BOOK OF ABSTRACTS

ProCardo Final Symposium
Cultivating Sustainable Futures in a Changing Climate

4 de Setembro de 2026, Vairão, Portugal

Funded by:

GreenUPorto (UID/PRR/05748/2025) is funded by FCT and PRR (https://doi.org/10.54499/UID/05748/2025; https://doi.org/10.54499/UID/PRR/05748/2025)

Welcome & Committee

It is with great pleasure that we welcome all participants to this symposium, dedicated to the closing of the ProCardo project. Over the past years, this project has brought together the teams from GreenUPorto, CEBAL, and UPV in a joint effort to understand the impact of climate change on the cardoon flower and, consequently, on the production of Queijo da Serra da Estrela DOP — one of the most emblematic products of Portugal's agri-food heritage.

This symposium's main goal is to present and discuss the results achieved by ProCardo, sharing the knowledge generated with the scientific community and other stakeholders interested in the subject. More than closing a project, we aim to open a space for dialogue: for this reason, this is also a moment to connect with other researchers working on climate change and its impact on crops, contributing to a broader and more enriching debate.

We thank all the authors, speakers, and participants for their contribution to this book of abstracts, and we wish everyone a productive and inspiring symposium.

Scientific Committee
Cláudia Pereira, GreenUPorto, INOV4AGRO
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Fátima Duarte, CEBAL/MED/CHANGE
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Abstracts Invited Speakers

Does climate change affect the expression of cardosins and cheese quality?

Miguel Sampaio[1], Ana F. Paulino[2], João Neves[1,3], João Monteiro[1], Idália Guerreiro[4], Justino Sobreiro[4], José Pissarra[1], Paulo Barracosa[5], Maria F. Duarte[2], Cláudia Pereira[1]

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Cynara cardunculus (cardoon) flower extracts are traditionally used as a natural coagulant in the production of Serra da Estrela cheese, with their milk-clotting activity mainly attributed to the aspartic proteases cardosin A (CdA) and cardosin B (CdB). As environmental conditions may affect plant physiology and protease expression, climate change could influence the availability and functionality of these enzymes, potentially affecting cheese production. Here, we investigated the response of the 3M cardoon ecotype to water stress, elevated temperature and combined heat and water stress, focusing on plant performance, flower composition and cardosin expression. CdA and CdB expression was analysed by RT-qPCR in different floral structures and developmental stages, and protein accumulation was assessed by Western blot. Flower extracts obtained under control and water-stress conditions were also used for Serra da Estrela DOP cheese production. Water stress affected plant growth, with a significant reduction in leaf dry weight and a tendency towards lower biomass and reproductive development. Cardosin expression was strongly dependent on stress condition and flower developmental stage. CdA expression increased under water stress in closed inflorescences, while reaching its highest levels under combined heat and water stress. CdB showed a similar induction under combined stress but was significantly reduced by water stress alone, highlighting a differential response to temperature and water availability. Both cardosins were detected in their active forms. Flower extracts from control and water-stressed plants also resulted in differences in curd characteristics and cheese yield, although no significant differences were detected in the final physicochemical properties of the cheeses. Overall, these results demonstrate that environmental stress can modify cardoon growth and cardosin expression, with potential consequences for the consistency of cardoon-based cheese production under future climate scenarios.

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Exploring Plant Science, Sustainability and Innovation

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A viticultura enfrenta desafios crescentes decorrentes dos efeitos das alterações climáticas, num contexto em que a necessidade de reduzir a utilização de produtos químicos convencionais exige o desenvolvimento de sistemas de produção mais sustentáveis e resilientes. Neste cenário, a ciência da biologia vegetal assume um papel fundamental na transformação do conhecimento sobre as respostas das plantas em soluções inovadoras para a gestão da vinha.

