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Interim Activity Report

Genetic diversity of *Patellifolia* species

(GeDiPa)

9 February 2015 – 31 December 2016

L. Frese, L. Bülow, S. Castro, M. C. Duarte, J. Iriondo, U. Lohwasser, J. Loureiro, N. Maxted, M. Nachtigall, M. Pinheiro de Carvalho, A. Santos Guerra, M. M. Romeiras, M.L. Rubio, E. Rey

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From left to right. The first four pictures show morphological variation within a *P. procumbens* occurrence growing on a site in northern Tenerife. The fifth picture was taken in La Palma and shows *P. patellaris*. © L. Frese, JKI.

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INTRODUCTION

Although the genus *Patellifolia* (syn. *Beta* section *Procumbentes*) consists of only three recognized species, several taxonomic problems still need to be solved. Wagner et al. (1989) doubted that *Patellifolia webbiana* (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams and *Patellifolia procumbens* (Smith) A. J. Scott, Ford-Lloyd & J. T. Williams are distinct diploid species. *Patellifolia patellaris* (Moq.) A. J. Scott, Ford-Lloyd & J. T. Williams was considered a tetraploid species and thus readily distinguishable from the diploid species until the detection of triploid forms within a *P. patellaris* locality. A survey of herbarium specimens carried out at a later stage of the GeDiPa project underpinned the need for investigations into the variation patterns of the species and the need for a reliable taxonomic key to the species. A sound classification of collected material is at the core of any genebank quality management system as stipulated by the AEGIS Quality System (AQUAS). Therefore, this action aims at a better understanding of the genetic diversity within the genus *Patellifolia* and at the establishment of taxonomic standard accessions to facilitate the classification of genebank accessions.

As *Patellifolia* species are very difficult to use for the genetic enhancement of cultivated beets, this action also aims at the establishment of a small but representative set of the Most Appropriate Accessions (MAAs). For that purpose, passport data from genebank holdings were compiled and the spatial distribution of the species was reviewed to enable the identification of MAAs for AEGIS and Most Appropriate Wild Populations (MAWPs) for the settlement of genetic reserves. The MAWP concept was described by Maxted et al. (2015).

This report describes milestones achieved from February until December 2015. Results of the genetic diversity study will be reported during 2016.

APPROACH

Project workshop

A project workshop hosted by the Universidad Rey Juan Carlos, Madrid (Spain) took place from 16 to 18 February 2015. During the meeting, a detailed guideline for data documentation and sampling of material for genetic and cytological analysis was discussed and agreed. Accessions considered typical for *P. patellaris, P. procumbens* and *P. webbiana* were selected to facilitate the categorization of collected material. These may serve as taxonomic standards in the future.

Gap analysis

Information on the geographic distribution of *Patellifolia* was extracted from the literature and from the information systems. This gap analysis yielded a summary table (Table 1, pages 7-9) with the three species (*P. procumbens*, *P. webbiana* and *P. patellaris*) and 25 geographic subunits. The potential distribution area was checked against known findings of occurrences.

Sampling

The GeDiPa partners marked out the areas where *Patellifolia* can occur and organized four field missions to collect samples. The interest was to locate the places and validate the literature data or the data documented in the information systems. Samples were collected in south-eastern Spain, Tenerife (Canary Islands), mainland Portugal and on the archipelagos of Madeira and Cape Verde. If mature seeds were available at the time of harvesting leaf samples (for genetic and cytological

analysis), these were collected to cover geographic gaps in the European *Patellifolia* holding. At the same time, leaf samples were harvested on approximately 800 individual plants in the natural habitat and prepared for SSR marker analysis. Also fresh leaves were taken from the same plants (a subset of the 800 samples) and were sent to the Centre for Functional Ecology (Department of Life Science, University of Coimbra) for flow cytometric analysis of the ploidy level of each individual, and to evaluate the occurrence of hybridization between *P. patellaris* and *P. procumbens.*

In addition to data on individual plants and probes, passport data on populations (population identifier) and seed collections (collection number) were also recorded, and further sent to the Julius Kühn-Institut (JKI), Quedlinburg (Germany) and compiled. During the collecting mission to Southeast Spain individual plants were geo-referenced.

The sampling procedure was as follows:

- For the molecular analysis, fresh leaf probes of 0.5-1 g from 20 (minimum) to 40 (maximum) individual plants were sent to the Julius Kühn-Institut, Quedlinburg (Germany).
- For the flow cytometric analysis, a branch with at least three leaves per individual was sent to Sílvia Castro, Centre for Functional Ecology, Department of Life Science, University of Coimbra (Portugal).
- For the herbarium specimens, at least two flowering plants were sent to M. Cristina Duarte, Instituto de Investigação Científica Tropical (IICT) at Lisbon (Portugal), for later identification.
- Seed samples were sent to the national genebank.

