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Beta patula population monitoring to promote *in situ* and *ex situ* conservation of these endangered beet CWR

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Geographic location

- Madeira Archipelago is a Portuguese Autonomous Region.
- The Archipelago is located in the Atlantic Ocean.
- The Region is part of the biogeographic Region of Macaronesia.







Madeira Region description

The region is composed by two sub-archipelagos: Madeira and Selvagens.

Total land area of 750 square kilometres.

Eight (8) islands, but only two inhabited: Madeira and Porto Santo

Eight (8) protected areas (parks, reserves and natura 2000 areas): 495 square kilometres.

Floristic data

3,200 plant taxa 1,204 spontaneous *taxa* 546 native taxa 228 endemisms









Madeira Flora additional data

The total number of spontaneous CWR reaches 413 taxa, including:

- 288 native CWRs.
- 97 endemic CWRs.

Four beet CWRs and one ruderal "crop" are present in the region territory:

- Beta maritima L. [= Beta vulgaris L. subsp. maritima]
- Beta patula Aiton *
- *Patellifolia patellaris* (Moq.) A.J. Scott, Ford-Lloyd & J.T. Williams
- Patellifolia procumbens (C. Sm.) A.J. Scott, Ford-Lloyd & J.T. Williams *
- Beta vulgaris L. [= Beta vulgaris subsp. vulgaris]







Early ISOPlexis Gene Bank (PRT 102) activities

B. patula is a rare endemic species of the Madeira's flora, occurring in a protected, but very restricted area.

Species is a member of the first beet genepool and a potential source of Beet Mild Yellowing Luteovirus (BMYV) resistance.

The *B. patula* was a target species of AEGRO project, whereas the ISOPlexis team started the study to establish its distribution, population baseline and to setup appropriated strategies for CWR conservation.

Main outcomes of AEGRO project

- An initial characterization of the species occurrence and population was made.
- A plan for the implementation of a genetic reserve was developed.





Beta patula habitat and places of occurrence







Establishing Beta patula population boundaries

Maps shows 2 places of occurrence (populations?).

Populations distribution, showing that species colonize different soil types (3 at least).







Establishing *Beta patula* population boundaries

The AEGRO study of population distribution allow us to determine that species occurs in a:

- Netto area: 105,000 m²
- Real area: < 80,000 m^{2*}
- A first population sizes estimation has obtained.







Ecogeographic conditions of *Beta patula* occurrence

Maps of the precipitation and temperature data (series of 20 years), showing that species occurs in **dry, saline and relative high temperature and radiation conditions.**







Beta patula conservation status and additional measures to undertaken Species populations are located in Ponta de São Lourenço and Desertas Islands Both sites are integrate Natura 2000 Network

- Ilheu do Desembarcadouro (ID) is a Partial Protected Area (PPA)
- Ilheu Chão (IC) is a Full Protected Area (FPA)







Beta patula studies in the framework of LIFE12.NAT.PT.000195 Project

These studies were included in 3 actions (WP) whose prupose was to increase our understanding of the *Beta patula* population dynamics, genetic diversity and conservation status. And include the task aiming to:

- a) Monitor of the ecological conditions at the *Beta patula* population sites;
- b) Implement a methodology to perform *Beta patula* field and lab studies;
- c) Analyze the plant diversity and species richness at the places of *Beta patula* occurrence;
- d) Analyze *Beta patula* population sizes and dynamics;
- e) Analyze the species genetic variability and its spatial display;
- f) Evaluate the impact of habitat restoration measure in the species conservation status;
- g) Delimit the genetic reserve boundaries;
- h) Establish a middle term protocol for *Beta patula* and other CWRs management.





Methodology used in Beta patula studies in Life Recover project

16 15 14

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- Fifteen quadrants (16 m² each)
- Randomly selection of 4 m² per quadrant
- Species accounts
- Beta patula specimens accounts
- Plant georeferenciation
- Specimens sampling for lab analysis.



