v. 28, n. 3, p. 380-386, 2006.

Oliveira, M. N. S.; Lopes, P. S. M.; Simões, M. O. M.; Pereira, E. G.; Pereira, E. G.; Ribeiro, L. M. Post-harvest quality of pequi (*Caryocar brasiliense* Camb.) collected from the planto rafter naturally falling off and subjected to slow and quick freezing. **Revista Brasileira de Fruticultura**, v. 39, n. 1, p. e-768, 2017. https://doi.org/10.1590/0100-29452017768

Ordóñez, J. A. **Tecnologia de Alimentos**. Porto Alegre: Artmed, 2005. 294p.

Orikasa, T.; Koide, S.; Okamoto, S.; Imaizumi, T.; Muramatsu, Y.; Takeda, J. I.; Shiina, T.; Tagawa, A. Impacts of hot air and vacuum drying on the quality attributes of kiwifruit slices. **Journal of Food Engineering**, v. 125, p. 51-58, 2014. https://doi.org/10.1016/j.jfoodeng.2013.10.027

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Pallai, E.; Szentmarjay, T.; Mujumdar, A. S. Secagem Em Leito De Jorro. Em: Mujumdar, A. (Ed). **Manual de industrial secagem**. Filadélfia: Taylor & Francisco Grupo, 2006. p. 363-384. http://dx.doi.org/10.1201/9781420017618.ch14

Pinto, M. R. M. R.; Paula, D. A.; Alves, A. I.; Rodrigues, M. Z.; Vieira, E. N. R.; Fontes, E. A. F.; Ramos A. M. Encapsulation of carotenoid extracts from pequi *(Caryocar brasiliense* Camb*)* by emulsification (O/W) and foam-mat drying. **Powder Technology**, v. 339, n.1, p. 939-946, 2018. https://doi.org/10.1016/j.powtec.2018.08.076

Rodriguez-Amaya, D. B. Estabilidade de carotenoides durante o armazenamento de alimentos. *In*: CHARALAMBOUS, F. (Ed.). **Estudos de vida útil de alimentos e bebidas**: aspectos químicos, biológicos, físicos e nutricionais. Amsterdã: Elsevier Science, 1993. p. 591-624.

Sahoo, M.; Titikshya, S.; Aradwad, P.; Kumar, V.; Naik, S. M. Estudo do comportamento de secagem e cinética de cor da secagem convectiva de fatias de inhame (*Dioscorea hispida*). **Industrial Crops & Products**, v. 173, 2022. https://doi.org/10.1016/j.indcrop.2021.114258

Santana, A. A.; Oliveira, R. A.; Kurozawa, L. E.; Park, K. J. Microencapsulation of pequi pulp by spray drying: use of modified starches as encapsulating agent. **Engenharia Agrícola**, v.34, n.5, p. 980-991, 2014. https://doi.org/10.1590/S0100-69162014000500017

Santos-Sánchez, N. F., Salas-Coronado, R., Hernández-Carlos, B.; Villanueva-Cañongo, C. Shikimic acid pathway in biosynthesis of phenolic compounds, In M. Soto-Hernández, R. García-Mateos & M. Palma-Tenango (Eds.), **Plant physiological aspects of phenolic compounds** London: Intech Open. https://doi.org/10.5772/intechopen.83815

Santos, B. O.; Tanigaki, M.; Silva, M. R.; Ramos, A. L. C. C.; Labanca, R. A.; Augusti, R.; Melo, J. O. F.; Takahashi, J. A.; Araújo, R. L. B. Development and Chemical Characterization of Pequi Pericarp Flour *(Caryocar brasiliense* Camb.*)* and Effect of in vitro Digestibility on the Bioaccessibility of Phenolic Compounds. **Journalof Brazilian Chemical Society,** v. 33, n.9, p. 1058-1068, 2022. https://doi.org/10.21577/0103-5053.20220022

Silva, D. F.; Rigueto, C. V. T.; Loss, R. A.; Guedes, S. F.; Carvalho, J. W. P. C. Tratamento osmótico na obtenção de lascas da polpa de pequi (*Caryocar brasiliense*) desidratadas. **Natural Resources**, v.7, n.1. p.1-8, 2017.https://doi.org/10.6008/SPC2237-9290.2017.001.0001

Silva, A. P. R.; Rocha, W. M.; Fernandes, D. M.; Finzer, J. R. D. Production of Pequi Powder by Drying Pulp. **Agricultural Sciences**, v. 9, n. 8, p. 1047–1057, 2018. https://doi.org/10.4236/as.2018.98072

Silva Júnior, M. A. V. **Modelagem e simulação da secagem convectiva de materiais alimentícios via método de elementos finitos**. 2023. Tese (Doutorado) – Universidade de São Paulo, Pirassununga, 2023. Disponível em: https://www.teses.usp.br/teses/disponiveis/74/74133/tde-02022024-101632/. Acesso em: 15 abr. 2025.

Singhal, S.; Rasane, P.; Kaur, S.; Singh, J.; Gupta, N. Thermal degradation kinetics of bioactive compounds in button mushroom (*Agaricus bisporus*) during tray drying process. **Journal of Food Process Engineering**, v.43, n.12, p.e13555, 2020. https://doi.org/10.1111/jfpe.13555

Souza, J. L. F. Farinha do mesocarpo do pequi (Caryocar brasiliense Cambess): cinética da secagem, propriedades nutricionais, funcionais e enriquecimento de iogurtes. 2015. 106 f. Dissertação (Mestrado em Alimentos) – Instituto Federal Goiano – Câmpus Rio Verde, Rio Verde, 2015.

Sousa, E. P.; Figueirêdo, R. M. F.; Gomes, J. P.; Queiroz, A. J. M.; Castro, D. S. Lemos, D. M. Modelagem matemática da secagem da polpa de pequi e determinação da difusividade efetiva. **Revista Brasileira de Engenharia Agrícola e Ambientental,** v.21, n. 7, 2017. https://doi.org/10.1590/1807-1929/agriambi.v21n7p493-498

Sousa, E. P.; Lemos, D. M.; Figueirêdo, R. M. F.; Gomes, J. P.; Queiroz, A. J. M. Influência da temperatura de secagem e espessura da camada na qualidade física e físico-química de pós de pequi. **Revista Caatinga**, v.34, n. 4, p, 945-956, 2021. https://doi.org/10.1590/1983-21252021v34n422rc

Silva, L. M. M.; Sousa, F. C.; Sousa; E. P; Mata, M. E. R. M. C.; Duarte, M. E. M. Modelos de predição da cinética de secagem dos grãos de guandu. **Brazilian Journal of Food Technology**. v.17, n. 4, p.310-318, 2014. https://doi.org/10.1590/1981-6723.3014

Silva, J. R. B.; Campos, A. R. N.; Santana, R. A. C.; Dantas, D. L.; Macedo, A. D. B.; Sousa, A. P. M.; Malaquias, A, B.; Albuquerque, T. N.; Silva, G. B.; Santos, A. X. Drying kinetics of Eggplant (*Solanum Melongena* L.*)* in na over. **Research Society and Development**, v. 11, n.4, p. 34111427319, 2022. https://www.doi.org/10.33448/rsd-v11i4.27319

Silva, A. G. F.; Costa, F. B.; Nascimento, A. M.; Sales, G. N. B.; Silva, J. L.; Silva Neta, A. M. S; Ribeiro, W. S.; Silva, T. I.; Feitosa, B. F. Spondias purpurea L. (Anacardiaceae) fruits flours at different maturation stages: Drying kinetics, mathematical modelling, characterization and correlation analysis, **Heliyon**, v. 11, n. 2, e41832, 2025. https://doi.org/10.1016/j.heliyon.2025.e41832.

Silveira, E. A.; Barcelo, R.; Lamas, G. C.; Rodrigues, P. P. O.; Chaves, B. S.; Protásio, T. P.; Rousset, P.; Ghesti. G. Biofuel from agro-industrial residues as sustainable strategy for CO2 mitigation: Statistical optimization of pequi seeds torrefaction. **Energy Conversion and Management**, v. 304, n.1, p.1-17, 2024. https://doi.org/10.1016/j.enconman.2024.118222

Schiassi, M. C. E. V.; Souza, V. R. D.; Lago, A. M. T.; Campos, L. G.; Queiroz, F. Fruits from the Brazilian Cerrado region: physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation. **Food Chemistry**, v. 245, p. 305-311, 2018. http://dx.doi.org/10.1016/j.foodchem.2017.10.104

Soares, C. T. **Secagem da polpa de pequi por liofilização.** 2018. Dissertação (Mestrado em Engenharia Agrícola) — Universidade Estadual de Campinas, Faculdade de Engenharia Agrícola, Campinas, SP, 2018.