Neste sentido, serão abordadas estratégias sustentáveis destinadas a aumentar a resiliência da videira fomentando, sempre que possível a economia circular. Particular destaque será dado à utilização da luz UV-C como uma abordagem inovadora de proteção da cultura. Combinando efeitos diretos sobre os agentes patogénicos com a indução dos mecanismos de defesa da planta, a aplicação precisa de UV-C está a ser estudada como uma potencial alternativa à utilização de pesticidas convencionais. A investigação integra ensaios laboratoriais, em estufa e em campo, visando otimizar a dose e o momento de aplicação e avaliar os seus efeitos no desempenho fisiológico da videira, na incidência e severidade de doenças, nos organismos não-alvo, na produtividade e na qualidade da uva.

Complementarmente, outra estratégia visa explora a valorização da lenha de poda da videira numa perspetiva de economia circular, investigando a sua utilização como recurso renovável para o desenvolvimento de produtos de valor acrescentado destinados à própria vinha, incluindo materiais biodegradáveis para controlo de infestantes e proteção das plantas, bioestimulantes obtidos a partir de compostos bioativos e biochar para melhorar a saúde do solo.

Keywords: Alterações climáticas, sustentabilidade, viticultura, fisiologia vegetal

Chestnut tree for the future: the potential of stress priming and mycorrhization

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Over the past years, climate change has emerged as a major threat to chestnut production, with increasing drought frequency and intensity, together with recurrent heat episodes, challenging the species' capacity to maintain growth and productivity. Despite its ecological plasticity, the response of chestnut to abiotic stress, particularly under combined stress conditions, remains insufficiently understood. The CC&NUTS project, funded by Fundação "la Caixa"/BPI & FCT, addresses this challenge by investigating (i) the responses of different chestnut genotypes to drought and high temperature and (ii) sustainable strategies to enhance plant resilience under climate change.

Within this framework, both ectomycorrhization and stress priming have shown promising potential. Mycorrhization with autochthonous fungi enhanced plant water status and strengthened antioxidant responses, contributing to improved acclimation to heat and drought. Stress priming, in turn, induced substantial hormonal and proteomic adjustments that helped preserve key physiological and metabolic processes during subsequent stress, ultimately reducing plant sensitivity. Importantly, the first year of field trials provided promising evidence of the effectiveness of priming under realistic environmental conditions. Primed plants under stress showed improved photosynthetic performance and growth, as well as enhanced reproductive and productivity-related traits, including flower and burr production. These findings support the potential of stress priming and mycorrhization as complementary and sustainable approaches to enhance chestnut resilience, helping to maintain plant performance and productivity under increasingly challenging climatic conditions.

Plant Biotechnology for Climate Resilience: Selected Case Studies

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Climate change is reshaping the environmental conditions in which crops and forest species grow, with drought and heat becoming increasingly important constraints, particularly in Mediterranean regions. Plant biotechnology can contribute to climate resilience not only by accelerating propagation and breeding, but also by revealing, creating and exploiting adaptive variation. This presentation highlights three complementary case studies illustrating these opportunities.
The strawberry tree (Arbutus unedo L.) is a relict element of the pre-glacial laurel forest flora and is widely distributed throughout Portugal across contrasting edaphoclimatic conditions. This diversity provides a valuable framework for analysing intraspecific variation in drought response. Studies with contrasting genotypes revealed marked differences in photosynthetic performance, recovery capacity, metabolomic profiles and chloroplast gene expression, demonstrating the importance of genotype in determining drought resilience and supporting the use of physiological and molecular traits for selection.
Tamarillo (Solanum betaceum Cav.), an Andean fruit crop with potential for diversification under Portuguese conditions, illustrates how biotechnology can also generate novel variation. Micropropagated tetraploid plants displayed improved water status and a generally better performance under water deficit than diploid plants, suggesting that induced polyploidy may provide useful material for breeding under increasingly water-limited environments.
In conifers, somatic embryogenesis provides both a propagation system and a powerful experimental model for studying stress responses. In Pinus halepensis, a Mediterranean species well adapted to drought and high temperatures, heat treatments applied during embryogenic induction triggered substantial proteomic and metabolic reprogramming. Related work in P. radiata further indicates that environmental conditions imposed during early embryogenesis can induce long-lasting molecular and physiological effects, supporting the concept of stress priming and memory.
Together, these examples show that plant biotechnology can act at different levels, from identifying naturally resilient genotypes to creating new variation and conditioning stress responses, thereby contributing to the development of plant material better prepared for future climatic constraints.

