BOOK OF ABSTRACTS

II SIMPÓSIO NACIONAL DE VALORIZAÇÃO DO CARDO

Seminário de Encerramento do Projeto MedCynaraBioTeC

Uma abordagem combinada para a valorização económica do cardo

MED CYNARA BIO | TEC

2023

23 MARÇO

Auditório IPBeja

Organização e Parceiros:

Cofinanciado por:

II Simpósio Nacional de Valorização do Cardo

Book of Abstracts

23 de Março 2023

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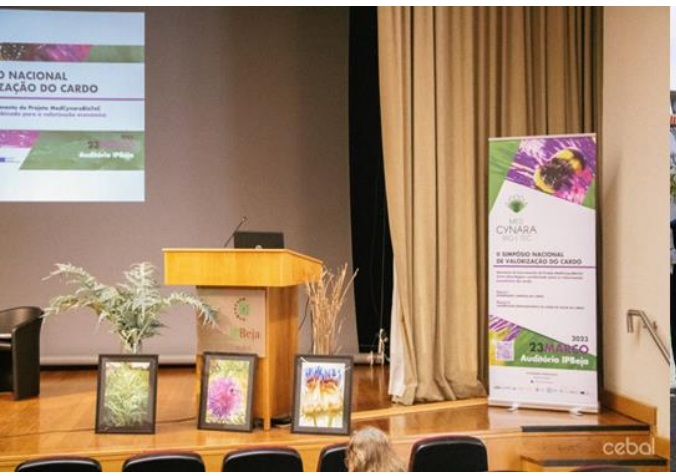

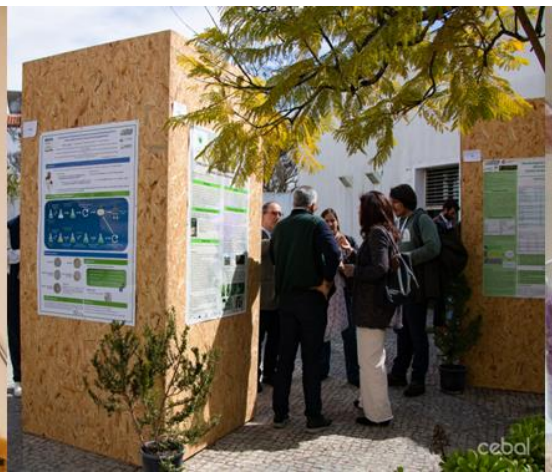

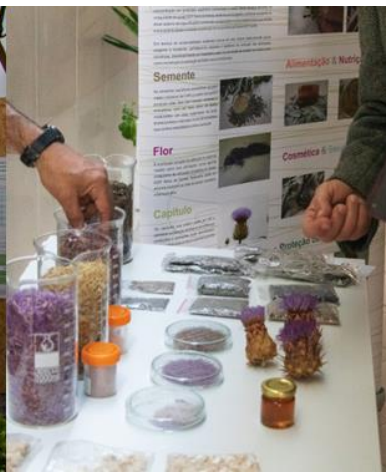

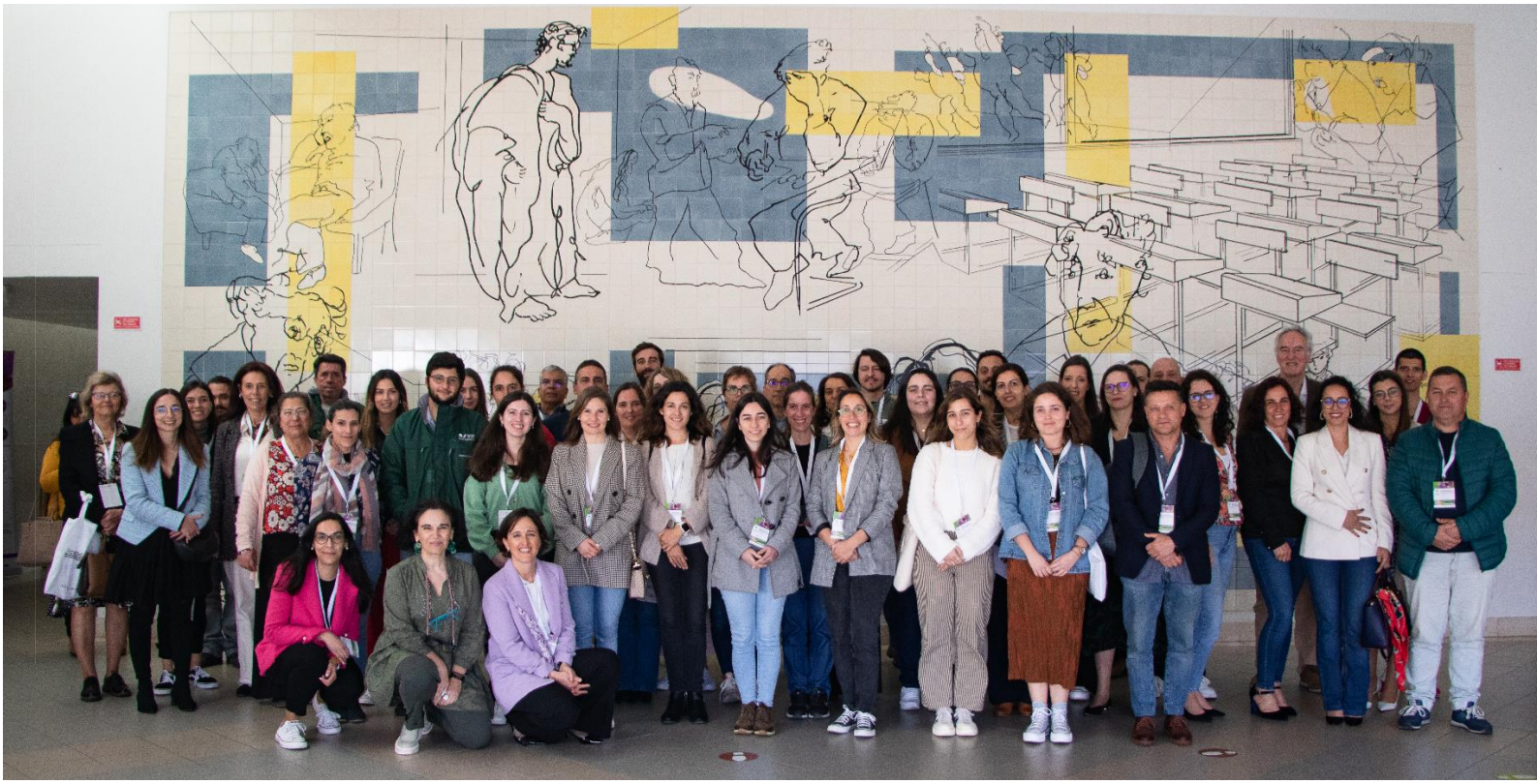

TITLE

eBook of Abstracts from the II Simpósio Nacional de Valorização do Cardo

Published by CEBAL – Alentejo Biotechnology Center for Agriculture and Agro-Food

This publication gathers all the communication (plenary, oral, and posters) done at the II Simpósio Nacional de Valorização do Cardo.

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WELCOME MESSAGE

Dear Colleagues,

On behalf of the Organizing Committee, it is our great pleasure to invite you to attend the II Simpósio Nacional de Valorização do Cardo, to held in Beja.

The II Simpósio Nacional de Valorização do Cardo will represent a very stimulating meeting and an unequalled opportunity for the scientists dealing with cardoon valorization, within different perspectives. The program will cover the most appealing and relevant topics of cardoon research, particularly focus within two major panels: I – The cardoon natural diversity including genetic, morphologic, and technological; II – The biotechnological valorization of cardoon and its added-value opportunities with a concept of circular bioeconomy.

The II Simpósio Nacional de Valorização do Cardo program will include a Plenary Lecture and several Oral Communications followed by discussion sections, as well as Poster Communications which will potentiate a time to discuss, and to promote networking.

We look forward to welcome as many of you as possible on this meeting. Hope you have a pleasant time in Beja.

The Chair of II Simpósio Nacional de Valorização do Cardo

Maria de Fátima Pereira Duarte Ricardo

ORGANIZERS

SUPPORTED

Project MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495)

ORGANIZING COMMITTEE

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Miguel Ferro (CEBAL, MED, CHANGE)

The II Nacional Symposium on Cardoon Valorization aims to promote the scientific and technologic knowledge regarding the genetic, the morphologic and biochemical diversity of cardoon, with attention to different circular economy strategies that bring more valorization and therefore economic cardoon valorization as a natural resource.
All the symposia will take place in the Auditório dos Serviços Comuns within the Instituto Politécnico de Beja.

23 Março |Auditório dos Serviços Comuns do Instituto Politécnico de Beja

8h30 **ABERTURA DO SECRETARIADO E RECEÇÃO DOS PARTICIPANTES**

9h30 **SESSÃO OFICIAL DE ABERTURA**

9h50 **APRESENTAÇÃO GERAL DO PROJETO MedCynaraBioTec**
Fátima Duarte |Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento (MED) & Instituto para as Alterações Globais e Sustentabilidade (CHANGE)

10h00 **SESSÃO PLENÁRIA**
Reflexão Sobre o Estado da Arte na Genómica do Melhoramento de Plantas
Octávio Paulo | Faculdade de Ciências, Universidade de Lisboa, CE3c & CHANGE
Moderador: **Liliana Marum** | CEBAL, MED & CHANGE

10h45 **PAUSA PARA CAFÉ & CHÁ e SESSÃO DE POSTERS**

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Moderadores: **Octávio Paulo** | Faculdade de Ciências, Universidade de Lisboa, CE3c & CHANGE e **Fátima Duarte** | CEBAL, MED & CHANGE

11h35 **Estudos Moleculares na Caracterização Genética do Germoplasma de *Cynara cardunculus***
Liliana Marum | CEBAL, MED & CHANGE

11h50 **Variabilidade Fenotípica em Populações Silvestres de *Cynara cardunculus* no Alentejo**
Anabela Belo | Departamento de Biologia, Universidade de Évora, MED & CHANGE

12h05 **Variabilidade dos Perfis Bioquímicos da Flor de *Cynara cardunculus***
Paulo Barracosa | Instituto Politécnico de Viseu, Centro de Estudos de Recursos Naturais, Ambiente e Sociedade (CERNAS)
Cristina Conceição | Departamento de Zootecnia, Universidade de Évora, MED & CHANGE

12h30 **Análise da Expressão Diferencial de Genes Envolvidos na Produção de Cinaropicrina em *Cynara cardunculus***
Ana Paulino | CEBAL, Faculdade de Ciências, Universidade de Lisboa, CE3c & CHANGE