Quadrado 1								
	Espécie	N						
	Beta patula	5	40					
	Suaeda vera	1	20					
	Mesembriathemum crystalinum	1	12					
	Senecio incrassatus	1	3					
	Sonchus oleraceus	3	3					
			78	%				
Quadrado 5								
	Espécie	N						
	Beta patula	4	32					
	Suaeda vera	2	16					
	Mesembriathemum crystalinum	1	2					
	Crepis divaricata	3	3					
			53	%				
Quadrado 10								
	Espécie	N						
	Beta patula	2	20					
	Suaeda vera	2	20					
	Mesembriathemum crystalinum	1	1					
	Phalaris maderensis	1	1					
	Sonchus oleraceus	3	2					
	Medicago serrata	5	6					
			50	%				
Quadrado 13								
	Espécie	N						
	Beta patula	4	20					*
	Suaeda vera	1	8			12		
	Mesembriathemum crystalinum	4	8					*
	Senecio incrassatus	2	2	*				
			38	%			1 7	





Monitoring the ecological conditions at the *Beta patula* population sites.







Analysing the plant diversity and species richness at the places of *Beta patula* occurrence

Introductory description of Ponta de São Lourenço

In Ilhéu Desembarcadouro (ID) *Beta patula* shows:

- Wider distribution and highest population sizes, with better chances to ensuring species viable population sizes;
- Cover a higher diversity of soil and landscape conditions.
- Coexist with higher plant diversity richness.

Plant diversity

At Ponta de São Lourenço 176 plant species occurs, including:

- 110 native species;
- 35 endemism;
- 31 introduced species.





Analysing the plant diversity and species richness at the places of Beta patula occurrence







Analysing the plant diversity and species richness at the places of *Beta patula* occurrence

Five years census data were used to determine the following indices:

- Corrected eveness
- Species richness
- Shannon-Winner diversity index
- Simpson index







Detrended correspondence analysis (DCA) of Beta patula sites



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Plant diversity results contribution to delimitate the Beta patula genetic reserve







Analyzing Beta patula population sizes and dynamics

Temporal distribution and variation plant specimens per quadrants



■ 2014 ■ 2015 ■ 2016 ■ 2017 ■ 2018





Assessing the role Beta patula seed bank on population dynamics.

The role of plant soil seeds bank in population effectives replacement has determined, through the aspiration of the topsoil in 15 m² established in the neighborhood of main census quadrants.

- Soil seed samples has cleaned and seeds accounted.
- Germination test of randomly subsamples of these seed has realized.
- Seeds viability has determine using triphenyl tetrazolium

data	altitude, m	nº seeds	weight, 100 seeds g	weight, 1,000 seeds g			
average	51	2,272	1.98	16.41			
max	93	5,073	4.31	31.43			
min	19	351	1.19	11.30			
Germination viability, %	10 to 15 (0.8%)						







Implementing combined measures for in situ and ex situ Beta patula conservation

The population of *Beta patula* was sampled along the linear transects. Seeds were collected directly from the plants and transferred to the laboratory to be included in the genebank germplasm collection.

Beta patula collection was created with 19 accessions, including:

- 15 accessions from the Ilheu do Desembarcadouro.
- 4 accessions from the Ilhéu Chão.

Stored in the ISOPlexis Genebank collection





ISOPlexis Genebank (Banco de Germoplasma ISOPlexis)

The genebank maintain *ex situ* and *in situ* collections of priority crop (mainly) and CWR genetic resources occurring in Madeira.

Documentation and Information System

Main Germplasm Collections

- Base collection for long time conservation.
- Active collection for research and farmers support.
- Breeding collection with improved varieties and lines.
- Field collection of tropical roots corps (sweet potato, taro, vegetative propagated species, etc.). Genebank database (ISOPex)

Includes records of 5330 (3370) accessions (access 6 of June 2018).