Keywords: drought stress, heat stress, polyploidy, somatic embryogenesis, stress priming

PrOryza: development of thermotolerant Luso rice – genetic and reproductive analysis

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Lourenço Palha[3], Cátia Soares[3,4], Ana Sofia Almeida[3], João Palhavã[5], Ana Marta Pereira[1]

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Climate change poses a major challenge to rice production, particularly in regions increasingly affected by heat stress. In Portugal, where rice consumption exceeds domestic production, increasing the resilience and productivity of locally cultivated varieties is essential to strengthen the sustainability of the rice sector. The PrOryza project aims to contribute to the genetic improvement of Portuguese rice (Oryza sativa L.) varieties by identifying and incorporating traits associated with heat tolerance while maintaining high productivity, with a particular focus on the Central Alentejo region (Vendas Novas & Montemor-o-Novo). The project combines the evaluation of reproductive performance, agronomic productivity, and genetic responses of five Portuguese-grown rice varieties under contrasting environmental conditions. During the first year, controlled-environment and field experiments assessed the effects of high temperature and different irrigation regimes, including conventional flooding and sprinkler irrigation, a more water-efficient strategy that may impose additional environmental stress. We evaluated reproductive traits associated with pollen tube growth, fertilisation, and grain production, and identified and analysed the expression of candidate genes previously associated with heat stress tolerance.
The results obtained so far are promising, revealing substantial variation among genotypes in their response to both heat stress and water-management conditions. Caravela and OP2115 displayed consistently positive responses, combining reproductive resilience under stressful conditions with favourable agronomic performance. These genotypes therefore represent promising candidates for further validation and for the integration of heat-tolerance markers into breeding programmes. Overall, PrOryza highlights the potential of combining phenotypic and genetic approaches to identify resilient rice genotypes adapted to the increasingly challenging climatic conditions of Portuguese rice-growing regions. The knowledge generated will support the development of more climate-resilient and productive varieties and contribute to the long-term sustainability of Portuguese rice production.

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Olive4Cereal - valorising an olive mill by-product as a biostimulant to enhance drought resilience in cereals

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The olive oil industry generates large amounts of by-products whose inadequate management can cause significant environmental impacts, while their chemical and nutritional composition offers considerable potential for agricultural valorisation. Olive4Cereal aims to transform concentrated olive mill wastewater, an underutilised by-product of olive pomace processing, into an innovative plant biostimulant to enhance drought resilience in cereals in the Alentejo region of Portugal.
After formulation development, the biostimulant will be characterised to assess its safety and gain insights into its potential mode of action. Its biostimulant activity will then be evaluated in wheat and barley under controlled drought conditions, focusing on plant growth and key physiological and biochemical responses. The results obtained under controlled conditions will subsequently be validated under field conditions in the Alentejo, complemented by the assessment of soil physicochemical properties and microbiome composition. Knowledge transfer and dissemination will be carried out throughout the project, engaging researchers, farmers, producers and other agricultural stakeholders, supporting the transfer of project outcomes into sustainable agricultural practices.
Olive4Cereal brings together complementary expertise in olive by-product processing and valorisation, plant stress physiology, and microbial ecology through a multidisciplinary consortium comprising researcher teams from UPorto, CEBAL and UMinho. By valorising an underutilised olive by-product to enhance cereal drought resilience and improve soil health, the project aims to contribute to more circular, resource-efficient and climate-resilient agricultural systems, particularly in Mediterranean regions.