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13h00 **ALMOÇO**

14h30 **PAINEL II – VALORIZAÇÃO BIOTECNOLÓGICA DA CADEIA DE VALOR DO CARDO**
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Fátima Duarte | CEBAL, MED & CHANGE

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Filipa Mandim | Instituto Politécnico de Bragança, Centro de Investigação de Montanha (CIMO), Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC)

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Teresa Brás | CEBAL, MED & CHANGE

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Helena Caiado | CEBAL, MED & CHANGE

16h15 **Cinaropicrina e a Pele: O que Aprendemos do Trabalho em Bancada?**
Andreia Gomes | Departamento de Biologia, Escola de Ciências da Universidade do Minho, Centro de Biologia Molecular e Ambiental

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Daniela Rosa | Universidade de Cádiz, CEBAL, MED & CHANGE

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Paulo Barracosa| Instituto Politécnico de Viseu, CERNAS

17h20 **SESSÃO DE ENCERRAMENTO**
Rui Marreiros | Presidente do CEBAL
Carmen Carvalheira | Vice-Presidente da Comissão de Coordenação e Desenvolvimento Regional do Alentejo
Isabel Ferreira | Secretária de Estado do Desenvolvimento Regional*

*- participação remota


OPENING SESSION

General Presentation of MedCynaraBioTec Project

Maria de Fátima Duarte

Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja, Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento (MED) & Instituto para as Alterações Globais e Sustentabilidade (CHANGE)

Email: fatima.duarte@cebal.pt

The enhancement of endogenous resources, mainly combining research with subsequent technological and economic valorization of primary production and agro-food sectors, emerges as one of the four pillars of the Strategy for Research and Innovation for a Smart Specialization (EREI). Currently, the Agroforestry sector represents 6% of the national gross domestic product (GDP), playing an important role in international trade, significantly contributing to the balance of Portuguese economy. Agriculture within Alentejo regions (Alto e Baixo), represents respectively, 10% and 7% of the Regional Product, and statistics suggest a continuous future growth, with the possibility of exploring new cultures, as well as to explore the ones already existing, but with poor cultivation tradition, and consequently with low added value, such as the case of Cynara cardunculus, commonly referred as cardoon. MedCynaraBioVal is a scientific strategy to improve the economic valorization of Cynara cardunculus, transforming an endogenous resource into an industrial crop, a natural source of several bioactive compounds, from which, cynaropicrin reveals to be the most abundant one. With great biological activity, cynaropicrin can be valorized with different industries, being the pharmaceutical and biotechnological options the ones with greater add-value. Within the present project we intend to explore the development of potential molecular markers to select the best cardoon genotypes for cynaropicrin production, leading to the basis of a future breeding program.

Acknowledgements or financial support

The present work was supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495).

PLENARY SESSION

Reflection on the state of the art of genomics crop improvement

Octávio S. Paulo

cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa P-1749-016 Lisboa

Email: octavio.paulo@fc.ul.pt

I presented a personal reflection on the state of art of genomics crop improvement with focus on *Cynara cardunculus*. Recent developments on the genome assembly of the Globe Artichoke with Hi-C technology[1] prepare the field for a much-needed pangenome of the species, including its varieties *sylvestris*, *altilis* and the several cultivars of *scolymus*. The pangenome is critical for any crop improvement that implements Genomics Assisted Breeding (GAB) using either Genome wide associations studies (GWAS) or Genomic selection (GS)[2]. A review on the papers on genetic diversity of the *Cynara cardunculus*, suggests that *sylvestris* from the Iberian Peninsula are probably on the origin of the *altilis* and *scolymus*[3,4] but a comprehensive study in Iberian, with more samples and from wide geographic range is needed to confirm this. This is particularly important to maintain crop wild relatives as a potential source of genetic variability and should include the other close species that evolve with *Cynara cardunculus* and have a different ecological niche *C. humilis*, *C. baetica*, *C. algarviensis*, and the vulnerable *C. tournefortii*.

Acknowledgements or financial support

The present work was supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495). Author also acknowledges FCT for the Project LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

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SESSION 1
CARDOON DIVERSITY AND GENETICS

MOLECULAR STUDIES IN GENETIC CHARACTERIZATION OF CARDOON GERMPLASM

Paulino A[1,2], Castro MM[1], Rosa D[1,3], Ferro AM[1,4], Faustino A[1,4,5], Brás T[1,4], Machado E[6], Cruz CP[7], Belo ADF[7], Nozes P[8], Portugal J[8,9], Ramôa S[8], Barracosa P[10], Mendonça D[11], Simões F[11], Duarte MF[1,4], Marum L[1,4]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), Beja, Portugal
[2]Centre for Ecology, Faculdade de Ciência, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal
[3]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.
[4]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal
[5]MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal
[6]MED-Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade de Évora, Ap 94, Évora, Portugal
[7]Ambiente e Desenvolvimento & Departamento de Biologia MED - Instituto Mediterrâneo para a Agricultura, Escola de Ciências e Tecnologia, Universidade de Évora, Ap 94, Évora, Portugal
[8]Departamento de Biociências/Instituto Politécnico de Beja (IPBeja), Beja, Portugal
[9]VALORIZA – Centro de Investigação para a Valorização dos Recursos Endógenos, Instituto Politécnico de Portalegre, Portalegre, Portugal
[10]Escola Superior Agrária de Viseu / Instituto Politécnico de Viseu (ESAV-IPV), Quinta da Alagoa, 3500-6060 Viseu, Portugal
[11]Instituto Nacional de Investigação Agrária e Veterinária I.P. (INIAV IP), Unidade Estratégica de Biotecnologia e Recursos Genéticos, I.P., Oeiras, Portugal

Email: liliana.marum@cebal.pt

The diversity of a plant species enables the development of new cultivars with specific and desirable traits. *Cynara cardunculus* L. (Cc) has been described with a huge variability at the biochemical and morphological levels[1]. This observation was also accompanied by the high genetic diversity detected recently in the south of Portugal in natural populations[2].

In this study, it was possible to evaluate the genetic diversity, geographic distribution, and population structure of *Cynara cardunculus* populations in different areas of Portugal. Using microsatelites markers, about 170 individuals of *Cynara cardunculus* from 19 different populations were identified and analysed. The total number of alleles ranged from 13 to 23 per loci. By geographic location sampled, the expected and observed means in heterozygosity were 0.61 and 0.59, respectively.

The Portuguese populations indicated a high level of genetic diversity at the species level. The highest proportion of genetic variation was identified within a geographic group, while variation was lower among groups. A significant genetic differentiation also existed between natural North-Alentejo geographic locations (Arraiolos, Évora, Monte da Chaminé), Viseu, and populations from Centro Hortofrutícola.

The present study reports the genetic diversity among different cardoon populations of Portugal. Thus, the generated data are a significant contribution to the knowledge of cardoon genetics, providing important information for future management and breeding programs of cardoon.

Acknowledgements

The present work was supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of Cynara cardunculus genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER039495). Authors also acknowledge FCT to Contrato – Programa to LM (CEECINST/00131/2018), PhD grant to A. Paulino (SFRH/BD/145383/2019) and D. Rosa (SFRH/BD/143845/2019), TB (SFRH/BD/110969/2015), Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and Project LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

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Phenotypic variation of wild *Cynara cardunculus* populations within Alentejo region

Belo ADF [1,2], Pinto-Cruz C [1,2], Machado E[1] and Duarte MF[3,4]

[1] MED – Mediterranean Institute for Agriculture, CHANGE – Global Change and Sustainability Institute, Environment and Development
[2] Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Évora, Portugal
[3] Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[4] MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal

Email: afb@uevora.pt

Cynara cardunculus L. is an extraordinary plant, native to the Mediterranean basin, and well adapted to its long hot and dry summers. It is a perennial plant with an annual growth cycle that includes a period of vegetative dormancy in the summer, after seed ripening. In Southern Portugal, *Cynara cardunculus* has a high natural phenotypical variability and, since this is a plant with multiple functionalities, it is important to know and document the variability of their wild populations. In this situation, the work developed (under ValBioTecCynara project), had two main objectives (1) the morphological characterization of wild populations and (2) the establishment of relationships between some of their genetic potential, namely the content of cardosin and cynaropicrin, and morphological characteristics, but also with environmental factors, specifically physical and chemical parameters of soil and climate.
We thoroughly accomplished the first objective, by quantifying 45 morphological traits of 13 *Cynara cardunculus* populations distributed along Alentejo region and estimate the relative importance of different environmental factors in predicting those traits. A large portion (45%) of the total variation showed by morphological characteristics was explained by climatic and soil characteristics variables. Nevertheless, an even larger portion of the variability between populations was not explained, due to the also large variability within populations, and this prevented us from further exploring the hypothetical relationship between cardosin and cynaropicrin contents and morphological traits. We are considering different approaches to this issue, namely, reproducing the data analysis with reproductive traits only, for they are the most conservative, and enlarging the screened geographical area.

Acknowledgements or Financial support

This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of ValbioTecCynara – Valorização Económica do Cardo (Cynara cardunculus): Variabilidade natural e suas aplicações biotecnológicas (ALT20-03-0145-FEDER-000038), and CynaraTeC – Transferência de Tecnologia para a Valorização do Cardo (ALT20-03-0246-FEDER-000067). Authors also acknowledge FCT for the Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

BIOCHEMICAL DIVERSITY OF CARDOON FLOWERS (*Cynara cardunculus* L.)