Sokhansanj, S.; Jayas, D. S. Drying of foodstuffs. *In*: Mujumdar, A. S. **Handbook of industrial drying**. New York: Marcel Dekker, 2006. p. 522-545.

Stepien, A. E.; Gorzelany. J.; Matlok, N.; Krzysztof, L.; Figiel, A. The effect of drying methods on the energy consumption, bioactive potential and colour of dried leaves of Pink Rock Rose (*Cistus creticus).* **Journal of Food Science and Technology**, v. 56, p. 2386-2394, 2019. https://doi.org/10.1007/s13197-019-03656-2

Pinto, B. D. S.; Melo, A. C. C. D.; Costa Junior, E. F. D.; Costa, A. O. S. D. Novel technologies combined with osmotic dehydration for application in the conservation of fruits: an overview. **Ciência Rural,** v.53, n.1, p.e20200935, 2022. https://doi.org/10.1590/0103-8478cr20200935

Vieira, J. N.; Silva, T. I. D.; Costa, F. B. D.; Macêdo, L. F.; Nascimento, A. M. D.; Sales, G. N. B.; Candeia, R. A. Postharvest Characterization of Passiflora cincinnata Fruit Pulp at Different Ripening Stages. **Brazilian Archives of Biology and Technology**, v. 67, n.1, p. e24230788, 2024. https://doi.org/10.1590/1678-4324-2024230788

Xu, K.; Alves-Santos, A. M.; Dias, T.; Naves, M. M. V. Grumixama (*Eugenia brasiliensis* Lam.) cultivated in the Cerrado has high content of bioactive compounds and great antioxidant potential. **Ciência Rural**, v. 50, n. 4, p.e20190630, 2020. http://dx.doi.org/10.1590/0103-8478cr20190630

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

CHAPTER VIII

FRUIT AND VEGETABLES HARVEST AND POSTHARVEST

M.Sc. Samuel Giovanny García-Castaño

Prof. Dr. Marcelo F. Pompelli

M.Sc. Andrés José Betin Ruiz

M.Sc. Marvin José Perneth-Montaño

10.37885/250619549

ABSTRACT

The study investigates the physiological, biochemical, and technological aspects of postharvest quality and preservation in fruits and vegetables, with an emphasis on minimizing losses and improving shelf life. Postharvest losses of up to 40% are highlighted as a major issue, driven by metabolic activities such as respiration, ethylene biosynthesis, and transpiration. The research aims to assess the effectiveness of various preservation techniques—including refrigeration, modified atmospheres, edible coatings, and intelligent packaging—by exploring their impact on product quality, industrial processing yield, and market viability. The methodology includes experimental practices evaluating soluble solids content in grapes, tomatoes, oranges, and mangos using refractometry, as well as respiration assessment through pH-indicator color change. Additionally, the research employs near-infrared (NIR) spectroscopy and chemometric analyses (PLSR and PCA models) to predict soluble solid content non-destructively. Key results demonstrate that soluble solids (°Brix) vary between fresh and processed samples and are critical indicators for determining optimal harvest time and industrial processing yield. Climacteric fruits showed higher respiration rates at room temperature, corroborated by faster bromothymol blue color changes. Moreover, the modified atmosphere coupled with refrigeration significantly extended shelf life in minimally processed vegetables, particularly cabbage, by reducing metabolic rates and dehydration. The study concludes that integrated postharvest technologies tailored to specific physiological traits and storage conditions are essential for ensuring quality, reducing waste, and increasing profitability. Furthermore, predictive models using continuous wavelength spectrometry and appropriate preprocessing techniques showed strong potential for industrial applications due to their robustness and scalability. The findings underscore the need for continued research to refine these technologies and address the complexities of fruit ripening and preservation across different cultivars and environmental contexts.

Keywords: postharvest physiology; soluble solids; climacteric fruits; modified atmosphere packaging.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

INTRODUCTION

It is estimated that from harvest to the arrival of the product on the consumer's table, there are qualitative and quantitative losses of up to 40% of the fruits and vegetables produced. These losses can be attributed to various factors, including improper handling, inefficient storage, and unfavorable transportation conditions. Post-harvest preservation of fruits, flowers, and vegetables, whether for short-term or long-term marketing, is essential to ensure greater profitability for producers and to reduce food waste. Since these products are living materials, they maintain metabolic activity even after harvest, which can lead to deterioration if appropriate preservation techniques are not applied. Post-harvest preservation techniques involve operations carried out from the selection of products in the field to their final consumption, with the primary goal of reducing or minimizing metabolic activity, particularly respiration, ethylene biosynthesis, and transpiration, thereby extending post-harvest shelf life.

Respiration is a natural process by which agricultural products consume oxygen and release carbon dioxide, heat, and water vapor. This process can be accelerated by factors such as mechanical damage, elevated temperatures, and high relative humidity, resulting in significant quality and quantity losses. Ethylene biosynthesis, a plant hormone, also plays a crucial role in the ripening and senescence of fruits and can accelerate deterioration if not adequately controlled. Various post-harvest preservation strategies have been developed and implemented to mitigate these effects and extend the shelf life of horticultural products. Refrigerated storage is one of the most common techniques, as lower temperatures reduce respiration rates and slow the growth of spoilage microorganisms. Additionally, using controlled or modified atmospheres and adjusting oxygen and carbon dioxide levels around the products can delay ripening and senescence, maintaining quality for extended periods. Eating coatings, such as natural waxes, have also been used to reduce transpiration and protect products from pathogens. These coatings form a semi-permeable barrier that decreases water loss and exposure to external agents.

The effective implementation of these techniques requires a deep understanding of each product's specific characteristics, including its physiology, susceptibility to pathogens, and response to different environmental conditions. Factors such as the cultivated variety, the stage of ripeness at the time of harvest, and climatic conditions during cultivation also influence the choice of the

most appropriate preservation strategies. Therefore, continuous research and developing innovative technologies are essential to improving post-harvest practices and reducing losses in the horticultural sector.

The adoption of post-harvest preservation techniques, such as refrigerated storage, controlled atmospheres, edible coatings, irradiation, and intelligent packaging, can substantially reduce these losses, ensuring better-quality products for consumers and higher profitability for producers. Integrating these practices and proper handling at all stages of the production chain is crucial to minimizing waste and promoting sustainability in the agricultural sector. Did you know that up to 40% of food is lost before it reaches your table? Imagine the taste, aroma, and vitality that go to waste! Each upcoming chapter unveils powerful strategies to preserve not just food but dreams, livelihoods, and hope. Get ready to be moved by solutions that turn waste into abundance and harvests into stories of success. Read on and be inspired to be part of this transformation!

Determination of Soluble Solids Content in Vegetables

The concentration of soluble solids (measured in Brix degrees) represents the content of sugars (mainly glucose, fructose, and saccharose), organic acids, and other smaller components directly related to the product's sweetness, whether vegetable or animal. In fruits, the content of soluble solids is a widely used characteristic to identify the point of harvest. In grapevines, the reading of the juice extracted from the berry indicates the point of harvest, having previously a reference value of the ripe grape for each cultivar [1]. In oranges, the higher the Brix degree, the lower the number of orange boxes to produce a ton of juice within the dilution pattern established for each variety [2]. In tomatoes, the higher Brix degree, the higher the yield and the lower the energy expenditure in the pulp concentration process [3]. The same occurs in sugarcane, where the delay in processing after cutting can reduce soluble solids values due to significant sucrose losses, contributing to low yields in the sugar-alcohol industry [4].