Acknowledgments
The authors acknowledge the support provided by the Promove Program, an initiative of the “la Caixa” Foundation in partnership with BPI, through the Olive4Cereal research project (PL25-00001). Additional funding for this study was received from national sources via FCT, under the projects UID/05748/2025 and UID/PRR/05748/2025 (https://doi.org/10.54499/UID/05748/2025; https://doi.org/10.54499/UID/PRR/05748/2025; GreenUPorto).

Decoding Tomato Responses to Silver: From Plant Stress Physiology to Crop Biotechnology

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The increasing use of silver in modern technologies, medicine, agriculture, and consumer products has contributed to its release into the environment, where it can accumulate in soils and negatively affect plant growth and development. Unlike essential potentially toxic elements such as copper, silver has no known beneficial biological function in plants. This study aimed to assess the effects of silver exposure on the growth and stress-related biochemical responses of *Solanum lycopersicum* Micro-Tom plants. Plants were grown for approximately 18 days on Murashige and Skoog medium before being transferred to a vermiculite/perlite substrate and exposed for 21 days to 0, 25, or 50 mg/L Ag supplied as silver nitrate in Hoagland nutrient solution. Plant biometric parameters and leaf biochemical markers associated with photosynthesis, oxidative stress, and antioxidant defence were subsequently evaluated.

Silver exposure significantly reduced total plant biomass, shoot and root growth, as well as chlorophyll a and b contents. The photoprotective pigments β-carotene and lutein also decreased with increasing silver concentrations. In contrast, hydrogen peroxide levels increased, reaching statistical significance at 50 mg/L Ag, indicating enhanced oxidative stress. Despite this increase, malondialdehyde levels decreased at the highest concentration, suggesting that lipid peroxidation and membrane damage were effectively limited. This response was accompanied by an increase in total thiols at 25 mg/L Ag and, notably, an increase in reduced glutathione at both concentrations. Conversely, proline levels decreased with increasing silver exposure.

Overall, silver negatively affected tomato growth and photosynthetic pigments while triggering oxidative stress. The increase in glutathione and thiol levels, together with reduced malondialdehyde accumulation, suggests that antioxidant defences, particularly the glutathione system, contributed to limiting oxidative damage. These findings highlight the potential of biotechnology to enhance metal tolerance through the manipulation of antioxidant and glutathione biosynthetic pathways.

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Propagation and intelligent conservation of Quercus lusitanica

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Quercus lusitanica (carvaliça) is a shrubby oak native to Portugal that plays a critical role in post-fire recovery of Atlantic forests, due to its ability to resprout through its unique rhizome formation, a rare trait in woody dicots. However, climate change threatens its survival, and efficient propagation methods are lacking so in this sense understanding this structure and developing reliable propagation methods are essential for restoration efforts. Moreover, there is very little information available about this species biology. Currently, a genomic sequencing effort has being carried out to characterize the populations genetic diversity, covering a large part of the species' distribution across Portugal. The project aims to (1) optimize vegetative propagation protocols; (2) describe the morpho-anatomy of the species; and (3) participate in the genetic diversity characterization effort.
Additional objectives include: Promoting the species’ use in urban and rural landscaping; Leveraging its charismatic appeal to engage students, the public, and policymakers in nature conservation action.