Barracosa PB[1,2], Conceição C[3,4]

[1]ESAV-IPV;
[2]CERNAS -Research Center for Natural Resources, Environment and Society;
[3]Departamento de Zootecnia, Universidade de Évora
[4] MED & Change

Email: pbarracosa@esav.ipv.pt & cristinaconceicao@uevora.pt

Cardoon (*Cynara cardunculus* L.) is a multi-purpose and versatile Mediterranean crop, adapted to climate change with a wide spectrum of potential applications due its added value as a rich source of fibres, oils and bioactive compounds. Cardoon flower extract is a vegetable coagulant used in the manufacture of some Mediterranean PDO cheeses, obtained exclusively from *C. cardunculus,* due to its extremely high concentration in cardosins. Selected cardoon plants established in the "Serra da Estrela" and Alentejo regions were analysed to evaluate biochemical characteristics based on flower composition and type of harvest. Six distinct cardoon genotypes (1M-6M) selected based on total and specific cardosin concentrations were used to investigate the relationship between a given genotype, proteolytic activity on caseins, coagulation properties and final texture of cheese. The cardosin profiles of each genotype, based on four main groups of cardosins A0, A1, A and B, were stable during the annual flower harvesting period and over all three years using ion-exchange chromatography and native-PAGE electrophoresis. Genotypes 1M and 2M were positively influenced by cardosin B and negatively by the proteolytic action over β-casein, in starting flocculation and gel consistency. Genotype 3M showed a slow micellar aggregation and low gel firmness along milk coagulation, which reflects in the smooth texture of cheese. Genotypes 4M, 5M and 6M, compared with others, presented the highest total concentration of cardosins, namely cardosin A, proteolytic action over K-casein, gel consistency, and the lowest time of coagulation which reflects in a firmer texture of the cheese matrix. The specific characterization of natural biochemical diversity of cardoon flowers and their influence on cheese texture seems to be of great interest since this knowledge will enable producers to predicted cheese properties within a wider range of texture to fulfil consumer demand. Phenolic profiles obtained by cardoon flowers showed significant differences among cardoon genotypes, but apigenin and caffeoylquinic acid derivatives were generally the major molecules in all samples. The results obtained are promising for the development of a plant breeding program based on biochemical and morphological characteristics in order to obtain the most adapted plant architecture for combined purposes related to specific cardosins composition, flower and plant biomass production, and ease of harvesting.

Acknowledgements or financial support

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DIFFERENTIAL EXPRESSION ANALYSIS OF GENES INVOLVED IN CYNAROPICRIN PRODUCTION IN *CYNARA CARDUNCULUS*

Paulino A[1,2], Fernandes I[2], Pires RC[1], Faustino A[1,3,4], Santos J[1,3], Brás T[1,3], Rosa D[1,3,5], Paulo OS[2], Duarte MF[1,3] and Marum L[1,3]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[2]cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Computational Biology and Population Genomics Group (CoBiG2), Campo Grande, 1749-016 Lisboa, Portugal
[3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal.
[4]MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.
[5]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

Email: ana.paulino@cebal.pt

The biochemical, morphological, and genetic variability of *Cynara cardunculus* L. (Cc) in southern Portugal is enormous[1,2]. Cc leaves are a natural source of numerous bioactive compounds, including high concentrations of sesquiterpene lactones (STLs), the most common of which is Cynaropicrin (Cyn)[3]. Transcriptomes of Cc genotypes were examined by comparing various biochemical profiles seen during 4 months (March - June) in 2020 and 2021, with reference to Cyn, collecting all samples when differing quantities of Cyn were obtained (May), in order to determine the molecular pathways necessary for STLs biosynthesis: (VH) samples with very high production (>60 mg/g DW), (VL) samples with very low production (40 mg/g DW), (H) samples with high levels (>40 mg/g DW), and L samples with low levels (<40 mg/g DW).
Total RNA was extracted from the Cc leaves for cDNA libraries biosynthesis. Stranded paired-end sequencing was performed on the DNBseq platform. The high-quality reads were mapped to the assembly of the Cc reference genome downloaded from The Global Artichoke Genome Database using STAR, and Htseq-count was used to count uniquely mapped genes. DESeq2 method was used for DEGs analysis. Differentially expressed genes were defined as genes with a log2 fold change (logFC) ≥ |2| and a False Discovery Rate (FDR) ≤ 0.05. High-quality sequencing generated an average of 24 million reads, of which an average of 91% were mapped. An average of 24 thousand genes were expressed in all plants. Differential expression analysis generated a total of 36 and 212 DEGs when comparing VH vs VL and H vs L, respectively. In both comparisons, most of the DEGs were more expressed in samples with a high amount of Cyn (VH vs VL: 78% (contrasting dataset); H vs L: 57% (all dataset)).
The results suggest that environmental factors have a significant impact on the regulation of cynaropicrin production. There were also DEGs found that were involved in photosynthesis, plant defense, and stress response. About 14 DEGs from the *Heat shot proteins* family were identified as involved in abiotic stress tolerance such as salt, drought, heat, and cold. Furthermore, 8 Transcription Factor (TF) genes from the *NAC* (3), *bZIP* (1), *LFY* (1), *AHL* (1), and *MYB* (2) families showed differential expression in H *versus* L. Concerning the biosynthetic pathway for STL synthesis, we can identify two crucial genes codifying the GAS (E.C. 4.2.3.23)

and GAO (E.C. 1.14.14.95) enzymes seem to positively and negatively regulate the cynaropicrin synthesis.

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This work is supported by Program Alentejo 2020, through the European Fund for Regional Development (FEDER) under the scope of MedCynaraBioTec – Selection of *Cynara cardunculus* genotypes for new biotechnological applications: the value chain improvement of cardoon, a well-adapted Mediterranean crop (ALT20-03-0145-FEDER-039495). Authors also acknowledge FCT for Contrato – Programa to L. Marum (CEECINST/00131/2018), Ph.D. grant to A. Paulino (SFRH/BD/145383/2019), D. Rosa (SFRH/BD/143845/2019), A. Faustino (UI/BD/153511/2022), and Project UIDB/05183/2020 to Mediterranean Institute for Agriculture, Environment and Development (MED), and Project LA/P/0121/2020 to CHANGE – Global Change and Sustainability Institute.

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SESSION 2
BIOTECHNOLOGICAL ENHANCEMENT OF THE CARDOON VALUE CHAIN

CARDOON VALORIZATION – A RETROSPECTIVE OF THE LAST 20 YEARS

Duarte MF[1,3]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal.

Email: fatima.duarte@cebal.pt

The present communication aims to give a chronological and scientific background on what is the recent story of cardoon valorization, within Europe, over the last 20 years.
When in 2018 the Commissioner Carlos Moedas presented cardoon, as an example of the bio-economy strategy he was trying to implement for a sustainable Europe, there were several R&D projects, in its majority developed in Italy, within the topic of cardoon valorization developed for years.
In the earl´s 2000 the main focus was the use of cardoon as an energetic crop, either through valorization of oil seeds for biodiesel production, or even as a biomass source with calorific output. Later in 2007 the project CYNARES[1] brought knowledge on genetic resources *of Cynara cardunculus*, more specifically on the genotype characterization, and call the attention to the importance of germoplasm conservation. In 2014 the operational group entitled GO-CARD[2] through a rural development plan of the Tuscany region developed cardoon as a low environmental impact crop for the redevelopment of marginal areas, aiming to evaluate the economic and environmental sustainability of the seeds supply chain. FIRST2RUN[3] aimed to demonstrate, at industrial scale, the techno, economical and environmental sustainability of different oil crops, among them *Cynara cardunculus*, grown in arid and/or marginal lands. The purpose of the valorization explored under this project was focused on extraction of vegetable oils to be further converted into biomonomers to further production of bio lubricants, cosmetics, bioplastics, additives through the integration of chemical and biotechnological processes. In 2019, the project CARDIGAN[4] focus on production of novel functional bioplastics. Different parts of the plant (seeds, leaves, and roots) are processed by using different approaches to obtain biopolymers, plasticizers and bioactive molecules for development of innovative packing materials. Most recently, in 2022 the project FIBSUN aims to address novel fiber value chains and ecosystems services from sustainable feedstocks, among them cardoon.
All the knowledge produced; all the technological advances are important building blocks for the establishment, in the near future, of a cardoon valorization circular bioeconomy strategy adapted to the different European rural realities, associated with regional development towards industrial and economic engagement.

Acknowledgments

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Cardoon vegetable tissues as a promising source of functional ingredients

Mandim, F.[1,2,3], Petropoulos, S.A.[4], Santos-Buelga, C.[3], Barros, L.[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal;
[2]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal;
[3]Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain;
[4]University of Thessaly, Department of Agriculture, Crop Production and Rural Environment, 38446, Volos, Greece.

Email: filipamandim@ipb.pt

Cynara cardunculus L. var. *altilis* DC., commonly known as cardoon, belongs to the Asteraceae family. This species is included in the Mediterranean diet and has several industrial applications (e.g., food, paper pulp, energetic, pharmaceutical, and cosmetic industries)[1]. Cardoon is a rich source of bioactive compounds and has low requirements in agronomic inputs, high biomass yields, and high adaptation to adverse conditions. Despite its multifaceted applications, tons of plant material are normally discarded and unexploited. This biowaste constitutes a rich source of valuable compounds, that can be explored as functional ingredients for diversified applications[1,2]. Their exploitation and proper use could contribute to the economic valorization of the species and their producing countries, being also essential for circular economic stimulation and environmental impact reduction. Cardoon vegetable tissues (heads, blades, seeds, bracts, and petioles) were collected in Greece throughout different developmental stages (PGS 1 – 9). The polyphenolic profile was analyzed by HPLC-DAD-ESI/MS. Antioxidant potential was studied through two cell-based assays: TBARS and OxHLIA. The anti-inflammatory activity was evaluated through the extracts' capacity to inhibit the formation of nitric oxide. Finally, the antiproliferative potential was also studied. Overall, the best results were obtained for the leaves, which exhibited a higher variety and concentration of phenolic compounds, as well as more interesting bioactive properties compared to the rest of the studied tissues. Contrarily, the seeds exhibited a low variety of phenolic compounds, as well as less promising antitumor and anti-inflammatory activities. The growth cycle showed an influence on the bioactivities studied, with younger tissues exhibiting higher levels of phenolic compounds, and intermediate ones standing out for the studied bioactivities. Further studies regarding the relationships between the observed potential and the identified compounds should be developed, to establish a correlation between the bioactivities and the chemical composition. Cardoon vegetable tissues demonstrated to be a rich source of functional ingredients with promising applicability in diversified industrial applications.

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Cynara cardunculus leaves as a source of cynaropicrin: from extraction to application and economic viability

Teresa Brás[a,b*], L.A. Neves[c], J. Crespo[c], Maria F. Duarte[a,b]

[a]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/ Polytechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[b]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Iinstitute, CEBAL, 7801-908, Beja, Portugal
[c]LAQV/ REQUIMTE, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

*Email: teresa.bras@cebal.pt

With an estimated biomass production that can range from 7.8 – 20.0 ton DW/ha, *Cynara cardunculus* (Cc) presents a high potential from the perspective of a complete plant exploitation, being a source of: lignocellulosic compounds, with leaves representing from 33.1% till 48.4% of the total biomass average weight[1,2]. Among the bioactive compounds that can be extracted from Cc, sesquiterpene lacontes are the most prevalent group detected (≈94.5 g/kg DW), mostly represented by cynaropicrin (≈87.4 g/kg DW)[3], with a known high biological potential. It is therefore challenging to consider the cynaropicrin extraction from Cc leaves, following a concept of environment-friendly extraction.