Effective wavelengths play a crucial role in the robustness of predictive models, particularly in evaluating fruit quality parameters such as soluble solids content (SSC). Models based on continuous wavelengths, such as CSMW-PLSR, demonstrated greater interpretability for new batches of kiwifruit compared to those based on discrete variables like CARS-PLSR [5]. These findings align with Brito*, et al.* [6], who emphasized that SSC is a key parameter influencing flavor and consumer acceptance in tomatoes. The use of partial least squares

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

regression (PLSR) models associated with portable Vis-NIR spectrometers proved efficient in predicting SSC in intact tomatoes, mainly when orthogonal signal correction (OSC) pretreatment was applied, resulting in low mean square errors and demonstrating the robustness of the method [6].

Furthermore, spectral analyses of tomatoes revealed significant spectral variations linked to pigments like lycopene and compounds associated with ripening stages [6]. This observation complements the work of Tian, Liu and Xu [5], who found that selecting continuous intervals minimizes issues related to peak displacement in spectra, ensuring more consistent predictions. Specifically, the spectral region between 840 and 1050 nm effectively discriminates SSC due to absorption peaks linked to water and carbohydrates. Similarly, the CSMW-PLSR model combined with skew correction was identified as the most efficient approach for predicting SSC in kiwifruit, reducing predictive error and improving the coefficient of determination, thus making it more suitable for industrial applications.

Integrating chemometric analyses, such as principal component analysis (PCA) and PLSR models, was essential for interpreting spectral data and selecting relevant wavelength ranges in both studies. Brito, *et al.* [6] highlighted that PCA revealed no significant clustering based on geographic origin, suggesting that variability among tomato samples stemmed from common factors like variety and ripeness. This complements Tian, Liu and Xu [5] conclusion that continuous wavelength-based models like CSMW-PLSR generalize better due to their ability to accommodate biological variability. The PLSR model yielded satisfactory calibration and prediction coefficients and mean square prediction errors around 0.32%, indicating its strong predictive capacity and robustness for commercial applications [6].

Regarding practical applications, both studies underscore the importance of robust, non-destructive methods for SSC determination to enhance quality control in agricultural products. Brito, *et al.* [6] demonstrated that Vis-NIR spectroscopy combined with PLSR and OSC preprocessing offers a rapid and accurate technique for analyzing SSC in market tomatoes without damaging the samples. This approach is highly applicable in industrial and commercial processes, ensuring the delivery of high-quality fruits to consumers. Similarly, Tian, Liu and Xu [5] adopting the CSMW-PLSR model with skew correction for kiwifruit, as it not only improves predictive accuracy but also requires fewer samples for adjustments, making it more practical and cost-effective.

Both studies also discuss the metabolic processes influencing SSC and their implications for harvest timing and industrial processing yields. The maturity phase of fruits affects spectral properties, as seen in tomatoes from Bahia State – Brazil, which exhibited higher absorption in the 500–570 nm range due to increased red pigment content [6]. This finding parallels the importance of accounting for biological variability in kiwifruit batches, where the CSMW-PLSR model proved capable of maintaining prediction accuracy despite such variations [5]. These insights highlight the necessity of understanding metabolic processes to optimize SSC applications in industrial contexts.

Finally, the convergence of findings from these studies suggests that adopting continuous wavelength-based predictive models, coupled with appropriate spectral preprocessing techniques, offers a robust and scalable solution for SSC determination in various fruits. While Brito, *et al.* [6] emphasized the advantages of Vis-NIR spectroscopy and OSC preprocessing for tomatoes, Tian, Liu and Xu [5] demonstrated that the CSMW-PLSR model with skew correction provides superior performance in predicting SSC in kiwifruit. Together, these studies illustrate how advanced spectroscopic techniques and chemometric analyses can be effectively applied across different agricultural products to improve quality control processes, ensuring accurate and consistent predictions despite inherent biological variability. Thus, the objective of these classrooms is to evaluate the content of soluble solids in grapes, tomatoes, oranges, and mangos, to compare the values with those obtained in the same products after industrial processing, and to understand the relationship between soluble solids, the point of physiological ripeness, the point of collection, post-harvest quality and yield in industrial processing; to review concepts related to the content.

MATERIALS

- 3 grape berries
- 1 orange fruit (different varieties or cultivars can be used)
- 1 mango fruit (different varieties or cultivars can be used)
- 100 ml of industrially processed grape, mango and orange juice
- 1 tomato fruit (different varieties or cultivars can be used)
- 1 portion of tomato pulp obtained from the supermarket
- 1 plastic sieve

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

- Glass beakers
- Pasteur pipettes
- Mixer or blender
- Refractometer

METHOD

Procedures

All materials tested must be in liquid form. Therefore, 5 to 10 ml of juice should be obtained from each fruit species or variety/cultivar. You can use a blender and filter the juice through a sieve if necessary.

When you have all the liquid samples, collect a portion from each with a Pasteur pipette and place one or two drops on the refractometer prism located at one end of the device. Adjust the focus and read, observing the device's display at the end of the prism. The reading is given directly in ° Brix.

Repeat the same procedure for each sample tested. Record the refractometer reading values in Table 1 and analyze them simultaneously at the resolution of the directed study.

Table 1. Vegetable products and their respective soluble solids values in ° Brix.

Product	Refractometer reader (ºBRIX)

FINAL CONSIDERATIONS

After completing this practice, the student is expected to have a clear understanding of the concept of soluble solids and the method and procedures used for their evaluation. Applying knowledge about this parameter in different contexts should also be clear: harvesting, post-harvest quality, and processing. In the first context, which refers to harvesting, the student must understand the importance of soluble solids as a parameter widely used to determine the harvest point of certain fruit species. In a second context, we must understand its implications for post-harvest quality, when values can interfere with the

quality (*i.e.* degree of sweetness) and yield (*i.e.* concentration of solids) of the final product. Finally, also its implications for the industrial processing of vegetable samples, the relationship between the values found and the yield in the food processing industry, and understanding that higher values mean higher income and savings depending on the case.

At the end of the experiment, the student can carry out his or her directed study through the following instructions. What does the soluble solids content represent and how is it assessed in practice? Comment comparatively on the ºBrix values observed in "fresh" juices and processed juices of the same species and answer how other fruits differ from tomatoes when comparing the "fresh" material with the processed material. Know the importance of the soluble solids content in the processed food industry. Know the relationship between the physiological ripening point, the point of harvest, and the soluble solids content of fruits. Sucrose is one of the sugars that form part of the soluble solids and represents a respiratory substrate. In practice, what can be expected in the ºBrix of sugar cane if there is a delay in its processing after harvest?

POSTHARVEST OF CLIMACTERIC AND NON-CLIMATERIC FRUITS

Introduction

Respiration is the primary physiological process involved in postharvest fruit. A maximum respiration rate at the beginning of fruit development and a subsequent reduction as development progresses, as occurs in grapes and oranges, which have this respiratory pattern, are called "non-climacteric" and are harvested at the point of consumption [7]. However, in some fruits, at the end of development, there is an increase in the respiratory rate, i.e., in the production of CO_2 (climacteric), associated with an increase in ethylene synthesis; these fruits are called climacteric fruits. Tomato, banana, mango, and papaya are climacteric fruits that continue the ripening process even after being harvested.

The ripening and shelf life of climacteric fruits are intrinsically linked to factors affecting respiration and ethylene metabolism, with shelf life being extended by inhibiting these processes [8]. Chen*, et al.* [9] complement this by highlighting ethylene's fundamental role as an essential plant hormone in physiological processes such as fruit softening and color changes. Both authors agree that manipulating ethylene, either by its application or inhibition, can control ripening. Kou and Wu [10] add that the traditional classification of fruits into

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

climacteric and non-climacteric, based on ethylene and CO_2 production patterns, may be oversimplified, as some non-climacteric fruits also respond to ethylene.

Furthermore, Chen, *et al.* [9] emphasize that ethylene perception occurs through transmembrane receptors (ETRs) in the endoplasmic reticulum, which act as negative regulators of the ethylene signaling cascade. Kou and Wu [10] also discuss how molecular mechanisms involved in ethylene signaling are conserved across plant species, with genetic studies in tomatoes revealing additional components in these pathways. The variation in ETR gene expression between climacteric and non-climacteric fruits suggests distinct regulatory mechanisms, with climacteric fruits requiring higher ethylene levels to trigger ripening. Paul, *et al.* [11] reinforce this complexity by stating that ethylene sensitivity varies across fruit tissues and developmental stages, driven by interactions with other hormones like auxin.