Proceeds certified under the standards 9001:2000 e 14001:2004







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100	ISOP 1397	Beta Patula	Beta patula	Portugal	Madeira Islands	Seed	ISOPLEXIS	Not Available	
0111	ISOP 1440	Beta Patula	Beta patula	Portugal	Madeira Islands	Seed	ISOPLEXIS	Not Available	
1000	ISOP 1441	Beta Patula	Beta patula	Portugal.	Madeira Islands	Seed	ISOPLEXIS	Not Available	
0001	ISOP 1520	Beta Patula	Beta patula	Portugal	Madeira Islands	Seed	ISOPLEXIS	Not Available	
111	ISOP 1549	Beta Patula	Beta patula	Portugal	Madeira Islands	Seed	ISOPLEXIS	Not Available	
100	ISOP 1552	Beta Patula	Beta patula	Portugal,	Madeira Islands	Seed	ISOPLEXIS	Not Available	
0	ISOP 1553	Beta Patula	Beta patula	Portugal,	Madeira Islands	Seed	ISOPLEXIS	Not Available	
(100)	ISOP 1554	Beta Patula	Beta patula	Portugal.	Madeira Islands	Seed	ISOPLEXIS	Not Available	
000	ISOP 1555	Beta Patula	Beta patula	Portugal	Madeira Islands	Seed	ISOPLEXIS	Not Available	
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10221	ISOP 2018	Beta patula	Beta patula	Portugal.	Madeira Islands	Seed	SOPLEXIS	Not Available	
1000	ISOP 2511	Beta patula	Beta patula	Portugal,	Madeira Islands	Seed	ISOPLEXIS	Not Available	
openet 1	ISOP 2512	Beta patula	Beta patula	Portugal,	Madeira Islands	Seed	ISOPLEXIS	Not Available	

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ISOPlexis





Analyzing Beta patula population sizes and dynamics

Early data about the *Beta patula* population data

Beta patula census show the following evolution of knowledge about the plant population

sizes:	Source	Plant census, Nº specimens	Observations			
	Directive of Habitats	< 50	Estimation of local authorities			
	AEGRO Project	± 5,000*	Estimation on basis of initial census			

Results of population census undertaken shows that *Beta patula* sizes and number of specimens are higher than initial AEGRO estimations.

Population	IC	ID
Average	2,917	16,906
min	250	5,873
max	6,500	31,078





Population sizes and dynamics results contribution to delimitate the Beta patula genetic reserve







0.96

Population sizes and dynamics results contribution to delimitate the Beta patula genetic







New Beta patula distribution and population boundaries



100 0 100 200 300 400 m







New Beta patula distribution and population boundaries







Analyzing the species genetic variability and its spatial display

The genetic studies aimed to determine species population genetic variability and its spatial display to delimit the genetic reserve boundaries.

The genetic study was based on the analysis of polymorphic SSRs.

- 32 SSRs used in beet studies has been identified.
- 14 polymorphic SSRs has been selected.
- 7 SSRs, according to bibliography shows to have linkage with plant traits (phenology, behaviour, tolerances).

The *Beta patula* genetic variability was determined in 134 individual samples. Plant polymorphism for 8 SSRs was obtained.











Analyzing the species genetic diversity and its spatial display

Population	Sample Size	PIC	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ID Average	120	0.869	0.6387	0.3613	0.1238	0.8762	0.8684	0.7809
IC Average	14	0.694	0.8671	0.1329	0.2379	0.7621	0.6934	0.7809



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Analyzing the species genetic diversity and its spatial display

Figure. Allelic variation distribution map created using Qgis for ID *Beta patula* population, with two possible selected areas for protection (represented in red and in green).





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diversity indices	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Η'	2.689	2.108	2.588	2.362	2.412	0.355	1.907	1.485
Ε'	0.841	0.690	0.829	0.802	0.742	0.150	0.795	0.473
D	0.184	0.289	0.189	0.242	0.221	0.889	0.286	0.493
diversity indices	Q9	Q10	Q11	Q12	Q13	Q14	Q15	-
Н'	1.727	2.943	2.258	2.499	0.972	1.914	1.954	-
Ε'	0.708	0.861	0.598	0.751	0.383	0.590	0.825	-
D	0 350	0 144	0 278	0.214	0.684	0.346	0 315	

H' = Shannon-Wienner diversity index (ecosystems' condition); E' = Corrected Eveness; D = Simpson Index (Inverted).