Plant extracts may include compounds and/or fractions with distinct biological potential. Although solvent resistant nanofiltration (SRNF) has been applied with success in the pharmaceutical industry, its utilization on natural compounds purification is still scarce mainly due to the lack of SRNF commercially available membranes. Based on this, the overall objective of this study was the optimization of methodologies for cynaropicrin from Cc leaves extraction, followed by fractionation by SRNF and its incorporation in a chitosan matrix for anti-inflammatory drug delivery for use in chronic skin wounds.

From the extraction methodologies studies, ultrasound assisted extraction combined with ethanol as extraction solvent, led to a cynaropicrin extraction yield of 55.00 ± 2.92 mg/g DW and an energy consumption of 0.027 kWh/$g_{cynaropicrin}$. Extraction optimization by response surface methodology allow the achievement of optimum extraction conditions (S/L – 1/27; T – 44ºC, Amplitude – 67%).

Fractionation with SRNF allowed a removal of sugars of 94% with a maximum cynaropicrin loss of 13.9% (mass values). Economic evaluation to total process, including extraction, revealed a total fixed investment of approximately 1.4 M€, with a pay-back of 4.58 years.

Enriched cynaropicrin extract incorporation on chitosan films, allowed films dense and thermal stable. A decrease on mechanical strength was observed with extract loading, however still acceptable for skin applications. Chitosan and cynaropicrin enriched extracts presented effect on interleukin-6 mediation, a good indicator in case of chronic wounds.

The alliance between the use of *Cynara cardunculus*, economic viable production process and application of cost-effective wound dressings revealed to present a high potential and can be applied as example for other biomasses.

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Valorization of natural extracts obtained from *Cynara cardunculus* L. var. *altilis* leaves and the assessment of their biologic effect in negative breast cancer

Helena Caiado[1,2], Ana Calçona[1], Maria M. Castro[1+], Teresa Brás[1,2], Maria F. Duarte[1,2]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[2]MED – Mediterranean Institute for Agriculture, Environment and Development, CEBAL, 7801-908, Beja, Portugal.
[+]Current address: Institute for Research and Innovation in Health, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.

Email: helena.caiado@cebal.pt

According to the Global Cancer Observatory data, in 2020, Breast Cancer was one of the top five most prevalent types of cancer in Portugal, accounting for 11.6 percent.
From all the existing breast cancer sub-types, triple negative breast cancer (TNBC), characterized by the absence of hormonal receptors (HER2, estrogen and progesterone), displays high rates of cancer relapse and tends to have a worst prognosis and poor survival rates associated.
Due to the nonexistence of these receptors, the fewer therapeutic lines for this sub-type of cancer are limited to surgery, chemotherapy or radiotherapy.
Thus, it is of great interest the discovery of alternative therapeutic strategies, such as, the use of bioactive compounds to could be used in combination with chemotherapeutic agents, that could contribute to the patient welfare.
Mediterranean variety *Cynara cardunculus L. alitis* (DC), besides to be known for the Iberian traditional cheese production, is also known by its immuno- and hepato- protective properties, as well as for cancer anti-proliferative properties, as demonstrated by our group in previous studies, by affecting the Akt signaling pathway, promoting cell cycle arrest, along the cell proliferation inhibition.
The aim of this study consisted in the valorization and evaluation of the biological effect of ethanolic *Cynara cardunculus L. alitis* (DC) leaves extracts in the combination with the chemiotactic agent 5-FU in an *in vitro* model of triple negative breast cancer (MDA-MB-231 breast cancer cell line).
Results demonstrated a regulation by these ethanolic extracts in several proteins in different signaling pathways, thus contributing to the great potential of using bioactive compounds extracted from natural plants as a natural complement to conventional cancer therapies.

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CYNAROPICRIN AND SKIN: WHAT WE'VE LEARNT IN THE LAB

Pereira A[1,2], Gonçalves A[1,2], Gonçalves C[1,2], Brás T[3,4], Duarte MF[3,4], Gomes AC[1,2]

[1] Centre of Molecular and Environmental Biology (CBMA) / Aquatic Research Network (ARNET) Associate Laboratory, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[2] Institute of Science and Innovation for Sustainability (IB-S), Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[3] Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[4] Mediterranean Institute for Agriculture, Environment and Development–MED & CHANGE – Global and Sustainability Institute, CEBAL, 7081-908 Beja, Portugal

Email: agomes@bio.uminho.pt

The skin is the largest organ of the human body, assuming several crucial functions for survival, such as protection against external aggressions, including infectious agents. Almost a third of the world's population is affected by skin diseases, which constitute the fourth most common cause of all human diseases. The development of robust and informative *in vitro* skin models is therefore extremely relevant and has numerous areas of potential application. Recently, our research group developed a model of co-culture with keratinocytes and fibroblasts, in order to mimic some of the cell interactions that occur in the skin and influence the skin's response to different stimuli.
This model was used to study the anti-inflammatory potential of ethanol extracts from *Cynara cardunculus* leaves and of cynaropicrin, the main sesquiterpene lactone present, and the results were compared with the responses of the same cells grown in isolation. This analysis demonstrated the bioactivity of both the extract and cynaropicrin, suggesting an opportunity to develop topical applications based on *C. cardunculus* extracts, which may contribute to a better and more natural treatment of inflammation. Furthermore, this study revealed that the interaction between the two types of cells tested significantly affects the response to an inflammatory insult. This substantiates the use of more complex cell culture models to understand the processes involved in the skin response to an external insult or in a pathology.

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Regarding the skin model development, the authors acknowledge the funding for the project Repel+: New solutions for mosquito repellence as an application for malaria control (project number 47036) from the Agência Nacional de Inovação, co-funded by the European Regional Development Fund (ERDF), through the Operational Programme for Competitiveness and Internationalisation (COMPETE 2020), under the PORTUGAL 2020 Partnership Agreement.

Sesquiterpene lactones enriched-fractions obtained from *Cynara cardunculus* extract diaultrafiltration

Rosa D[1,2,3], Brás T[1,2], Rial C[2], Varela RM[2], Maçãs B[4], Macías FA[2], and Duarte MF[1,2]*

[1]Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[2]MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal
[3]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz Spain
[4]INIAV - National Institute for Agrarian and Veterinarian Research, Estrada Gil Vaz, Ap. 6, 7350-901 Elvas, Portugal

Email: daniela.rosa@cebal.pt

Emerging worldwide need to find alternatives to synthetic herbicides, more economically and environmentally sustainable, for sustainable weed control has prompted considerable interest in exploiting natural sources as potential herbicides[1]. A natural source for weed control is provided by allelochemicals, among them, Sesquiterpene Lactones (SL) represent an interesting group of compounds with broad phytotoxic activity demonstrated against weed species[2,3,4]. Purification of these compounds using conventional methods are expensive, time-consuming, and by using noxious solvents. Membrane processing technology has proven to overcome these disadvantages and to be a suitable method to bioactive compounds purification[5]. The aim of this study was *Cynara cardunculus* ethanolic leaves extract fractionation by membrane technology for SL-enriched fractions obtention with enhanced phytotoxic activity, using ultrafiltration and nanofiltration membranes. Distinct membranes were studied in terms of solvent volumetric flux (Jv) and compounds of interest rejection (cynaropicrin and chlorophylls) at different transmembrane pressures in total circulation mode to select the one(s) giving better separation efficiency. Among membranes studied, Suez™GH (MWCO – 2000 Da) and Duramem®200 (MWCO – 200 Da) membranes were selected for sequential assay on diafiltration mode for high and low molecular weight compounds removal, respectively. Results showed that Suez™GH was more efficient in terms of SL purification than Duramem®200 with a concentration enhancement to double, approximately. So, an innovative approach was tested, based on the SL permeate collection during time using Suez™GH membrane. The experiment was performed in diaultrafiltration mode at transmembrane pressure of 18 bar, collecting permeate and feed samples every 24h for 8 days. Results showed that after 96 hours, SL purity had the highest improvement from 35.9% (initial feed) up to 71%, in average. Phytotoxic activity was evaluated against *Portulaca oleracea* and results showed enhancement in phytotoxic activity specially in terms of shoot growth inhibition. Results obtained confirms the applicability of membrane technology to produce SL-rich fractions with relevant phytotoxic activity. Environmental, and economical sustainability of the overall approach highlights the potential to be used in phytochemical industry to develop a bioherbicide SL-based. As for future work, it is of our interest to develop an effective formulation for further application.

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POSTER SESSION

Poster 1

Genetic, Morphological and Biochemical Characterization of *Cynara carduncus* from Alentejo Region

MM. Castro[1], AM. Ferro[1], T. Brás[1], D. Mendonça[3], F. Simões[3], E. Machado[4], E. Almeida[4], AF. Belo[4,5], C. Pinto-Cruz[4,5], L. Marum[1], MF. Duarte[1]

[1] CEBAL- Centro de Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal
[2]LAVQ/REQUIMTE, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[3]Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Unidade Estratégica de Biotecnologia e Recursos Genéticos, I.P., Avenida da República, Quinta do Marquês Edifício da Ex-Estação Florestal Nacional, 2184-505 Oeiras, Portugal
[4]ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Instituto de Formação e Investigação Avançada, Universidade de Évora. Pólo da Mitra, Ap. 94, 7006-554 Évora.
[5]Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, 7002-554 Évora, Portugal

Email: teresa.bras@cebal.pt

Cynara cardunculus (*Cc*) is a Mediterranean species used as a multipurpose crop, not only in food but also as solid biofuel, as a source of paper pulp, while the seeds are extracted for oil and the flowers employed in cheese production. Furthermore, *Cc* leaves have been used in folk medicine given to their hepatoprotective and choleretic actions due to their phenolic and terpenic composition. The main goal of this work was to address the natural cardoon genetic, morphological and biochemical variabilities within different natural occurring geographic populations. It was studied a collection of 4 *Cc* geographic populations, comprising 23 individuals distributed across Alentejo region, South of Portugal. Genetic diversity of the population was determined using 10 microsatellite markers, previously identified. To the study of plants morphological profile were evaluated 32 morphological descriptors. Previously, our research group reported cynaropicrin (Cyn) as the main compound detected in *Cc* leaves-lipophilic extracts (CLE)[1], which belong to sesquiterpene lactones family well recognized by several biological activities. Biochemical profiles, specifically Cyn quantification, was assess by HPLC. Results of morphological characterization, genetic diversity and biochemical profiles will be presented. ValBioTecCynara represents a combined strategy to address the natural cardoon genetic, molecular, morphologic, and biochemical variabilities, to identify individuals with certain and specific required profiles.