Additionally, Kou and Wu [10] argue that biochemical parameters such as sugar content, acidity, starch, and pectin levels are critical in fruit ripening beyond ethylene. These aspects are assessed through methods like gas chromatography and spectrophotometry. Alexander and Grierson [12] elaborate on two ethylene production systems: system 1, responsible for basal levels, and system 2, associated with ripening through auto-induction. The transition between these systems remains under investigation, with stress factors like wounding influencing ethylene production. These intricate networks highlight the need for precise analytical methods to distinguish ripening patterns accurately. Moreover, these authors assert that the classical distinctions between climacteric and non-climacteric fruits are increasingly viewed as oversimplified, as some fruits exhibit mixed behaviors depending on genotype or cultivar. Chen, *et al.* [9] support this perspective, noting that alternative signaling pathways may exist, mainly when ETR-CTR interactions are non-essential, as observed in some tomato varieties. Such findings emphasize the necessity for integrated approaches considering ethylene-dependent and ethylene-independent pathways to fully understand ripening mechanisms.

Understanding the physiological and molecular mechanisms governing fruit ripening requires a nuanced perspective incorporating genetic, biochemical, and environmental factors. Kou and Wu [10] stress that integrated approaches considering antioxidant enzyme activity, volatile compound production, and gene regulation are vital for optimizing postharvest quality. Similarly, Chen, *et al.* [9] argue that the traditional binary classification does not adequately capture

the diversity of ripening processes. Therefore, further research is essential to explore these complex regulatory networks and develop advanced postharvest technologies that enhance fruit quality and shelf life.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

If the aqueous phase contains a pH indicator, color changes can indicate variations in CO_2 released. In this method, the fruits are placed in an airtight medium, and during their respiration, the CO_2 released meets the pH indicator, which, after some time, has altered its color. Bromothymol blue is the indicator used and acquires a blue color in an alkaline medium, green in a neutral medium, and yellow in an acidic medium. Therefore, it is possible to qualitatively evaluate respiration and the effect of factors that interfere with respiration by analyzing the time required to change in bromothymol blue under each condition. Thus, this class has the following specific objectives: to qualitatively compare respiratory activity in climacteric and non-climacteric fruits subjected to different temperatures; to correlate respiration with the post-harvest life of climacteric and non-climacteric fruits; to review concepts related to the content.

Materials

- Climacteric fruits such as bananas, tomatoes or avocados in maturation process
- Non-climacteric fruits such as oranges and grapes
- HCL 0.1 M
- NaOH 0.1 M
- Bromothymol blue solution 0.01 g L-1
- Carbonated mineral water
- Containers that can be sealed tightly (for example: glass desiccators or large lids and transparent glasses with lids, capable of holding the fruits)
- Small test tubes
- small glass (3 cm x 1 cm) and transparent
- Micropipettes and pointers
- Crepe tape

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

- Refrigerator or cold chamber

Procedures

Provide four containers (desiccators or large glass) and label the following:

- Container 1: Climacteric fruits + room temperature.
- Container 2: Climacteric fruits + refrigerator or cold room temperature.
- Container 3: Non-climacteric fruits + room temperature.
- Container 4: Non-climacteric fruits + refrigerator or cold room temperature.

Place a tomato and banana in the two climacteric fruit containers and an orange and a bunch of grapes in the two non-climacteric fruit containers. Place 1 mL of bromothymol blue in small bottles and push the bottom of each container with crepe tape to hold them in place. Close the containers and write the time on the lid of the containers with a label. Pack one container of each group (climacteric and non-climacteric) in the refrigerator and one at room temperature. Wait at least 12 hours, note and write down the color changes of the indicator, adopting a system of conventions for the color variations of the indicator solution. Write the results in the results table (table 2).

After that, perform the following procedures using the bromothymol blue indicator:

- Test 1
- Test 2
- Test 3

Following all the data in Table 2.

Test 1

Add a drop of 0.1 M HCl to test tube 1, containing 1 to 2 mL of the indicator, and observe what happens. Write down the change and explain the result.

Test 2

In the same test tube, add 0.1 M NaOH, drop by drop, until a new color changes. Write the change and explain the result.

Test 3

Add a few drops of carbon dioxide-containing water to another test tube containing the same amount of the indicator. Write down the change and explain the result.

Then record all the results in table 2.

Table 2. Table to fill in the values found.

Treatment	Color change	Time lapsed	Observations
Test 1			
Test 2			
Test 3			
Chamber 1			
Chamber 2			
Chamber 3			
Chamber 4			

* Indicate with an initial and final arrow. Eg...: blue → yellow

Analyze and discuss the results concomitantly with the resolution of the study conducted concerning the subject.

Final considerations

The time required to change the color of an indicator solution (bromothymol blue) resulting from the change in the pH of the medium promoted by the CO2 released from the respiration of the fruits was analyzed. With this experiment, the student is expected to understand that the faster color change in the treatment containing climacteric fruits is because these fruits have a peak in respiratory activity at the end of their development; that is, when they are ripening, they have a higher respiratory rate than non-climacteric fruits. The faster color change in fruits is because the respiratory rate is higher at room temperature than in fruits kept refrigerated or cold. It should also be noted that the method of evaluating respiratory activity is qualitative (not quantitative) because the value of the respiratory rate of fruits in any treatment is not known; it is only

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

known that this respiration is higher or lower in a given condition, concerning the other, which could be observed utilizing the time necessary for the alteration in the color of the indicator.

At the end of the experiment, the student can carry out a directed study using the following instructions: Differentiate climacteric fruits from non-climacteric fruits. Report on the importance, in practice, of knowing the climacteric of a fruit. What is the interval of fruit development when you want to know its climacteric? Discuss the results of the practice, making clear the difference between the time necessary for the color change of the indicator substance in containers containing:

a) climacteric fruits, concerning non-climacteric;

b) Fruits kept at room temperature, compared to those kept in the refrigerator

c) What is the role of low temperatures in the post-harvest shelf life of fruits?

EFFECT OF MODIFIED ATMOSPHERE AND MINIMAL PROCESSING ON VEGETABLE PRESERVATION

Minimally processed foods (MPF) are those that, once harvested, are subject to cleaning, washing, sorting, peeling, cutting, and packaging operations. Usually, these are marketed in polystyrene foam trays wrapped in plastic films. The mechanical damage caused by minimal processing promotes increased metabolic processes, such as respiratory activity and ethylene production, leading to loss of quality and reduced shelf life concerning the same unused product. Among the techniques frequently used to prolong the shelf life of MPF is the modified atmosphere (MA), associated or not, with the refrigerated atmosphere, which aims to minimize the intensity of metabolic processes by using conditions that allow the reduction of normal metabolism, avoiding rapid deterioration. Modifying the atmosphere around the plastic films provides a micro atmosphere inside the plastic, with relative humidity and a higher concentration of CO_2 and a lower one of O_2 than the external environment. While increasing relative humidity decreases the transpiration rate and, consequently, water loss, increasing CO_2 concentrations and reducing available O_2 decreases the effects of ethylene, and refrigeration reduces respiratory activity, increasing the shelf life of AMP. The expression "modified atmosphere" refers to the technique promoting altering

the concentration of CO_2 and O_2 gases inside the packaging (relative to that of the external atmosphere) without their specific values being known.

The AMP segment is growing worldwide, mainly due to its practicality, which consumers highly desire. As a result, the undergraduate student needs to know the metabolic processes influenced by the minimal processing of vegetables or fruits and the technologies used to conserve AMP.

The objectives of this class are to evaluate the effect of a modified atmosphere, whether associated with refrigeration, in the preservation of minimally processed vegetables. To relate the technologies associated with preserving minimally processed foods to the post-harvest metabolism of these materials. To understand the use of technologies associated with the preservation of minimally processed foods in the context of marketing the consumption of these foods in Brazil per example.

MATERIALS

- Green cabbage leaves
- Rectangular ice box 18 cm wide by 22 cm long
- PVC film, 12 to 15 μm thick
- Common plastic bags used for refrigerator food storage
- Identification brush
- Identification labels
- Sodium hypochlorite 2%
- Distilled water
- Scale
- Centrifuge
- Cold chamber or refrigerator

Procedures

Wash cabbage leaves in running water just after harvesting so that no organic matter remains due to dirt from the field. Divide into two batches: one for minimal processing (leaves will be chopped) and one for intact (leaves will remain whole). If processing is not possible soon after harvesting, place the

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

leaves on trays with the petiole submerged in water and store for 4 to 8 hours in a refrigerator at 6ºC until processing.