Development of SSR markers

Microsatellite primers were developed to promote studies on the relationship between the three species of the genus *Patellifolia* and the patterns of genetic diversity within species. 543 MB representing 72,453 single sequences with an average size of 7499 nt of the unpublished genome assembly Papro-1.0 from the *P. procumbens* accession BGRC 35335 (renamed by the genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, (Germany), as BETA 951) were screened for SSRs using SciRoKo v3.4 software (Kofler et al. 2007) and default search parameters. We were able to identify 3648 SSRs. A subset of 53 was validated using 24 individuals of *P. procumbens, TPH0604151144* from Tenerife, Punta del Hidalgo (Spain); of *P. webbiana, graisl1* from Gran Canaria, La Isleta (Spain); and of *P. patellaris, AZO2403151630* from Murcia, La Azohía, Playa de la Azohía (Spain).

RESULTS

Taxonomic standards

Standard accessions for different purposes were defined, including the BETA 534 (BGRC 57667), Almeria, Carboneras, 5 km N of Playa del Algarrobico (Spain) which is the international molecular standard named *P. patellaris*, and BETA 951 (BGRC 35335) named *P. procumbens* of which the collecting site is unknown. BETA 951 was used for the sequencing of the *P. procumbens* genome and thus was considered a reference standard.

Gap analysis and collection to close gaps

The *Patellifolia* species occurs in Portugal, Spain, Cape Verde, Morocco, Algeria and possibly Italy. The distribution area was divided in 26 geographic subunits, including eco-geographic zones,

province, island or even islets (Table 1). We assumed that occurrences within these subunits are spatially isolated and thus represent a specific fraction of intraspecific diversity. While *P. patellaris* can potentially be found in the 26 geographic regions, there are good reasons to assume that *P. procumbens* an *P. webbiana* can only be found in 15 and 1 of these geographic units, respectively (Table 1).

Breeding researchers need a set of well described and readily available accessions. A *Patellifolia* AEGIS collection should therefore be composed by one seed accession from each of the 26, 15 and 1 targeted geographic units of *P. patellifolia*, *P. procumbens* and *P. webbiana*, respectively. Therefore, for each geographic subunit, accessions with passport data recorded according to the FAO/Bioversity Multi-Crop Passport Descriptors (MCPDs) (Alercia et al. 2012) were identified in genebank information systems and selected as MAA candidates, resulting in 7 MAA candidates for *P. procumbens* and 14 candidates for *P. patellaris*. The set of AEGIS accessions can be completed by accessions held by the United States Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA-ARS-NPGS).

Table 2 (page 9) lists MAA candidates and describes action to be undertaken before accessions can be nominated by the holding genebanks. Accession B0591 (holder genebank GBR003 [School of Biological Sciences, The University of Birmingham, UK])[1] was regenerated by the JKI in 2015. Accession numbers BETA 882, BETA 862, BETA 622, BETA 928, and BETA 534 (holder genebank DEU146 [Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research]) also belong to the set of 14 MAA candidates of *P. patellaris*. The latter five have been nominated as Multilateral System (MLS) accessions and are safety-duplicated at Spitzbergen (holder NOR051 [Svalbard Global Seed Vault, Norway]). In conclusion, out of 42 accessions needed for building a representative AEGIS *Patellifolia* collection, 22 accessions are stored in genebanks or have been collected recently and would in principle be eligible.

The International Treaty calls for the implementation of complementary conservation actions. Information on recommended genetic reserve sites for *Beta* and *Patellifolia* is available at: www.agrobiodiversidad.org/aegro. *Ex situ* and *in situ* conservation actions can be organized for accession ISOP (holder PRT102 [Banco de Germoplasma – Universidade da Madeira, Portugal]). The sample was collected within the Parque Natural da Madeira – Ponta de São Lourenço (Portugal), PTMAD0003. The establishment of a genetic reserve site for *Beta patula* Aiton is being planned by the responsible authorities (Pinheiro de Carvalho et al. 2012), which would also cover the site of *P. procumbens*. A genetic reserve site for *P. webbiana* within the protected Area Marítima de La Isleta (Spain), ES7010016, was also proposed. Accession B0614 (*P. procumbens*) and B0597 (*P. patellaris*), holder GBR003, were once collected near Punta del Hildalgo, Tenerife (Spain). This site is located just outside the protected area ES0000109 (Anaga, Bird Directive). The site would be very valuable for the *in situ* conservation of wild beet genetic diversity. *Beta macrocarpa* Guss., *P. patellaris* and *P. procumbens* all occur in the area. The latter two species seem to cross and form a hybrid swarm as indicated by the high morphological diversity (see composite photo of the cover page) and the occurrence of di-, tri- and tetraploid plants.