Keywords *Cynara cardunculus*; biochemical profiles; genetic diversity; cynaropicrin.

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Poster 2

R68G as putative marker associated with antiproliferative effect on a breast cancer cell model

Ana Ferro[1,2], Patrícia Ramos[3], Maria Miguel Castro[1], Ângela Guerra[1,3], Teresa Brás[1,4], Helena Oliveira[5], Armando Silvestre[3], Rafael Lozano[6], M. Margarida Oliveira[7], Maria F. Duarte[1,2], Sónia Gonçalves[1,8]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), Beja, Portugal;
[2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Évora, Portugal;
[3]CICECO – Instituto de Materiais de Aveiro e Departamento de Química da Universidade de Aveiro, Aveiro, Portugal;
[4]LAQV, REQUIMTE, FCT, Universidade Nova de Lisboa, Caparica, Portugal;
[5]CESAM e Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal;
[6]Centro de Investigación en Biotecnología Agroalimentaria (BITAL) Universidad de Almería, Almeria, Spain;
[7]Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa, Oeiras, Portugal;
[8]Wellcome Trust Sanger Institute, Cambridge, UK.

Email: fatima.duarte@cebal.pt

Breast cancer is one of the most frequent cause of female cancer death[1]. Triple-negative breast cancer (TNBC), currently does not have a specific target treatment, and the chemotherapy approaches frequently used are not completely efficient[2]. Therefore, it is important to develop new TNBC therapeutic strategies, as preventive or complementary approaches. Natural bioactive compounds have been the basis of several new pharmaceutical products. *Cynara cardunculus* L. (Cc) is well known for its therapeutic activities, and its use in folk medicine since ancient times. Cc represents a natural source of terpenic compounds, with the predominant molecule being cynaropicrin (Cyn), a sesquiterpene lactone, with recognized biological properties, such as anti-tumor[3]. The *germacrene A synthase gene* (GAS) is involved in the initial step of Cyn biosynthesis, and GAS expression levels showed a correlation with Cyn content[4]. The present work aimed to identify the new allelic variant in GAS gene involved in Cyn biosynthesis, and correlate them with improved biological activities, such as the antiproliferative effect of Cc leaves-derived lipophilic extracts (CLE), and Cyn, using the human TNBC MDA-MB-231 cell line, as model. Using high resolution melting, nine haplotypes were identified and the putative impact of the identified allelic variants in GAS protein was evaluated by bioinformatics tools. MTT viability assay was assessed, flow cytometry for cell cycle analysis and western blotting for AKT molecular signalling and cell cycle markers were studied. In our work, we identified, in a *C*c population, one allelic variant R68G in *GAS* gene sequence with significant associations with MDA-MB-231 antiproliferative cells activity. Our results demonstrated that CLE (IC_{50} 10,39 µg/mL) and Cyn (IC_{50} 6,19 µg/mL) prevent MDA-MB-231 cell growth, with 48h incubation time. After CLE and Cyn treatment, the relative expression levels of important G2/mitosis checkpoint proteins, namely $p21^{Waf1/Cip1}$, p-Try15-CDK1 and cyclin B1, were significantly higher in MDA-MB-231 cells, which may be related to G2 cell cycle arrest. Cyn and CLE decreased significantly the relative expression of p-Ser473-Akt in MDA-MB-231 cells, comparatively to control. Thus, in this work we identified the allelic variant in GAS (R68G) associated with biological proprieties in CLE and it was showed the promising potential of CLE and Cyn as a natural-modulator of proliferation within TNBC in vitro cells.

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Poster 3

MedCynaraBioTec: a strategy to select *Cynara cardunculus* genotypes for new biotechnological applications – the value chain improvement of cardoon, a well-adapted Mediterranean crop

L. Marum[1,2], T. Brás[1,3], M. Castro[1], AF. Paulino[1], C. Cruz[2], ADF. Belo[2], M. Regato[4], I. Costa[4], O. Paulo[5], MF. Duarte[1, 2]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal;
[2]ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal
[3]LAQV/ REQUIMTE, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal
[4]Escola Superior Agrária de Beja, Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal;
[5]Faculdade de Ciências da Universidade de Lisboa, 1700, Lisboa, Portugal.

Email: fatima.duarte@cebal.pt & liliana.marum@cebal.pt

The Agroforestry sector represents currently 6% of the national gross domestic product, playing an important role in international trade, significantly contributing to the balance of Portuguese economy. Agriculture within Alentejo regions represents respectively, 10% and 7% of the Regional Product, and statistics suggest a continuous future growth, with the possibility of exploring new cultures, as well as to explore the ones already existing, but with poor cultivation tradition, and consequently with low added value, such as the case of *Cynara cardunculus*, commonly referred as cardoon.

MedCynaraBioVal is a scientific strategy to improve the economic valorization of *Cynara cardunculus*, transforming an endogenous resource into an industrial crop, a natural source of several bioactive molecules, from which, cynaropicrin reveals to be the most abundant one. With great biological activity, cynaropicrin can be valorized within different industries, being the pharmaceutical and biotechnological option the ones with greater add-value. Within the present project we intend to explore the development of potential molecular markers to select the best cardoon genotypes for cynaropicrin production, leading to the basis of a future breeding program. The chemical and genetic characterization of different mapped *Cynara cardunculus* geografical locations, and half-sib families, were previously established by us. Transcriptomic studies and a genotyping-by-sequencing (GBS) approach will be assessed to provide dense genome-wide marker coverage. Furthermore, we intent to disclose the potential of genome-wide association studies in the genetic improvement of cardoon.

Regarding the bioactivities, we aim to comprehend the potential effect of *Cynara cardunculus* leaves extracts (CLE), and cynaropicrin, in cell signaling mechanisms, apoptosis/necrosis, in vitro models of metastatic breast cancer. The biological relevance of CLE, and cynaropicrin, will be compared with tested clinical drugs. Moreover, the in vitro assessment of drug combinations with CLE, and cynaropicrin will further clarify mechanisms of co-action, which will then be tested and studied in an in vivo xenograft model. This research project also aims to assess new potential uses of CLE and/or cynaropicrin by studying two putative application with agro-industrial and biotechnological relevance: herbicidal potential and development of functional foods.

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Poster 4

RNA-SEQ REVEALS DIFFERENTIAL EXPRESSION PROFILES AND FUNCTIONAL ANNOTATION OF GENES INVOLVED IN CYNAROPICRIN SYNTHESIS IN CARDOON

Paulino A[1,2], Pires RC[1], Fernandes I[2], Santos J[1,3], Brás T[1,3], Rosa D[1,3,4], Paulo OS[2], Duarte MF[1,3] and Marum L[1,3]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[2]cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Computational Biology and Population Genomics Group (CoBiG2), Campo Grande, 1749-016 Lisboa, Portugal
[3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal.
[4]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

Email: ana.paulino@cebal.pt

Cynara cardunculus L. (Cc) is a versatile crop representing a natural source of sesquiterpene lactones, namely cynaropicrin[1]. Portugal has a great natural variability of this plant at morphological, biochemical, and genetic levels[2]. Previously, great variability in cynaropicrin content was obtained in plants from different genotypes and different collection periods[3]. This work aimed to identify differentially expressed genes (DEGs) in Cc plants with different levels (high *versus* low) of cynaropicrin, to further understand the molecular mechanisms underlying this sesquiterpene lactone synthesis.

For transcriptome analysis, total RNA was successfully extracted from Cc leaves, using RNAqueous™-4PCR Total RNA Isolation Kit (Invitrogen™). cDNA libraries were prepared for transcriptome sequencing. Stranded paired-end sequencing was performed on Illumina Sequencers. The quality analysis of sequencing data was performed using FastQC and MultiQC. The high-quality reads were mapped to the assembly of the Cc reference genome of Cc downloaded from The Global Artichoke Genome Database using STAR. DESeq2 method was used for DEGs analysis. High-quality sequencing generated an average of 24 million reads, of which an average of 91% were mapped to the Cc reference genome. An average of 24 thousand genes were expressed in all plants, either with high or low production of cynaropicrin. Differential expression analysis generated a total of 36 DEGs, with an average of 86% of DEGs being annotated according to the reference genome, which was mainly downregulated. The results suggest that the production of cynaropicrin has a regulation mainly related to environmental factors. However, DEGs involved in stress and genes related to photosynthesis and cellular respiration were also found. We can highlight the *HSP70* and *HSP17.3-B* genes involved in increasing tolerance to abiotic stresses, including salt, drought, heat, and cold. Among the expressed genes related to cellular respiration, we can focus on *ATPA* and *COX1*. Related to photosynthesis, *MT-CYB*, and *psaB* are examples of differentially expressed genes.

This study reveals new insights into the transcriptional regulation of cynaropicrin biosynthesis in Cc, being a necessary step for better conservation of the wild cardoon gene pool, and more efficient use for future Cc breeding programs.

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Poster 5

Degradability of sesquiterpene lactones high expressed in *C. cardunculus* leaves

Rosa D[1,2,3], Brás T[1,3], Rial C[2], Varela RM[2], Maças B[4], Duarte MF[1,3], Macías FA[2]

[1]Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[2]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz Spain
[3]MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908 Beja, Portugal
[4]INIAV - National Institute for Agrarian and Veterinarian Research, Estrada Gil Vaz, Ap. 6, 7350-901 Elvas, Portugal

Email: daniela.rosa@cebal.pt

The emerging worldwide need to find alternatives to synthetic herbicides. Considerable interest has been devoted to natural sources as potential herbicides[1]. Sesquiterpene Lactones (SL) with high expression in *C. cardunculus* leaves (≈ 95g/kg dry weight) represent an interesting group studied for their broad phytotoxic activity[2,3,4]. In the context of final agronomic use, factors such as biodegradability and chemical structure, must be taken into consideration[5].

The present study aimed to evaluate the degradability of main SL present in *C. cardunculus* leaves extract (CCLE): aguerin B, cynaropicrin and grosheimin, described with higher phytotoxic activity [3]. CCLE aqueous solution was studied in terms of compounds degradability over time in water, standard soil, and sterilized soil. Experiments were made at room temperature and samples collected during 24h. SL quantification was performed using UHPLC-MS/MS[6]. SL pure compounds degradability were also studied at the same conditions.