For minimal processing, remove the veins from the leaves and cut into slices 1 to 3 mm thick. Immerse the material from both batches (whole and chopped leaves) in water cooled to 5ºC with ice, containing 200 mg L^{-1} of active chlorine, for 10 minutes. Then rinse in cold water at 5ºC to remove excess chlorine. Dry the whole leaves and centrifuge the minimally processed cabbage for 10 minutes using a small household centrifuge with a speed equivalent to 800 x g. Separate two portions of approximately 100 g of minimally processed cabbage leaves and one portion of approximately three whole leaves.

Procedures

- The number of whole leaves should be sufficient to weigh about 100 g. Subject the portions to the following conditions:
- In the ordinary plastic bag: three whole leaves, which will serve as a reference.
- In the ordinary plastic bag: three chopped leaves.
    - Styrofoam tray 1: Three whole leaves wrapped with plastic film;
    - Styrofoam tray 2: three chopped leaves wrapped with plastic film.
- Weigh and store materials in the refrigerator for seven days. After seven days, visual aspects such as coloration and hydration are evaluated, and the weight is assessed to assess dehydration.
- Analyze and discuss the results concomitantly with the resolution of problems related to the directed study.

FINAL CONSIDERATIONS

It is expected that after the practice, the student will understand the meaning of minimally processed foods (MPF), and modified atmosphere (MA), as a process and technology used in the post-harvest preservation of fruits and vegetables, as well as to realize their interrelations and metabolic and physiological aspects related to post-harvest deterioration that give rise to minimal processing. It is also important to use these technologies in the market context

of the production/consumption relationship of minimally processed foods in a spatial and temporal context.

At the end of the experiment, the student can carry out directed study through the following instructions: Conceptualize the expressions "minimal processing" and "modified atmosphere" and present a relationship between them. Based on the effects of modified atmosphere and minimal processing on vegetables used in the practice, discuss how these techniques influence the post-harvest life of minimally processed foods. Study how atmosphere modification can be effective for conserving marketed cabbage. What are the effects of minimal processing on the postharvest shelf life of a vegetable? Changing the atmosphere extends the shelf life of minimally processed food. How does minimal processing decrease a food's shelf life relative to the same unprocessed food? What is the relationship between ethylene, CO_2, O_2, and modified atmosphere?

REFERENCES

1. Botti, C.; Franck, N.; Prat, L.; Ionnnidis, D. The effect of climatic conditions on fresh fig fruit yield, quality and type of crop. **Acta Hortic** 2003, 605, 37-42.

2. Auler, P.A.M.; Fiori-Tutida, A.C.G.; Scholz, M.B.S. Qualidade industrial e maturação de frutos de laranjeira "valência" sobre seis porta-enxertos. **Rev Bras Frutic** 2009, 31, 1158-1167, doi:10.1590/S0100-29452009000400033.

3. Raupp, D.S.; Gardingo, J.R.; Schebeski, L.S.; Amadeu, C.A.; Borsato, A.V. Processamento de tomate seco de diferentes cultivares. **Acta Amazon** 2009, *39*, 415-422, doi:10.1590/S0044-59672009000200021.

4. Oliveira, M.D.S.; Tosi, H.; Sampaio, A.A.M.; Vieira, P.F.; Santiago, G. Aaliação de duas variedades de cana-de-açúcar submetidas a diferentes tempos de armazenamento. **Pesq Agropec Bras** 2005, 34, 1435-1442.

5. Tian, S.; Liu, W.; Xu, H. Improving the prediction performance of soluble solids content (SSC) in kiwifruit by means of near-infrared spectroscopy using slope/bias correction and calibration updating. **Food Res Int** 2023, 170, 112988, doi:10.1016/j.foodres.2023.112988.

6. Brito, A.A.; Campos, F.; Nascimento, A.R.; Corrêa, G.C.; Silva, F.A.; Teixeira, G.H.A.; Cunha-Júnior, L.C. Determination of soluble solid content in market tomatoes using near-infrared spectroscopy. **Food Control** 2021, 126, 108068, doi:10.1016/j.foodcont.2021.108068.

7. Concha, C.M.; Figueroa, N.E.; Poblete, L.A.; Oñate, F.A.; Schwab, W. Methyl jasmonate treatment induces changes in fruit ripening by modifying the expression of several ripening genes in Fragaria chiloensis fruit. *P***lant Physiol Bioch** 2013, 70, 433-444, doi:10.1016/j.plaphy.2013.05.039.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

8. Alonso-Salinas, R.; López-Miranda, S.; Pérez-López, A.J.; Acosta-Motos, J.R. Strategies to delay ethylene-mediated ripening in climacteric fruits: implications for shelf life extension and postharvest quality. **Horticulturae** 2024, 10, 840, doi:10.3390/horticulturae10080840.

9. Chen, Y.; Grimplet, J.; David, K.; Castellarin, S.D.; Terol, J.; D.C.J., W.; Luo, Z.; Schaffer, R.; Celton, J.M.; Talon, M.; *et al.* Ethylene receptors and related proteins in climacteric and non-climacteric fruits. *Plant Sci* **2018**, *276*, 63-72, doi:10.1016/j.plantsci.2018.07.012.

10. Kou, X.; Wu, M. Characterization of climacteric and non-climacteric fruit ripening. In **Plant Senescence: Methods and Protocols, Methods in Molecular Biology**, vol. 1744, Guo, Y., Ed.; Berlin: Springer Science, Business Media, LLC, 2018; pp. 89-102.

11. Paul, V.; Pandey, R.; Srivastava, G.C. The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—An overview. **J Food Sci Technol** 2012, 49, 1–21, doi:10.1007/s13197-011-0293-4.

12. Alexander, L.; Grierson, D. thylene biosynthesis and action in tomato: a model for climacteric fruit ripening. **J Exp Bot** 2002, 53, 2039–2055, doi:10.1093/jxb/erf072.

CHAPTER IX

HOW TO MEASURE OSMOTIC POTENTIAL ON NON-BIOLOGICAL MATERIAL AND XYLEM TRANSPORT IN THE FLOWER SEGMENT

Prof. Dr. Luis Eliécer Oviedo Zumaque

Prof. Dr. Marcelo F. Pompelli

M.Sc. Samuel Giovanny García-Castaño

10.37885/250619550

ABSTRACT

This material details two practical classes designed to enhance understanding of plant water dynamics through experimental visualization—the first practice employed hydrogel beads to explore the osmotic potential and water movement under varying NaCl concentrations. By observing weight changes in beads, students grasped the relationship between osmotic gradients and water flow, supported by a calibration curve validating the experimental setup. The second practice demonstrated xylem water transport using white roses and dyed solutions. The dye movement through the petals visualized the cohesion-tension mechanism and the role of transpiration, emphasizing the influence of environmental factors such as light intensity. Both practices provided an interactive framework for understanding complex physiological concepts, bridging theory, and observation. Key insights included regulating water balance, the interaction between transpiration and photosynthesis, and the plant's response to osmotic stress. These exercises highlighted plant physiological processes' interconnectedness and ecological significance, offering students a comprehensive learning experience. Integrating these practical methods into plant physiology curricula underscores their value in fostering technical skills and conceptual understanding.

Keywords: environmental adaptation; osmotic gradients; plant water balance; transpiration mechanisms; xylem transport.

HOW TO MEASURE OSMOTIC POTENTIAL ON NON-BIOLOGICAL MATERIAL

Pure water's water potential (Ψ_w) is the quantification of the free energy expressed per unit of pressure (usually expressed in megapascals – MPa). It is based on pure water, the water potential of which is equal to zero. The Ψ_w can be broken down into four other components: osmotic potential, pressure potential, gravitational potential, and matric potential. The osmotic potential represents the effect of dissolved solutes on the free energy of water. In other words, the simple presence of a solute (salts or molecules with hydrophilic groups) in water promotes a rearrangement between the electrical charges of the solute (total or partial charges) and the water, as they disturb the network of hydrogen bonds between water molecules, reducing the free energy.