Sampling

New populations were found in Southeast Spain, Portugal and Cape Verde. Table 3 (page 11) provides an overview of the material sampled in 2008 and in 2015. Not all of the populations visited where as rich in number of individuals as expected, so not all the populations have the same size of individuals available for analysis.

[1] Institute codes of holding genebanks according to FAO WIEWS

Development of SSR markers

Twenty-five SSRs proved to be polymorphic in the three species except for marker *JKIPat16* which is specific for *P. webbiana*. The number of alleles varies between the three *Patellifolia* species. Altogether, the 25 polymorphic SSR loci bear 87, 187, and 227 alleles in *P. patellaris, P. procumbens*, and *P. webbiana*, respectively. The number of alleles per locus within a species ranges from 1 to 8 (*P. patellaris*), 2 to 15 (*P. procumbens*) to 4 to 15 (*P. webbiana*). With these new 25 markers, the populations of the genus *Patellifolia* stored in genebanks will be characterized. The sequencing of the SSRs is in progress. When finished, a primer note will be submitted for publication and the information will be made publicly available.

RECOMMENDATIONS

1. BETA 534 (BGRC 57667) can be used as taxonomic standard for *P. patellaris* and BETA 951 (BGRC 35335) for *P. procumbens*. The standards should be used by genebank curators to categorize accessions, and when the taxonomic problems will be solved, to classify the accessions.

2. Genebank curators should focus seed multiplication efforts on those accessions that were identified as MAAs. The genebank at Madrid could initiate the recollection of *P. webbiana* at La Isleta. Accessions held by the Spanish and Portuguese genebanks need to be nominated as MLS first.

3. All *Patellifolia* accessions shown in Table 1 that were already included in the MLS should be included in AEGIS.

4. There is a clear need for closing geographic gaps in *ex situ* holdings and a need for the establishment of genetic reserves for *Patellifolia* species.

5. A set of 25 polymorphic genetic markers is available and can be used to study different aspects of genetic diversity in *Patellifolia*. It seems that, in particular, *P. procumbens* populations growing in the natural habitat are much more diverse than accessions kept in genebanks. It can be assumed that the seed regeneration of the hard-seeded species caused genetic bottleneck and loss of genetic variation in the accession. A comparison of the amount of genetic diversity kept in genebanks with the amount of genetic diversity available in a number of geographically distinct sites of *P. procumbens* and *P. patellaris* would show how much of the diversity was captured in the past by collectors and is currently being conserved *ex situ*. Such investigation would contribute to the quality of managing genetic diversity of *Patellifolia*.

6. A significant number of *Patellifolia* accessions are being conserved by the USDA-ARS-NPGS and other partners outside Europe. We suggest therefore setting up a joint EU/USA project dealing with aspects outlined in recommendation 5.

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ANNEXES

Genetic diversity of *Patellifolia* species (GeDiPa)

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Table 1. Gap analysis and recommendation of MAAs

Aim:
One accession per species per known distribution area

Rationale:
Breeding researchers do not need many accessions, but a well described set of accessions. The AEGIS *Patellifolia* holding should facilitate access to a set of accessions that represents all known distribution areas (islands / provinces) of the species.

Selection criteria for accession numbers:
Passport data with description of collecting site

1a. *P. procumbens*

Country	Location		*procumbens*	Accession	Holder	Multi-lateral System	Dupli-cation Site	Proposed Genetic Reserve
Portugal (PRT)	Madeira	Madeira, main island	?					
		Ilheu do Desembarcadouro	x	ISOP 1510	PRT102			Parque Natural da Madeira - Ponta de São Lourenço - PTMAD0003
		Ilheu Chao	x					
		Ilheu Farol	x					
		Porto Santo	x	ISOP 1902	PRT102			
		Salvage Islands	x					
	PRT Mainland	Setúbal						
		Faro						
Cape Verde Republic (CPV)	Cape Verde	São Vicente						
Spain (ESP)	Canary Islands	El Hierro	x					
		Fuerteventura	?	B0594	GBR003			
		Gran Canaria	x	B0554	GBR003			
		La Gomera	x	B0628	GBR003			
		Lanzarote	?					
		La Palma	x	B0618	GBR003			
		Tenerife	x	B0614	GBR003			Anaga (Bird Directive) ES0000109
	ESP Mainland	Almería						
		Málaga						
		Murcía						
		Alicante						
Morocco (MAR)		Zone E						
		Zone C						
		Zone D (Aglou)						
		Zone D (Agadir)						
Algeria (DZA)			?					
Italy (ITA)		Linosa	x					
	Number of geographic subunits with *Patellifolia*		**15**					