Results demonstrated that both CCLE and pure SLs were stable in water with decrease less than 15±2%, except for pure cynaropicrin with 25±4% decrease after 24h. The same behavior wasn´t observed on standard soil, with a 97±2% and 97±3% reduction after 24h for CCLE (cynaropicrin and aguerin B) and 93±3% for pure cynaropicrin. Regarding degradability in sterilized soil, a less pronounced decrease was observed for aguerin B, and cynaropicrin (CCLE) (41±3%) compared to cynaropicrin (pure) (63±4%), after 1 h. Nevertheless, after 24h, 87±12% and 96±4% reduction was observed, respectively, for cynaropicrin and aguerin B. Regarding grosheimin (CCLE) a lower degradability was achieved (62±9%) after 24h.

Results obtained evidence a possible chemical degradation of SL on both soils, being more pronounced within the latest, most probably due to concomitant microbial degradation. Further studies are being conducted, to understand the mechanisms underlying the SLs degradation in soil, namely chemical characterization of SL degradation products, as well as the assessment of their phytotoxic activity.

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Poster 6

Effect of *Cynara cardunculus* L. var. *altilis* leaves extracts in oncogenic target proteins associated to triple negative breast cancer

Helena Caiado[1,2], Maria M. Castro[1+], Teresa Brás[1,2], Maria F. Duarte[1,2]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal ;
[2]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal;
[+]Current address: Institute for Research and Innovation in Health, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal.

Email: helena.caiado@cebal.pt

Breast cancer is one of most prevalent types of cancers in Portugal, with an estimated incidence rate accounting for 11.6 percent both genders in 2020 according to the Global Cancer Observatory data[1], were triple-negative breast cancer (TNBC) represents around 15 percent from all the breast cancer sub-types and is mainly characterized by the absence of hormonal receptors (HER2, estrogen and progesterone), displaying high rates of cancer relapse and poor survival outcome.

Due to the nonexistence of these receptors, therapeutic approaches for this sub-type of cancer are limited to surgery, chemotherapy or radiotherapy, where unfortunately, not all the patients have a positive response or present adverse side effects allied to these treatments.

Therefore, it is of great interest the discovery of alternative therapeutic strategies, such as, the use of bioactive compounds that could ameliorate or contribute to the patient welfare.

Mediterranean specie *Cynara cardunculus L.* comprise three varieties, were *Cynara cardunculus L. alitis* (DC), besides to be known for the Iberian traditional cheese production[2] is also known by its immuno- and hepato- protective properties[3].

In previous studies, it was shown the anti-proliferative effect from the lipophilic extracts derived from the *Cynara cardunculus L. alitis* (DC) leaves by affecting the Akt signaling pathway and cell cycle arrest, along with cell proliferation inhibition[4].

In the light of these findings, the aim of this study was to evaluate the effect of ethanolic *Cynara cardunculus L. alitis* (DC) leaves extracts in several oncogenic targets associated to breast cancer using The Proteome Profiler Human XL Oncology Array Kit, in the triple negative MDA-MB-231 breast cancer cell line.

Results demonstrated a regulation by these ethanolic extracts in several proteins in different signaling pathways, contributing to the great potential of using bioactive compounds extracted from natural plants as a natural complement to conventional cancer therapies.

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Poster 7

GENOME-WIDE TRANSCRIPTOMIC ANALYSIS REVEALS NOVEL GENES INVOLVED IN CYNAROPICRIN SYNTHESIS IN *CYNARA CARDUNCULUS*

Paulino A[1,2], Pires RC[1], Fernandes I[2], Faustino A[1,3,4], Santos J[1,3], Brás T[1,3], Rosa D[1,3,5], Paulo OS[2], Duarte MF[1,3] and Marum L[1,3]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[2]cE3c – Centre for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Computational Biology and Population Genomics Group (CoBiG2), Campo Grande, 1749-016 Lisboa, Portugal
[3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal.
[4]MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.
[5]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, 11510 Puerto Real, Cádiz, Spain.

Email: ana.paulino@cebal.pt

Cynara cardunculus L. (Cc), commonly named cardoon, is a Mediterranean plant from the Asteraceae family, which has gained growing interest for its natural source of sesquiterpene lactones (STLs), namely cynaropicrin (Cyn), the major SLT presented in Cc leaves[1]. Portugal has a huge natural variability of Cc at morphological, genetic, and biochemical levels[2,3].

To identify the molecular mechanisms essential for STLs biosynthesis, transcriptomes of Cc genotypes were analyzed by comparing different biochemical profiles observed over 4 months (March – June) in regard to Cyn, collecting all samples when contrasting amounts of Cyn were achieved (May): (HP) samples with a peak of high production, (LP) samples with a peak of low production, (AH) samples with constant high levels and AL samples with constant low levels.

Total RNA was extracted from the Cc leaves for cDNA libraries synthesis. Stranded paired-end sequencing was performed by the DNBseq platform. The raw reads were pre-processed to remove low-quality reads and adaptors contamination yielding a set of high-quality reads which were then mapped with STAR v.2.7.19a against the assembly version 2.0 of the Cc genome from The Global Artichoke Genome Database. Unique mapped reads were used for differential gene expression analysis, which was performed using DESeq2. Differentially expressed genes were defined as genes with a log2 fold change (logFC) ≥ |2| and a False Discovery Rate (FDR) ≤ 0.05. The differential expression analyses yield a total of 36 and 212 DEGs when comparing HP vs LP and HP+AH vs LP+AL, respectively. In both comparisons, most of the DEGs were more expressed in samples with a high amount of Cyn (HPvsLP: 78%; HP+AH vs LP+AL 57%).

Several DEGs involved in stress and related to photosynthesis and cellular respiration were identified suggesting that environmental factors have an essential role in the regulation of Cyn production. Considering the potential biotechnological, agronomic, and pharmaceutic potential use of Cc plants, the relevance of this study is substantially high-level.

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Poster 8

Cynara cardunculus as a source of cynaropicrin – extraction and purification

T. Brás[1,2], A.F.C. Paulino[1], D. Rosa[1], L.A. Neves[2], J.P.G. Crespo[2], MF. Duarte[1,3]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal;
[2]LAQV/ REQUIMTE, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal;
[3]ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: teresa.bras@cebal.pt

Cynara cardunculus (cardoon) is a perennial plant, from the Asteraceae family, exhibiting a vigorous growth, with great adaptation to Mediterranean climates. Within the different plant parts, leaves represent about 35.4% dry weight of the total plant, and are a great source of natural compounds, with known biological potential, and high applicability in food and pharmaceutical industries. Cynaropicrin (CYN), a guaianolide sesquiterpene lactone, was firstly found in *Cynara cardunculus* var. *altilis* (cardoon) leaves, by Ramos et. al. with a content of approximately 87.5% of dried weight (DW). Considered as the main compound responsible for the artichoke bitter taste, it is an interesting compound due to its large availability on cardoon leaves and its biological potential. In general, extraction and separation processes to recover bioactive compounds typically need high working times, involving exhausting procedures, high energy consumptions and use toxic organic solvents. Recent studies from our research group, underlined the contribution of cavitation effect, within ultrasound assisted extraction (UAE) with ethanol as extraction solvent, as the best methodology for CYN extraction. The extraction method achieved presented a reduction of 99% of extraction time, a 30% increase on CYN extraction yield and reduction of 97% of energy consumption, expressed in kWh/g CYN extracted, when compared to the conventional CYN extraction methodology described.
Chemical characterization of the obtained extract showed that, sesquiterpene lactones may represent 45% of the extract, where CYN is the major compound. However, UAE-derived cardoon leaves extracts may also present 31% of compounds with low biological potential, namely monosaccharides. Nanofiltration of ultrasound assisted ethanolic extract from *Cynara cardunculus* leaves showed to be an effective process for the removal of monosaccharides, and recovery of CYN from the extract. Two final streams were obtained within the purification and concentration process, a permeate rich in monosaccharides, and a concentrate stream with higher CYN content.
Interaction between UAE and ethanol followed by fractionation by nanofiltration lead to an innovative optimized CYN extraction and purification process, with low environmental and economic impact, allowing a *Cynara cardunculus* valorization with potential application on agro food, pharmaceutical or cosmetics industries.

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Poster 9

Morphological and genetic diversity in *Cynara carduncus* L.

Jacqueline Santos[1,2], Maria Castro[1], Daniela Rosa[1,2], Ana F. Paulino[1], Liliana Marum[1,2], Maria F. Duarte[1,2], Anabela Belo[2], Carla P. Cruz[2]

[1]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal.
[2]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, Beja, Portugal.

Email: fatima.duarte@cebal.pt

Cynara cardunculus is a herbaceous perennial crop known from ancient times. During the last three decades, this thistle has intensively been researched and recently became a commercial crop for biofuel production.

During the last three decades, *Cynara cardunculus* L (cardoon) has intensively been researched and recently became a commercial crop for its multifaceted industrial applications, with pharmacological and nutritional properties. For different purposes, flowers and leaves as well as total biomass are harvested in the wild, managed in situ and cultivated populations. Due to its biological importance, it's critical to gain knowledge of its diversity, especially in the management in situ of wild populations aiming to enhance the abundance of plants with desirable phenotypes.

The present study aimed to evaluate the morphological and genetic variation of *Cynara cardunculus* L. We analyzed by comparing morphological diversity indices (IMD, based on Simpson's index) and SSR markers of 35 individuals of *C. cardunculus* from 5 wild populations of the Portuguese Alentejo. Morphological diversity (IMD) was similar among the population ranging from 0.298 to 0.318. However, Herdade da Revilheira and Herdade de São Romão populations showed averaged higher genetic variation than Monte da Chaminé, Herdade do Peral and Jerumenha.

The results illustrate that wild *Cynara cardunculus* populations are important reservoirs of variation and are crucial for the general maintenance of diversity. These populations may play a principal role in designing strategies for conservation and will provide valuable information for future management of *C. cardunculus* germplasm.

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Poster 10

Bioherbicide Cynara: weed control through natural resources for sustainable agriculture

D. Rosa[1], T. Brás[1,2], A. Paulino[1], F. A. Macías[3], B. Maçãs[4] MF. Duarte[1,5]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), Beja, Portugal.
[2]LAQV/ REQUIMTE, FCT, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal
[3]Allelopathy Group, Department of Organic Chemistry, INBIO Institute of Biomolecules, Campus de Excelencia Internacional Agroalimentario (ceiA3), University of Cádiz, Avda. República Saharaui, s/n, 11510 Puerto Real, Cádiz Spain.
[4]INIAV - National Institute for Agrarian and Veterinarian Research, Estrada Gil Vaz, Ap. 6, 7350-901 Elvas, Portugal
[5]ICAAM (Instituto de Ciências Agrárias e Ambientais Mediterrânicas), Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal.