This practical course includes a complete review of the topics covered in theoretical classes. The sensible course's main objective is to show how water balance and the direction of water flow occur as a function of differences in water potential, varying only the osmotic component of the medium. To this end, students must evaluate the influx and efflux of water in hydrogel beads, generally made of acrylamide and acrylate copolymer. They are placed in different media with decreasing osmotic potentials. The student would come to understand more easily the concept of water balance with the use of beads pre-equilibrated in water ($\Psi_w = 0$; equal to that of distilled water in which they are found) and the visualization of dehydration when placed in a medium with a lower water potential (due merely to the osmotic effect), or even hydration at different speeds when the dehydrated beads are inserted into different NaCl solutions or distilled water. Students will have the opportunity to discuss not only the direction of the flow but also the speed of this flow. This practice is integrated with the objectives of the Plant Physiology course for undergraduate students who would begin to evaluate with more remarkable skill the direction and flow of water in plant cells. The simplicity and observation of this practice are the key to understanding an abstract concept and aim to facilitate the understanding of water balance and relations.

This method can also be used to measure the osmotic potential of a solution with an unknown osmotic potential or even of the cell juice of a plant if it can be extracted, such as the cell juice of a sugar bed, orange juice, or the juice of another fruit that can be prepared without the addition of sugar or water.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Thus, plants with succulent juices or stems are easy to determine, but plants with fruits with a lot of pulp or those from which the cell juice cannot be extracted are not recommended. To do this, you need to have the weight x osmotic potential calibration curve that we will create in the first part and compare this calibration curve with the weight of the gel ball obtained after calibration with the cell juice of unknown osmotic potential.

MAIN GOAL OF THIS TECHNIQUE

Reinforce the concept of water balance;

Verify that water flow depends on the difference in Ψ_w between the different media;

Verify that water flow is always towards the lowest Ψ_w;

Verify that both influx and efflux can occur;

MATERIALS

- Hydrogel beads
- 50 mL beakers
- NaCl solutions (0, 0.125, 0.25; 0.5; 0.75, 1.0; 1.5, 2.0, 3.0, and 5.0 M)
- Distilled or bi-distilled water (better)
- Analytical balance

METHOD

Osmotic potential calibration curve

First, we will prepare 750 mL of a 5 M NaCl solution. We must take 219.19 g of NaCl (preferably analytical grade) and dissolve all the powder in a beaker in a bit of water (400 or 450 mL), preferably using an orbital shaker. Since we are preparing a super concentrated solution, we may need to heat this solution to dissolve all the salt. In this case, we recommend using water at a temperature of ~70ºC or an orbital shaker with heating (preferable).

Once the salt has completely dissolved, transfer the solution to a 500 mL volumetric balloon topped with water. Fill another 250 mL volumetric balloon with distilled water. Afterward, combine both solutions in a 1 L flask, preferably

brown or covered with two layers of aluminum foil. Label this solution as NaCl 5 M. It is recommended that data be added to prepare this solution.

Once we have the 5 M solution and want to prepare the other solutions, we must use a simple dilution formula:

$$M = m_1 \, x \, v_1 = m_2 \, x \, v_2$$

Where M is the molarity of the final concentration, m1 is the molarity of the mother solution, v1 is the volume to be pipetted from the mother solution, m2 is the desired molarity, and v2 is the desired volume. To exemplify, we will prepare 200 mL of a 1 M NaCl solution:

$$5 \cancel{M} \, x \, v_1 = 1 \cancel{M} \, x \, 200 \, mL$$

$$v_1 = \frac{200 \, mL}{5}$$

$$v_1 = 40 \, mL$$

We must pipette 40 mL of the 5 M stock solution to make a 1 M NaCl solution. Then pipette 40 mL of 5 M solution directly in a newly graduated balloon and then complete with 160 mL of distilled water. To do other solution follow the Table 1.

Table 1. How to prepare all NaCl concentrations.

Molarity	Mother solution (mL)	Water (mL)
5	200	0
3	120	80
2	80	120
1.5	60	140
1	40	160
0.75	30	170
0.5	20	180
0.25	10	190
0.125	5	195
0	0	200

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Once you have labeled all solutions, separate four 50 mL beakers for each NaCl solution and fill them with the appropriate osmotic solutions as previously prepared. It is recommended that the beakers also be labeled (Fig. 1).

Figure 1. The hydrogel beads are preferably monochromatic; however, if colored, try to join all the same colors of hydrogel beads in each Becker.

Separate 40 sets of hydrogel beads, four sets for each saline solution. Each set should contain five beads. It is not recommended to insert more than five, as the solution may not be enough to inflate all five beads or may be too much at higher concentrations.

Regardless of osmotic concentration, each set of 5 balls must be weighed on an analytical balance. Their weight and the type of beaker they will be placed in should be noted in the laboratory notebook.

After weighing the 40 sets of 5 balls, insert the balls into their respective solutions and leave them in the solution for long enough to reach equilibrium with the solution. The recommended time is between 1.5 and 2 hours at 25ºC. This time can be reduced to higher temperatures and increased to lower temperatures. The ambient temperature usually influences this imbibition process, so it should be carried out in a germination chamber or a location with a temperature adjusted and calibrated to 25ºC.

During this time, the differential absorption of water by the balls at different osmotic concentrations will be observed. At the end of this time, the balls should be removed from the beaker carefully so as not to burst. The balls become very slippery during this process, so a laboratory spoon would be helpful in this process. Remove each set of balls, dry them lightly, without removing the water from the outside of the balls, and weigh the sets again. The laboratory notebook should correctly note the initial and final weight values.

Each osmotic concentration prepared with NaCl must have its osmotic potential calculated. To do so, use the following formula:

$$\Psi_s = -RTCi$$

Where R is the perfect gas constant (0.00820574587 L MPa mol^{-1} K^{-1}), T is the absolute temperature in Kelvin or K (ºC + 273.15), C is the solute concentration of the solution (mol L^{-1}), and i is the solute dissociation coefficient (*e.g.* NaCl = 1.8; sucrose = 1; $CaCl_2$ = 2.4, and KCl = 1.8). Thus, let's complete Table 1 with the respective osmotic potential of all NaCl solutions.

Table 2. All NaCl concentrations and their theoretical[1] osmotic potential.

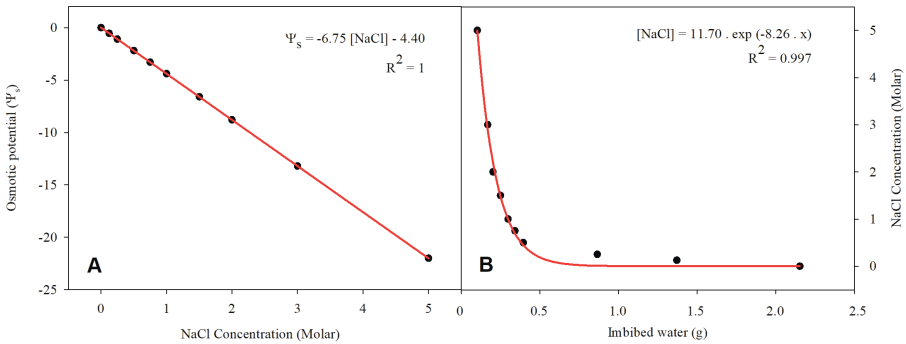
Molarity (C)	Mother solution (5 Molar; mL)	Water (mL)	R	T	i	Ψ_s
5	200	0	0.008206	298.15	1.8	-22 MPa
3	120	80	0.008206	298.15	1.8	-13.2 MPa
2	80	120	0.008206	298.15	1.8	-8.8 MPa
1.5	60	140	0.008206	298.15	1.8	-6.6 MPa
1	40	160	0.008206	298.15	1.8	-4.4 MPa
0.75	30	170	0.008206	298.15	1.8	-3.3 MPa
0.5	20	180	0.008206	298.15	1.8	-2.2 MPa
0.25	10	190	0.008206	298.15	1.8	-1.1 MPa
0.125	5	195	0.008206	298.15	1.8	-0.55 MPa
0	0	200	0.008206	298.15	1.8	0 MPa

To check if the calibration was good, plot the molar concentration on the x-axis and the osmotic potential on the y-axis and draw a trend line that should be 1 or very close to 1 (Fig. 2A)

[1] Theoretical because in nature, other features would be influenced by the osmotic potential, and here, we don't use the true constant of perfect gases nor exact temperature. To do it, please take place an osmometer, an expensive laboratory equipment.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

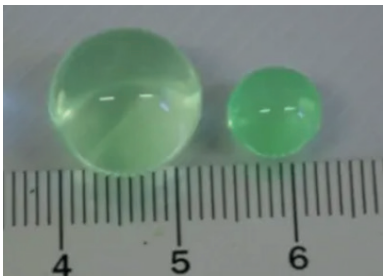
Figure 2. Calibration curve with NaCl and osmotic potential.