= species does likely not occur in the subunit ? = not sure if species occur in the subunit

1b. *P. webbiana*

Country	Location		*webbiana*	Accession	Holder	Multi-lateral System	Dupli-cation Site	Proposed Genetic Reserve
Portugal (PRT)	Madeira	Madeira, main island						
		Ilheu do Desembarcadouro						
		Ilheu Chao						
		Ilheu Farol						
		Porto Santo						
		Salvage Islands						
	PRT Mainland	Setúbal						
		Faro						
Cape Verde Republic (CPV)	Cape Verde	São Vicente						
Spain (ESP)	Canary Islands	El Hierro						
		Fuerteventura						
		Gran Canaria	x					Area maritima de La Isleta - ES7010016
		La Gomera						
		Lanzarote						
		La Palma						
		Tenerife						
	ESP Mainland	Almería						
		Málaga						
		Murcía						
		Alicante						
Morocco (MAR)		Zone E						
		Zone C						
		Zone D (Aglou)						
		Zone D (Agadir)						
Algeria (DZA)								
Italy (ITA)		Linosa						
	Number of geographic subunits with *Patellifolia*		**1**					

= species does likely not occur in the subunit

1c. *P. patellaris*

Country	Location		*patellaris*	Accession	Holder	Multi-lateral System	Dupli-cation Site	Proposed Genetic Reserve
Portugal (PRT)	Madeira	Madeira, main island	x	BETA 882	DEU146	y	NOR051	
		Ilheu do Desembarcadouro	?					
		Ilheu Chao	?					
		Ilheu Farol	?					
		Porto Santo	x	ISOP2834	PRT102			
		Salvage Islands	?					
	PRT Mainland	Setúbal	x	FM-1	Genebank, Braga			
		Faro	x	PS-1	Genebank, Braga			
Cape Verde Republic (CPV)	Cape Verde	São Vicente	x	CV.SV.RSJ-1 or CV.SV.BG-1 (*)	Genebank, Braga			
Spain (ESP)	Canary Islands	El Hierro	x					
		Fuerteventura	x	B0591	GBR003			
		Gran Canaria	x	BETA 862	DEU146	y	NOR051	
		La Gomera	x	BETA 622	DEU146	y	NOR051	
		Lanzarote	x	BETA 928	DEU146	y	NOR051	
		La Palma	x	B0617	GBR003			
		Tenerife	x	B0597	GBR003			Just outside of Anaga (Bird Directive) ES0000109
	ESP Mainland	Almería	x	BETA 534	DEU146	y	NOR051	
		Málaga	x	ESP/2015/JMI&LdH-2	Genebank, Madrid			
		Murcía	x	ESP/2015/MLRT&PS-1	Genebank, Madrid			
		Alicante	x	ESP/2015/PFG&EL-1	Genebank, Madrid			
Morocco (MAR)		Zone E	x					
		Zone C	x					
		Zone D (Aglou)	x	W6 44512	USA008			
		Zone D (Agadir)	x					
Algeria (DZA)			x					
Italy (ITA)		Linosa	?					
	Number of geographic subunits with *Patellifolia*		**26**					

? = not sure if species occur in the subunit = will these seeds be made available as MLS / AEGIS accessions?

(*) (CV.SV.RSJ-1) and (CV.SV.BG-1): both accessions have been collected on Cape Verde. The legal status of the accessions still needs to be clarified.