Email: daniela.rosa@cebal.pt

Weeds represent a negative impact to crop plants productivity, on average, weeds can lower crop productivity by 34%. The intensive use of synthetic herbicides in the last decades not only has led to herbicide-resistant weeds development, but also the bioaccumulation of synthetic compounds in the environment resulting in health and ecological concerns. Sesquiterpene lactones (SL), abundantly present in *Cynara cardunculus* leaves (Ccl), have recently been described for its allelopathic effect on common target species showing strong evidences of their phytotoxicity and potential use as bioherbicides. The main objective of this PhD proposal is to develop a green bioherbicide based on Ccl extract, rich in SL to be used in olive orchards, grapevines and cereals production. Ccl extract will be prepared by ultrasound assisted extraction methodology, followed by membrane nanofiltration technology, to obtain Ccl- derived SL rich fractions. SL identification and quantification will be monotorized by HPLC-DAD in fractions obtained, with special attention to cynaropicrin, grosheimin and deacylcynaropicrin. Simultaneously, phytotoxicity activity, using wheat coleoptile bioassay, will be performed to select SL fractions with higher activity. Then, selected fractions based on the phytotoxicity activity will be further assessed against a panel of weed species commonly found on agriculture crops mentioned. Formulations will be developed considering type, extract stability in solution and ecological excipients. The formulations produced will be first tested in vitro against weeds, in which case phytotoxicity will also be evaluated at different stages of the plant development in order to observe physiological effects. Furthermore, the formulations will be also tested on crop fields to verify phytotoxicity potential.

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Novel fibre value chains and ecosystem services from sustainable feedstocks (FIBSUN): a perspective

Fonseca DFS, Martins, LM, Chagas R

Food4Sustainability – Associação para a Inovação no Alimento Sustentável, Zona Industrial, 6060-182 Idanha-a-Nova, Portugal

Email: daniela.fonseca@food4sustainability.org

Novel fibre value chains from sustainable feedstocks are accelerating rural bioeconomy and industrial competitiveness while achieving climate mitigation and biodiversity targets. As a pivotal component in the global nexus of environment-economy, sustainable feedstocks offer the promise of increasing the resilience of production systems. However, under-developed biomass processing methods and business models, and poor understanding of the multiple benefits of novel applications currently restrict the implementation of sustainable value chains. Aiming to support the development of resilient and competitive production systems, the project FIBSUN will develop sustainable fibre value chains in European industries. A series of pilots using underexploited biomass will ensure the development and validation of robust and transferable solutions such as insulation rolls and boards, composites for cars, bioconcrete and textile yarn. In Portugal, the pilot will capitalize on the potential of fibres from thistle (*Cynara cardunculus* L.) to set the stage for a new generation of textiles, increasing the sustainability of the production process and taking advantage of this plant to provide soil health and increase biodiversity of marginal lands. Furthermore, FIBSUN will verify the feasibility of the pilots for upscaling, and yield numeric proof of their characteristics, compared to equivalent conventional materials. Finally, FIBSUN will showcase the implementation of biorefinery processes in realistic industrial environments and facilitate further uptake of piloted practices and processes by relevant stakeholders across diverse value chains. Keywords: fibre value chains, thistle, underexploited biomass, bioeconomy, bio-based products.

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Cardoon seed a new lipid source to improve the fatty acid composition of fat and reduce the methane emissions in ruminants

Patrícia Lage[1,2], Alexandra Francisco[3,4,5], Cristina Conceição[2,6,7], Eliana Jerónimo[1,2,6]

[1]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL) / Instituto Politécnico de Beja (IPBeja), Beja, Portugal
[2]MED – Mediterranean Institute for Agriculture, Environment and Development, Portugal
[3]Instituto Nacional de Investigação Agrária e Veterinária, Polo de Investigação de Santarém (INIAV-Fonte Boa), Vale de Santarém, Portugal
[4]CIISA - Centro de Investigação Interdisciplinar em Sanidade Animal, Lisboa, Portugal
[5]AL4AnimalS – Associate Laboratory for Animal and Veterinary Sciences, Portugal
[6]CHANGE – Instituto para as Alterações Globais e Sustentabilidade, Portugal
[7]Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Évora, Portugal

Email: patricia.lage@cebal.pt & eliana.jeronimo@cebal.pt

Improving the nutritional value of products and reducing environmental impact are the main targets of current research in ruminant nutrition. The ruminant digestive metabolism differs from that of other domestic animals by the microbial digestion that occurs in the reticulum-rumen, a particularity that strongly affects the fatty acid composition of fat and results in the production of methane, a potent greenhouse gas. Several nutritional strategies can be adopted to improve the fatty acid composition of ruminant fat and reduce digestive methane emissions by ruminants.

Dietary supplementation with lipid sources rich in polyunsaturated fatty acids is successfully applied for both purposes.

Cardoon seed (*Cynara cardunculus* L.) is a very interesting oilseed for application in ruminant nutrition, particularly as a novel lipid source of polyunsaturated fatty acids. Cardoon seed has high contents in ether extract (250 g/kg dry matter (DM)), composed mainly of unsaturated fatty acids such as linoleic acid (18:2n-6, 57% total fatty acids) and oleic acid (c9-18:1; 28% total fatty acids)[1]. The utilization of cardoon seed in ruminant nutrition is little explored and its effectiveness to improve the fatty acid composition of ruminant products and mitigate methane production is not yet known. In this context, we are studying the use of cardoon seed in ruminant nutrition to develop feeding strategies for lambs to increase health-promoting fatty acids in animal fat and reduce methane production without compromising animal performance.

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Poster 13

CYNARA CARDUNCULUS L. FLOWERS AND THE CARDOSINS VARIABILITY: A PROBLEM OR AN OPPORTUNITY?

Cristina Conceição[1,3]*, Elsa Lamy[3], Ana Fonseca[3], Inês Caeiro[3], Maria F. Duarte[2,3]

[1]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Zootecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal;
[2]Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/ Instituto Politécnico de Beja (IPBeja), 7801-908 Beja, Portugal;

[3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE –
Global Change and Sustainability Institute Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal;

*Email: cristinaconceicao@uevora.pt

Production of milk and cheese from small ruminants in Mediterranean countries has a socio-economic relevance, mainly in rural areas. Cynara cardunculus flowers aqueous extracts have been widely employed in manufacture of sheep and goat cheeses, being legally required in several PDO cheeses in the Iberian Peninsula (Spain, Portugal) and also Italy. Several aspartic proteinases, named cardosins A-H have been identified which have specific ability for milk coagulation as well the residual cheese enzymes reveal specific proteolytic action on the cheese casein with technologically and sensorial cheese consequences. The aim of this study was to evaluate the enzymatic profile variability among Cynara cardunculus ecotypes and the variability within each ecotype (individual flowers).
Individual flowers of four Cynara cardunculus ecotyptes (SCL, ALV, MC, HSR) were selected from two experimental fields (CEB and CEM). The aqueous extracts were prepared, and a total of hundred and five aqueous extracts of C. cardunculus pistils have been analysed by native-PAGE (12,5%).
The technique makes it possible to discriminate the main forms of cardosin (A, B and unprocessed forms of cardosin A). The results showed an enormous variability in the enzymatic profile among the ecotypes and individual plants. The SCL ecotype showed profiles predominantly without cardosin A (89%-CEB and 52% CEM). The results show the importance of evaluating the profile of cardosins, based on individual plants, in order to select a batch of plants with the appropriate profile for each type of cheese.

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Characterization of antitumorigenic potential of cynaropicrin in triple negative breast cancer cell line

Ferreira I[1,2], Brás T[3,4], Duarte MF[3,4], Gomes AC[1,2]

[1]Centre of Molecular and Environmental Biology (CBMA) / Aquatic Research Network (ARNET) Associate Laboratory, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[2]Institute of Science and Innovation for Sustainability (IB-S), Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[3]Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/Polythechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[4]Mediterranean Institute for Agriculture, Environment and Development–MED & CHANGE – Global and Sustainability Institute, CEBAL, 7081-908 Beja, Portugal

Email: inesferreira239@gmail.com

Breast cancer is the most common cancer worldwide, responsible for 1 in 6 cancer-related deaths and causing 25% of female cancer diagnoses. Patients with breast tumors expressing hormone and growth receptors like ER, PR, and HER2, face favorable prognoses and have more therapeutic options, compared to patients with tumors that do not express these receptors (designated triple negative breast cancer (TNBC)). TNBC tumors tend to develop fast, metastasize early, and have a high recurrence when compared to other types of breast cancers. Alternative anticancer drugs must be developed with less toxic but more potent effects than currently existing drugs. *Cynara cardunculus* L. (CC) has been shown to have anticancer properties in TNBC. Cynaropicrin, a sesquiterpene lactone present in cardoon leaves, is known to have antitumor effects by downregulating the phosphorylation of AKT protein, which is involved in cellular growth and development. The present study evaluated the antitumorigenic potential of CC and cynaropicrin in BJ-5ta fibroblasts and MDA-MB-231 TNBC cell lines. Our results suggest that MDA-MB-231 and BJ-5ta cell lines have different behavior when cultured in 2D *versus* 3D models. CC leaf extract and cynaropicrin increased the cytotoxicity of TNBC spheroids in a dose-dependent manner (IC50 = 20.09 ± 6.00; 4.01 ± 0.44 μg/ml, respectively), without affecting the viability in fibroblasts. Interestingly, the extracts possibly enhance the disaggregation of TNBC spheroids. These results indicated that cynaropicrin-rich extract has great potential to be incorporated into a natural-based TNBC treatment or as an adjuvant. However, further research is required to comprehend the mechanisms of action of CC leaf extract and cynaropicrin, particularly in the 3D model.

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Poster 15

Development of biomaterials based on cynaropicrin extracted from a plant for wound dressing applications

Marta J. Lopes[a,*], Teresa Brás[b,c], Fátima Duarte[b,c], Luísa A. Neves[a]

[a]LAQV/REQUIMTE, Departamento de Química, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica
[b]Alentejo Biotechnology Center for Agriculture and Agro-food (CEBAL)/ Polytechnic Institute of Beja (IPBeja), 7801-908 Beja, Portugal
[c]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal

*Email: luisa.neves@fct.unl.pt

Skin, the largest body organ, has as main function to serve as a barrier to harmful environments preventing pathogens to enter the body[1]. Compared to systemic delivery, localized controlled release systems reduce undesired side effects, such as toxicity and suboptimal delivery and provide spatio-temporal control over the drug dosage directly at the wound site. Besides that, it protects the drug from metabolic deactivation, and maintain the drug concentration at a desired level over a prolonged period of time[2]. Besides drug delivery, appropriate and immediate coverage of the wound area with an adequate dressing is essential for wound protection in order to accelerate wound healing. Their primary function is to keep the wound dried, allowing evaporation of wound exudates and preventing the entry of harmful bacteria into the wound. In recent years, wounds treatment has been revolutionized based on a better understanding of the underlying molecular and cellular abnormalities that prevent wound healing[3].