Now calculate the amount of water absorbed in each set of balls, weighing the final weight subtracted from the initial weight. Please do this for the four repetitions, calculate an average and a standard deviation, and plot the values of water absorbed with its equivalent NaCl concentration, as shown in Figure 2B.

In this experiment, we analyzed that the more concentrated the saline solution, the less water the hydrogel beads absorb (Fig. 3), and the lower the final weight in water when discounted from the initial weight.

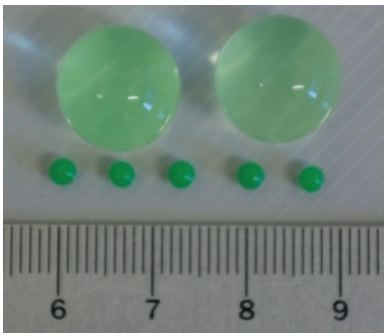
Figure 3. The hydrogel beads were calibrated with 0 M and 2 M NaCl.



In addition to checking the role of the negative pressure potential in the hydration of the hydrogel beads, we will do the opposite. To do this, we will need hydrogel beads left to hydrate for at least 3 to 4 hours in distilled water to ensure that they are super-filled and cannot increase in volume due to the negative pressure potential. Then, we will insert some of these super-filled beads into the most different osmotic solutions and their osmotic potentials calculated to see, after a while, how the cell, represented here by the hydrogel beads, loses water to the circulating medium, if the latter has a lower osmotic potential than the osmotic potential of the beads.

First, take the beads, weigh them, and measure their diameter. For this step, a caliper would be great, but a ruler is also fine. Then, do the same procedure after 2 hours, when the "cells" stabilize with the surrounding medium having an osmotic potential more negative than the osmotic potential of the "cells" (Fig. 4).

Figure 4. The hydrogel beads were calibrated with 0 M NaCl (above) and after calibrated with 5 M (lower) NaCl.



DISCUSSION

The results presented in this first classroom effectively demonstrate the principles of osmotic potential and water movement using hydrogel beads, a practical and visually intuitive model system. This experiment illustrates how osmotic gradients influence water flow, simplifying understanding of fundamental physiological concepts such as water balance and regulation. Hydrogel beads, initially equilibrated in distilled water ($\Psi_w = 0$), were immersed in solutions of varying NaCl concentrations. Consistent with established plant physiology principles, water moves from regions of higher water potential to lower water potential [1]. The beads lost water in solutions with more negative osmotic potentials, reflected in their reduced weight. Beads in lower-concentration solutions (*e.g.*, 0.125 M NaCl) retained most of their water, while those in higher concentrations (*e.g.*, 5 M NaCl) exhibited significant dehydration. This direct relationship between solute concentration and water movement aligns with theoretical models and demonstrates the practical application of osmotic potential.

The calibration curve generated during the experiment confirmed the linear relationship between NaCl concentration and osmotic potential, with an R^2 value near 1. This finding supports the validity of the experimental setup and its ability to model osmotic processes accurately [1]. Additionally, transferring fully hydrated beads into more negative osmotic solutions demonstrated water efflux, showing

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

how equilibrium is achieved when osmotic potential differences exist between the beads and the surrounding solution. These results have broader implications for understanding plant responses to osmotic stress. In saline environments, for example, reduced soil water potential limits plant water uptake, challenging their ability to maintain hydration and turgor pressure [2]. The behavior of the hydrogel beads under varying osmotic conditions mirrors how plant cells respond to similar stresses, offering insights into water regulation mechanisms. This understanding is vital for addressing issues like irrigation efficiency and crop performance in saline soils [3].

From an educational perspective, using hydrogel beads provides a simple and effective way to visualize osmotic principles. Their response to osmotic gradients is a tangible model for water movement in plant cells, bridging theoretical concepts and hands-on learning. This approach enhances engagement and facilitates comprehension of complex physiological processes [4]. Summarizing, this practice highlights the essential role of osmotic potential in determining water movement. By employing hydrogel beads, the experiment provides a clear and accessible demonstration of water dynamics, reinforcing theoretical principles and their practical applications in plant physiology and environmental management.

WATER TRANSPORT IN XYLEM

Water moves through the soil-plant-atmosphere continuum in a dynamic process governed by transpiration, a critical mechanism in plant physiology. Transpiration entails water vapor loss from plant surfaces, primarily through the stomata, which are small openings on the leaves. This process is driven by a gradient in pressure potential between the moist air spaces within the leaves and the relatively drier atmospheric conditions outside. As water vapor exits the leaf through the stomata, it generates a pull or tension that draws water upward from the soil, through the roots, and into the plant's vascular tissues, specifically the xylem. The phenomenon of transpiration serves multiple indispensable biological functions. Firstly, it facilitates the upward transport of water, ensuring that essential nutrients dissolved in the soil solution are distributed to various parts of the plant, including leaves, stems, and fruits. Secondly, transpiration contributes to thermoregulation by reducing leaf temperature through evaporative cooling. This cooling effect is crucial for preventing heat stress,

which can adversely impact photosynthetic efficiency and overall plant health. Thirdly, transpiration supports the maintenance of turgor pressure within cells, essential for structural support and stomata opening, thus enabling the gas exchange necessary for photosynthesis.

These two processes are closely interrelated due to the intricate connection between transpiration and photosynthesis. During photosynthesis, stomata must open to allow the intake of carbon dioxide (CO_2), a key substrate for sugar production. However, this opening also facilitates water loss through transpiration. The balance between water conservation and CO_2 uptake is finely regulated by the plant, depending on environmental conditions such as light intensity, temperature, humidity, and soil moisture availability.

This lesson aims to visually demonstrate the movement of water within the xylem, highlighting the process of cuticular transpiration, which occurs directly through the plant's cuticle—a waxy layer on the leaf surface. Although cuticular transpiration constitutes a smaller fraction of total water loss than stomatal transpiration, it underscores the multifaceted pathways through which water dynamics operate within plants. Furthermore, understanding the mechanisms underlying transpiration has profound implications for agricultural practices and ecological sustainability. By studying how plants adapt to water scarcity and regulate transpiration under varying environmental conditions, researchers and agronomists can devise strategies to enhance crop resilience to drought. Insights into the soil-plant-atmosphere continuum directly inform innovations such as breeding for water-use efficiency and optimizing irrigation schedules. Then, transpiration is not merely a process of water loss; it is an intricate physiological phenomenon integral to plant survival and productivity. This visual exploration of xylem water movement will provide a deeper appreciation of the interconnected processes sustaining life in the plant kingdom, emphasizing the critical role of water in supporting both plant growth and ecological balance.

MAIN GOAL OF THIS TECHNIQUE

Understand the Process and Mechanisms of Transpiration

Examine the Interrelationship Between Transpiration and Photosynthesis

Apply Knowledge to Agricultural and Ecological Contexts

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

MATERIALS

- White rose
- Glass beaker
- Scissors
- Distilled water

Blue food coloring or other contrasting color, except green

METHODS

Place a drop of food coloring in a 25 mL test tube and fill it with distilled water (Fig. 5). Using dyes with stronger tones, such as blue, violet, or red is advisable. This makes it easier to see the color of the petals. Since this practice is quite simple, it is possible to make several color combinations to obtain more varied results.

Figure 5. Some materials used in this classroom.

Using scissors, make a cross-sectional cut in the stem of two white roses (it can also be another type of white flower), approximately 10 cm from the corolla (Fig. 6). The cut should be made inside a beaker with distilled water to prevent air from entering the xylem vessels.

Figure 6. Some materials used in this classroom.

Quickly transfer the rose to the test tube with dye and observe the petals' color change. If possible, expose the plant material to sunlight so that the transpiration process is faster. The color change can be observed after 30 minutes (Fig. 7).