Table 2. List of MAA candidates

Species	Accession number	Population ID	Holder	Action to be taken	Number of candidates
P. procumbens	ISOP 1510		PRT102	Check seed quality and quantity, add to MLS/AEGIS	
	ISOP 1902		PRT102	Check seed quality and quantity, add to MLS/AEGIS	
	B0594		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	B0554		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	B0628		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	B0618		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	B0614		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
					7
P. patellaris	BETA 882		DEU146	Add to MLS/AEGIS	
	ISOP2834		PRT102	Check seed quality and quantity, add to MLS/AEGIS	
		(FM-1)	Genebank, Braga	Seed multiplication, then add to MLS/AEGIS	
		(PS-1)	Genebank, Braga	Seed multiplication, then add to MLS/AEGIS	
		CV.SV.RSJ-1 or CV.SV.BG-1 (*)	Genebank, Braga	Clear legal status. If available as MLS/AEGIS sample increase seed sample.	
	B0591		GBR003	Add to MLS/AEGIS	
	BETA 862		DEU146	Add to MLS/AEGIS	
	BETA 622		DEU146	Add to MLS/AEGIS	
	BETA 928		DEU146	Add to MLS/AEGIS	
	B0617		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	B0597		GBR003	Check seed quality and quantity, add to MLS/AEGIS	
	BETA 534		DEU146	Add to MLS/AEGIS	
		ESP/2015/JMI&LdH-2	Genebank, Madrid	Check seed quality and quantity, add to MLS/AEGIS	
		ESP/2015/MLRT&PS-1	Genebank, Madrid	Check seed quality and quantity, add to MLS/AEGIS	
		ESP/2015/PFG&EL-1	Genebank, Madrid	Check seed quality and quantity, add to MLS/AEGIS	
					14

(*) (CV.SV.RSJ-1) and (CV.SV.BG-1): both accessions have been collected on Cape Verde. The legal status of the accessions still needs to be clarified.

Table 3. Overview on collected leaf and seed samples

Population identifier	Country code	Site location	Species	Number of individual leaf samples	Seed sample
CV.SV.RSJ-1	CPV	Cape Verde, São Vicente Island	*patellaris*	14	Yes
CV.SV.BG-1	CPV	Cape Verde, São Vicente Island	*patellaris*	6	No
PS-1	PRT	Faro	*patellaris*	28	Yes
FM-1	PRT	Setúbal	*patellaris*	4	Yes
PSM2704151447	PRT	Madeira	*patellaris*	2	Yes
PSM2704151525	PRT	Madeira	*procumbens*	-	Yes
PSM2704151633	PRT	Madeira	*patellaris*	-	Yes
PSM2704151128	PRT	Madeira	*patellaris*	-	Yes
PSM2704151706	PRT	Madeira	*patellaris*	-	Yes
PSM2704150948	PRT	Madeira	*patellaris*	-	Yes
PSM2704151044	PRT	Madeira	*procumbens*	-	Yes
PSM2704151848	PRT	Madeira	*patellaris*	-	Yes
PSM2704151936	PRT	Madeira	*patellaris*	17	Yes
FXM0707151719	PRT	Madeira	*patellaris*	23	Yes
CLM0707151601	PRT	Madeira	*patellaris*	17	Yes
CTM0707151415	PRT	Madeira	*patellaris*	15	Yes
MOR0903151000	ESP	Alicante	*patellaris*	20	No
CNE2303151030	ESP	Murcia	*patellaris*	40	No
AZO2403151630	ESP	Murcia	*patellaris*	25	No
PCA3003151000	ESP	Málaga	*patellaris*	28	Yes
CGO3103151000	ESP	Málaga	*patellaris*	35	No
BAL2104150900	ESP	Almería	*patellaris*	40	Yes
STE2104151800	ESP	Almería	*patellaris*	40	Yes
COL2005151000	ESP	Alicante	*patellaris*	13	No
HS	ESP	El Hierro	*patellaris*	46	No
S	ESP	La Gomera	*patellaris*	42	No
H	ESP	La Gomera	*patellaris*	42	No
PPL	ESP	La Palma	*patellaris*	26	No
TLG	ESP	Tenerife	*patellaris*	25	No
TPM	ESP	Tenerife	*patellaris*	22	No
TES	ESP	Tenerife	*patellaris*	22	No
TPA	ESP	Tenerife	*patellaris*	25	No
TLS	ESP	Tenerife	*patellaris*	25	No
TAL	ESP	Tenerife	*procumbens*	23	No
TBA	ESP	Tenerife	*procumbens*	25	No
TPS	ESP	Tenerife	*procumbens*	22	No
TPC	ESP	Tenerife	*procumbens*	30	No
TGA	ESP	Tenerife	*procumbens*	25	No
TPH0604151144	ESP	Tenerife	*procumbens*	25	No
TPH0604151200	ESP	Tenerife	*patellaris*	8	No
TJ0604151815	ESP	Tenerife	*patellaris*	6	No
TB0604051905	ESP	Tenerife	*procumbens*	7	No