In a constant need to improve patient life quality and decrease ambulatory costs, efforts have been made, in the last two decades, towards the design of new materials for wound dressing, considering the different phases involved. Current research has shown benefits associated with the incorporation of bioactive materials on opposition to those that are inert, due to their biocompatibility, biodegradability, non-toxicity, as well as ability to interact with the biological environment and to influence cellular functions as proliferation, or histoarchitectural tissue organization[4]. Previous studies revealed the anti-inflammatory effect of chitosan - based wound dressings loaded with cynaropicrin enriched extract from *Cynara cardunculus* leaves. However, low cynaropicrin release was achieved[5].

The aim of this work is the development of biomaterials with anti-inflammatory response based on a biopolymer – chitosan or pectin – and cynaropicrin extracted from *Cynara cardunculus,* for wound dressings applications. The films prepared are characterized in terms of their physical and chemical properties, morphology, hydrophilicity, mechanical and thermal properties. Finally, a preliminary assessment of the biological properties of the films prepared will be carried out.

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Management and maintenance of a trial of *Cynara cardunculus* L.

Regato, M[1].; Guerreiro, I[1].; Paulino, A[2].; Duarte, F[2].

[1]Instituto Politécnico de Beja – Escola Superior Agrária de Beja, Beja
[2]CEBAL – Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo, Beja

The test field of *Cynara cardunculus* L. is installed in the Horticultural Center of the Higher Agricultural School.
The climate of the region where the *Cynara cardunculus* L. field is located is an Mediterranean climate, with hot and dry summers and moderate winters.
The annual average temperature oscillates between 15.9 and 16.3°C. The highest values of temperature occur in the months of July and August, with frequent occurrence of temperatures above 35°C. As *Cynara cardunculus* L. is a plant with high resistance to heat and water stress, it grows normally in almost the entire region of Alentejo.
During the spring and summer months there is water shortage, which leads to irrigation at this time of year
The soil where the *Cynara cardunculus* L. field is located belongs to the black clay family.
The plants used for planting the *Cynara cardunculus* L. test field came from seeds selected from the populations, which showed a concentration of cynaropicrin greater than 40 mg/g DW.
The *Cynara cardunculus* L. trial was installed in two phases, one in April 2016 and the other in March 2017, with a 2 m x 1 m compass.
The maintenance tasks of the *Cynara cardunculus* L. test field, carried out through the MedCynaraBioTec Project, were irrigation, weed control and crop protection against pests and diseases.
Taking into account the characteristics of the plants, in recent years, the irrigation has only been carried out when strictly necessary and at times of greater water stress. On average, in the months of May, June and September, there are one or two waterings per week. In the months of July and August, sometimes three waterings per week can be carried out. Normally the watering time does not exceed two hours.
The weed control is carried out mechanically in mid-March and June of each year. Annually, between mid-October and mid-November, the plants are cut with a machine to promote new plant growth. No disease is detected yet.
The most common pests are snails, slugs, aphids, some coleopterans, *Cassida d*eflorata and stem borer. From what could be observed, the pests that cause the most damage to the crop are *Cassida deflorata* and the borer, which can completely devastate it.

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Comparative study of coagulation kinetics of thistle and microbial rennet in sheep's milk from Baixo Alentejo

S Gomes[1,2], I Pina[3], J Dias[2,3], J Caeiro[3], J Martins[3], F Reboredo[2], N Alvarenga[1,2], APL Martins[1,2]

[1]UTI-INIAV, Quinta do Marquês, 2780-157 Oeiras, Portugal
[2]GeoBioTec, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.
[3]Escola Superior Agrária, Instituto Politécnico de Beja, 7800-295 Beja, Portugal

Email: sandra.gomes@iniav.pt

Milk coagulation is the essential step in the cheese-making process, for which the preparation of aspartic protease has been used for thousands of years. The Iberian Peninsula has the largest variety and production of cheeses using extracts of thistle flower as a coagulant, normally produced on an artisanal scale. These extracts present small differences in the coagulation of milk, compared to coagulation with rennet or chymosin, important for the cheese properties that are defined right in the coagulation of milk. The main objective of this test was to evaluate the coagulation kinetics of sheep's milk, as an additional and useful tool for controlling the manufacture of traditional cheeses.

Twenty-eight samples of sheep's milk, from four producers of sheep's milk in Baixo Alentejo, were used to monitor the milk coagulation process using an aqueous extract of *Cynara cardunculus* L. and using a fermentation produced chymosin (Maxiren DS , DSM Gist-Brocades, 2600 MA Delft, The Netherlands). Analysis of the chemical composition (fat, protein, lactose, total solids and non-fat solids), pH and acidity over six collection dates was also performed using Milkoscan 133B (Foss Electric, HillerØd, Denmark), potentiometry and titration (NP-470, 1983), respectively. The technological behavior of milk during coagulation was evaluated by determining the coagulant activity according to the ISO 23058/IDF 199 standard (ISO/IDF, 2006), and by Optigraph (Alliance, Frépillon, France), based on real-time measurement of the attenuation of the near infrared signal caused by micellar aggregation. All assays were performed in duplicate.

Preliminary results confirm the results obtained in previous works [1,2], where it was observed that the nature of the coagulant influences the coagulation properties of sheep's milk, with an impact on cheese making. In the essays using thistle extract, it was found that the curd tended to be less firm than that obtained with chymosin, which may be related to the greater nonspecific proteolytic activity of the aspartic proteases in the thistle flower. Although the clotting time and the properties of the curd may be related to the specificity of the coagulant, other factors such as milk composition may affect these properties, which should be considered in studies to monitor the evolution of the curd. These results reinforce the need to control the different technological factors involved in the coagulation process and which will have an additional effect on cheese quality.

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LIST OF PARTICIPANTS

Plenary Lecture Speaker

Name	Email
Otávio S. Paulo	ofpaulo@fc.ul.pt

Lectures Speakers

Name	Email
Ana Paulino	ana.paulino@cebal.pt
Anabela Belo	afb@uevora.pt
Andreia Gomes	agomes@bio.uminho.pt
Cristina Conceição	cristinaconceicao@uevora.pt
Daniela Rosa	daniela.rosa@cebal.pt
Fátima Duarte	fatima.duarte@cebal.pt
Filipa Mandim	filipamandim@ipb.pt
Helena Caiado	helena.caiado@cebal.pt
Liliana Marum	liliana.marum@cebal.pt
Paulo Barracosa	pbarracosa@gmail.com
Teresa Bras	teresa.bras@cebal.pt

List of Participants

Name	Email
Alexandra Peixoto	alexpeix@net.sapo.pt
Ana Barrocas	abcreative@outlook.pt
Ana Faustino	ana.luisa.faustino@gmail.com
Ana Inês Costa Ramos Abreu	Anaabreu188@hotmail.com
Ana Margarida Calçona	ana.calcona@cebal.pt
Anabela Gonçalves	ferneves57@gmail.com
Antónia Teresa Zorro Nobre Macedo	atmacedo@ipbeja.pt
Antonio Martins	aamartins@fe.up.pt
Carmen Carvalheira	carmen.carvalheira@ccdr-a.gov.pt
Clara Freitas	clarasfreitas99@gmail.com
Cláudia Marinho	claudia.marinho@cebal.pt
Cláudia Sofia Pereira	cpereira@fc.up.pt
Cristiano Rodrigues	sr.cristianorodrigues@gmail.com
Daniela Fonseca	daniela.fonseca@food4sustainability.org
David Passos Morgado Franco Frazão	david.frazao@cebal.pt
Eliana Jerónimo	eliana.jeronimo@cebal.pt
Fátima Carvalho	secretariado.presidencia@ipbeja.pt
Fernando Neves	ferneves57@gmail.com
Flor Torres	flor.torres@natureconsultants.pt
Hugo Alves	Ha@enac.pt
Idália Guerreiro Costa	idalia.costa@ipbeja.pt
Inês Caeiro	ines_caeiro_@hotmail.com
Inês Costa	ines_costa5@hotmail.com
Inês Ferreira	ic.ferreira@campus.fct.unl.pt
Inês Ferreira	inesferreira239@gmail.com
Joana Fernandes	esav3413@esav.ipv.pt

João Dias	joao.dias@ipbeja.pt
Joao Portugal	jportugal@ipbeja.pt
José Pissarra	jpissarr@fc.up.pt
Justino Sobreiro	justino.sobreiro@ipbeja.pt
Kevin Ramani	kevinramani@hotmail.com
Leonel Guerreiro	herdadeboavista@gmail.com
Letícia Fialho	leticia.fialho@cebal.pt
Liliana Cachucho	liliana.cachucho@cebal.pt
Lucas Almeida	lucas1202almeida@gmail.com
Luís Martins	luis.martins@food4sustainability.org
Manuela Miguel Antunes	manuela.m.a@hotmail.com
Maria da Conceição Fernandes	maria.fernandes@cebal.pt
Maria de Fátima Bravo Fragoso	Fatimafragoso11@gmail.com
Maria Isabel Patanita	ipatanita@ipbeja.pt
Maria Leonor Padrão Novais	leonorpnovais@gmail.com
Mariana Regato	mare@ipbeja.pt
Marta Lopes	mdj.lopes@campus.fct.unl.pt
Miguel Sampaio	miguelfsampaio@hotmail.com
Mírian Mendes Pereira	mirian_mendesp@hotmail.com
Nuno Filipe Mendes Batista	nuno-n.f.m.b@hotmail.com
Olinda Guerreiro	olinda.guerreiro@cebal.pt
Patrícia Coelho Lage	patricia.lage@cebal.pt
Paulo Aguiar	pjgaguiar@gmail.com
Pedro Silva	pedrosilva8000@hotmail.com
Rafael Larguinho	rafaellarguinho@gmail.com
Rita Inácio	rita.inacio@ipbeja.pt
Rui Marreiros	rui.marreiros@cm-beja.pt
Rui Melo	rmiguelmelo@gmail.com
Samuel Celestino	Samuelcelestino93@gmail.com
Sandra Marcelino	sandramarcelino@ipb.pt
Sónia Silva	Smargsilva@hotmail.com
Teresa Mata	inegi.up.pt
Tiago Silva	tiagoalexandre2009@hotmail.com
Tiago Sousa	tiago.sousa@cebal.pt