Figure 7. Rose was inserted into a test tube with food coloring.

Over time, you will verify a change in the color of the rose petals, depending on the color of the dye. This phenomenon occurs due to the petals' cuticular transpiration, which shows water transport through the xylem vessels. It is important to note that the color change in flowers with their leaves on will be slower.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

Figure 8. Difference between a flower placed in water and another placed in a solution with dye.

DISCUSSION

The results from these classroom illustrations and analyses provide a vivid demonstration of water transport through the xylem and the process of transpiration in plants. Using white roses and colored dyes, the experiment underscores how water, carrying dissolved substances, moves upward through the plant's vascular system and highlights its significance in plant physiology. When the stems of white roses were placed in test tubes containing distilled water mixed with contrasting dyes, a gradual color change in the petals was observed. This phenomenon visually confirmed the functionality of the xylem, the plant's specialized tissue responsible for water transport. Water absorption begins at the cut end of the stem. It moves upward through the xylem vessels due to the cohesion-tension mechanism, a principle well-documented in plant physiology literature [5]. As water travels, it carries the dye, depositing it in the petals, making the abstract concept of water transport directly observable.

This experiment also demonstrates transpiration's critical role in driving water movement. Transpiration creates a negative pressure potential in the leaves due to water vapor loss through the stomata, which is the pulling force for water uptake from the solution. This process is influenced by environmental conditions such as light intensity, temperature, and humidity, which regulate stomatal opening and water flow [6]. Roses exposed to sunlight exhibited faster color changes, correlating with increased transpiration rates caused by higher light intensity and temperature. The petals' color intensity intensified over time, illustrating water flow and uptake dynamics. Initially faint, the color deepened as water carrying the dye continued to reach the petals. This progressive change aligns with the understanding that water uptake and transport rate vary with the plant's physiological state and external conditions [7].

Furthermore, the comparison between flowers in plain water and those in dyed solutions highlighted the use of dye as a non-inhibitory visual tracer for water movement. The dyed solutions did not alter the xylem's functionality, demonstrating that water and solutes are efficiently transported without obstruction. This confirms findings in earlier studies that solutes at low concentrations, such as those in food dyes, do not significantly impact xylem flow [8].

Cuticular transpiration was another important aspect demonstrated in this experiment. While stomatal transpiration is the primary driver of water movement, the petals' gradual color change suggests a secondary water loss pathway through the cuticle. Cuticular transpiration contributes to the overall water balance in plant tissues, particularly in conditions where stomata remain closed [2]. The practical implications of these observations are profound. Understanding the mechanisms of xylem transport and transpiration informs agricultural and horticultural practices, particularly in water management and stress mitigation strategies. For instance, water uptake efficiency under varying conditions can guide irrigation techniques, optimizing water use in drought-prone areas [9]. Additionally, visualizing how environmental stressors like light and humidity influence water transport helps refine controlled-environment agriculture systems, such as greenhouses, to enhance crop productivity.

This practice also serves as an invaluable educational tool. It bridges theoretical concepts of plant water relations with hands-on experimentation, allowing students to observe and analyze these processes directly. The use of accessible materials, such as white roses and food coloring, makes this experiment easily replicable in educational settings, offering a tangible way to understand complex physiological processes. Also, practice 2 provides a compelling visual and experimental demonstration of plants' water transport and transpiration mechanisms. By tracing the movement of dye through the xylem and observing its deposition in the petals, this exercise reinforces key physiological principles and their practical applications. The findings align with established scientific understanding, highlighting the elegance and efficiency of plant systems. This practice enhances student engagement and deepens their comprehension of the interconnected processes governing plant water dynamics.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

CONCLUSIONS

The two practical classes on plant physiology effectively demonstrated critical processes essential for understanding plant water dynamics. The first practice illustrated osmotic potential and water movement using hydrogel beads as a model. The experiment showcased the direct relationship between solute concentration, osmotic potential, and water flow by immersing beads in NaCl solutions of varying concentrations. This hands-on approach facilitated comprehension of fundamental concepts such as water balance, osmotic gradients, and their role in water movement. The linear relationship observed in the calibration curve further validated the experimental setup, highlighting its educational and practical relevance for understanding osmotic stress and plant water regulation.

The second practice focused on water transport through the xylem using white roses and dyed solutions. The observed color changes in the petals visualized the cohesion-tension mechanism and the role of transpiration in driving water upward through the plant. Environmental factors such as light intensity were shown to influence transpiration rates, reinforcing the interconnectedness of physiological processes like transpiration and photosynthesis. Including cuticular transpiration as a secondary water loss pathway added depth to understanding water dynamics in plant tissues.

These practical classes combined theoretical knowledge with experimental visualization, enabling students to engage with complex physiological concepts tangibly. The methodologies and observations provided valuable insights into plant adaptation to environmental challenges, including osmotic stress and water scarcity. These practices underscore the significance of water dynamics in plant growth and survival, fostering a deeper appreciation of plant physiological processes and their ecological implications.

DIRECT STUDY

How does the concentration of NaCl in the solution affect the weight of hydrogel beads, and what does this demonstrate about osmotic potential?

Expected Answer: Higher NaCl concentrations lead to greater water loss from hydrogel beads, reducing their weight. This demonstrates that water moves from areas of higher water potential (inside the beads) to areas of lower water potential (outside in the saline solution), following the osmotic gradient.

Why is the calibration curve between NaCl concentration and osmotic potential important in this experiment?

Expected Answer: The calibration curve validates the relationship between solute concentration and osmotic potential, ensuring experimental accuracy. A linear correlation confirms theoretical predictions, allowing for precise quantification of unknown osmotic potentials based on observed water movement.

How does the dye movement in white roses demonstrate the cohesion-tension mechanism in xylem?

Expected Answer: The dye moves upward through the xylem vessels due to the cohesion-tension mechanism, where water molecules adhere to xylem walls and to each other, creating a continuous column. This movement is driven by transpiration, which creates a negative pressure gradient.

What role does light exposure play in the rate of color change in the petals, and what does this indicate about transpiration?

Expected Answer: Light exposure accelerates the rate of color change in the petals, indicating an increase in transpiration. Light stimulates stomatal opening, increasing water vapor loss and enhancing the upward pull of water and dissolved dye through the xylem.

REFERENCES

1. Holbrook, N.M. Water and Plant Cells. In **Plant Physiology and Development, 6th edition**, Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., Eds.; Sinauer Associates Inc.: Oxford, 2015; pp. 83-98.

2. Kerstiens, G. Water transport in plants: Mechanisms of control under environmental stress. In **Handbook of photosynthesis**, Pessarakli, M., Ed.; CRC Press: New York, 2006; pp. 545–561.

3. Chaves, M.M.; Pereira, J.S.; Maroco, J.; Rodrigues, M.L.; Ricardo, C.P.P.; Osorio, M.L.; Carvalho, I.; Faria, T.; Pinheiro, C. How plants cope with water stress in the field? Photosynthesis and growth. **Ann Bot 2002**, *89*, 907-916.

4. Salisbury, F.B.; Ross, C.W. **Plant physiology**; Wadsworth Publishing: Belmont, CA, 1992.

5. Assmann, S.M. Solute transport. In **Plant Physiology, 5th edition**, Taiz, L., Zeiger, E., Eds.; Sinauer Associates Inc.: Sunderland, 2010; pp. 131-159.

ISBN 978-65-5360-838-2 - www.editoracientifica.com.br

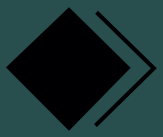
6. Hopkins, W.G.; Hüner, N.P.A. **Introduction to plant physiology**; John Wiley & Sons: London, 2004.

7. Nobel, P.S. **Physicochemical and environmental plant physiology**; Academic Press: New York, 2009.

8. Dunford, S. Translocation in the Phloem. In **Plant Physiology 5th edition**, Taiz, L., Zeiger, E., Eds.; Sinauer Associates Inc.: Sunderland, MA, USA, 2010; pp. 271-303.

9. Cernusak, L.A. Gas exchange and water-use efficiency in plant canopies. **Plant Biol** 2020, 22, 52-67, doi:10.1111/plb.12939.

INDEX



científica digital

ISBN 978-655360838-2