Female Signalling in the Transmitting Tract: Insights from Arabidopsis to Tomato

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Fruits and seeds, which sustain the global food supply, arise from plant reproduction, one of the most heat-sensitive stages of plant development. Rising global temperatures threaten fertilization efficiency, crop yield and food security [1]. The reproductive process begins when a pollen grain (PG) lands on a stigma, and germinates a pollen tube (PT) which grows along the pistil tissues towards the ovule[2]. Most PT growth occurs along the transmitting tract (TT) a female tissue deeply embedded in the pistil. PT-pistil interactions are extensively studied, but a significant gap remains: how the TT contributes for this dialogue and how this translates to crops with more complex reproductive architectures. Tomato *(Solanum lycopersicum*) has emerged as one of the most valuable model crop species with increasing economic and nutritional interest as well as extensive genetic resources [3]. However, unlike Arabidopsis, where the PT travels a comparatively short distance to reach the ovules, tomato pistils are considerably longer, requiring the PT to complete a larger distance and more sustained journey through the TT before fertilization can successfully occur. This anatomical difference raises the question of whether TT-derived signals existent in Arabidopsis are conserved in crop species with different reproductive structures, such as tomato. To address this, the *ntt* (*no transmitting tract*) Arabidopsis mutant was used as a genetic tool to dissect TT-specific signalling pathways [4,5], and RNA-seq was performed on wild-type (WT) and *ntt* pistils to identify differentially expressed genes (DEGs) associated with TT function. To begin translating this knowledge to a crop species, an *in silico* analysis identified the candidate *Solyc11g062060* as a putative NTT ortholog in tomato, to be functionally validated using CRISPR/Cas9.

Acknowledgments

This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos, from the CRISPit Project MSCA-2021-SE-01 (GA ID: 101086293) and the PrOryza project, PL24-00035 (Programa Promove 2024, Fundação La Caixa/BPI e Fundação para a Ciência e Tecnologia). JT received funding from FIDELIDADE - Impact Center for Climate Change, grants for master students. AMP received funding from Fundação para a Ciência e Tecnologia through the 2023.07134.CEECIND.

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Abstracts Posters

P1 - ProCardo: Impact of Climate Change on Cardosins' Expression and Serra da Estrela

Cheese Quality

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Cynara cardunculus (cardoon) flower extracts are essential for producing Serra da Estrela cheese due to milk-clotting capacity, mainly attributed to the aspartic proteases cardosins A and B (CdA and CdB). These enzymes are influenced by the environmental conditions, and climate change may alter their activity and expression. The goal of this project is to characterize the expression and activity of cardosins from plants under abiotic stress conditions and to evaluate the impact of such alterations on cheese quality and characteristics. To achieve this, inflorescences were collected from plants grown under control, drought and heat conditions. Expression of the genes encoding these cardosins were quantified by RT-qPCR across different floral structures and developmental stages of two ecotypes, and western blot was used to assess whether the observed transcriptional patterns were reflected at the protein level. In the 3M ecotype, combined heat and drought stress induced the expression of both cardosins, whereas drought stress alone increased CdA but reduced CdB expression in closed stigmas. Water stress had limited effect in 1M ecotype. Western blot confirmed the presence of both proteins in the active form. These results show how climate stress affects the expression of cardosins, encouraging strategies to preserve traditional cheese production.

Acknowledgments

Funded by Project "ProCardo - Cardosinas and Serra da Estrela cheese: protection against climate change" (PL23-00022), supported by the Promove Program of the 'La Caixa' Foundation in collaboration with BPI, and national funds via FCT (Foundation for Science and Technology) through the Strategic Projects UID/05748/2025 and UID/PRR/05748/2025, DOI https://doi.org/10.54499/UID/05748/2025 and https://doi.org/10.54499/UID/PRR/05748/2025Replace this paragraph with the abstract text (typically 200–300 words). Briefly describe the background, objective, methods, key results, and conclusion of the work. Keep the formatting of this paragraph — justified alignment, single paragraph, no subheadings — unless the event's guidelines state otherwise.

P2 - endoLEGUME - Exploring seed endophytic microbiota as a strategy to enhance chickpea resilience to combined drought and heat stress

Mariana Lebre[1,2]; Sofia Spormann[2]; Filipa Sousa[2]; Pedro Mateus[2]; Maria Martins[2]; Bruno Sousa[2]; Juan Ignacio Vilchez[3]; Isabel Duarte[4]; Fernanda Fidalgo[2]; Cristiano Soares[2]

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Climate change is increasing the frequency and intensity of extreme climatic events, particularly droughts and heatwaves, posing a major threat to Mediterranean agricultural productivity in Mediterranean regions. Among grain legumes, chickpea (Cicer arietinum L.) is an important source of dietary protein; yet Portuguese production remains critically dependent on imports (~80%) and increasingly vulnerable to climate change, particularly in the main producing region of Alentejo. Plant-associated seed endophytes represent a promising tool to enhance host tolerance to abiotic stress through diverse physiological and biochemical mechanisms. The endoLEGUME project aims to explore the seed-associated endophytic microbiota of chickpea to develop a sustainable bioinoculation strategy to enhance tolerance to combined drought and heat stress. The first stage of the project involves characterizing the responses of chickpea varieties displaying contrasting levels of tolerance to drought and heat stress. Preliminary assays were conducted to establish suitable growth conditions and stress treatments. The results showed that water deficit negatively affected plant growth, with droughtstressed plants exhibiting reduced shoot length and lower shoot and root biomass. Root biomass was particularly affected under co-exposure to water deficit and high temperatures. In parallel, the endophytic microbial communities present in the seeds of selected genotypes will be characterized to investigate the relationship between stress tolerance and seed microbiota composition. Subsequently, a microbial consortium comprising selected seed endophytes with plant-beneficial traits will be formulated and optimized for seed inoculation. Its effectiveness in improving plant performance and stress resilience will be evaluated under controlled environmental conditions and validated under field conditions. Overall, this project is expected to advance understanding of the physiological mechanisms underlying chickpea tolerance to abiotic stress and assess the potential of seed endophytes as climate-adaptation tools. Ultimately, the project may support the development of sustainable bioinoculants for chickpea and other legume crops, contributing to more resilient agricultural systems under future climate scenarios.

Acknowledgements

This work was partially supported by national funds via the Foundation for Science and Technology (FCT) through strategic programs within INOV4AGRO (LA/P/0126/2020), GreenUPorto (UID/05748/2025, UID/PRR/05748/2025), Green-it Bioresources for Sustainability (UID/04551/2025, UID/PRR/04551/2025) and LS4FUTURE Associated Laboratory (LA/P/0087/2020). The authors want to acknowledge the funding provided by the 'La Caixa' Foundation, in collaboration with BPI and FCT through the research project endoLEGUME (PD25-00055).

P3 - Genome-wide characterization of genetic diversity and population structure of Ceratonia siliqua across Morocco

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The carob tree (*Ceratonia siliqua L.*) is a long-lived, dioecious Mediterranean species of major ecological and economic importance and is increasingly recognized as a strategic resource for climate-resilient agroecosystems. Morocco represents a key center of diversity for the species in the western Mediterranean, yet genome-wide assessments of its genetic structure across the country remain limited and geographically incomplete. Leaf samples were collected from 213 Ceratonia siliqua individuals representing natural, semi-natural, and cultivated populations across major regions of Morocco. Whole-genome resequencing was performed following genomic DNA extraction, library preparation, and paired-end sequencing on the AVITI platform. High-quality SNPs were identified using a reference-based variant-calling pipeline followed by stringent filtering. Genome-wide variation was investigated using population genomic approaches to assess genetic structure, admixture patterns, nucleotide diversity, genetic differentiation, and isolation by distance. Results revealed weak but detectable genetic structure, with most individuals exhibiting admixed ancestry. Southwestern and northern populations showed partial differentiation, whereas central and Middle Atlas populations were largely admixed. Genetic differentiation between the non-admixed groups was moderate, suggesting limited but ongoing gene flow. Nucleotide diversity varied among regions, with higher diversity observed in admixed individuals and certain southern-central populations. A significant but weak isolation-by-distance pattern was detected, indicating that geographic separation contributes modestly to genetic differentiation. Overall, Moroccan carob exhibits moderate population structuring combined with extensive admixture, reflecting a dynamic evolutionary history shaped by geographic and demographic processes. These findings refine current understanding of carob population structure in Morocco and highlight the importance of conserving genetically diverse populations to support future breeding, genetic resource conservation, and climate adaptation strategies.

Keywords: Ceratonia siliqua L., SNP diversity, population structure, admixture

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