

Proposal

1. Problem statement.

In the management of collections of plant genetic resources of many species the taxonomic classification is often not sufficient to identify duplicate accessions.

Is the *Brassica rapa* model of MAA's selection criteria valid to *Brassica oleracea* in the Iberian collection?

2. Justification and rationale

The *Brassica oleracea* Iberian collection is an important source of genetic materials for Food and Agriculture. Portuguese Coles, “tranchuda” cabbage and “galega” kale are a rather unique but diversified group of vegetables that can only be found in Portugal and Spain (Galicia), or in regions with a strong Iberian influence.

The Portuguese *Brassica* collection preserved in Banco Português de Germoplasma Vegetal (**BPGV, INRB, I.P.**), which passport information of the collection is included in the CCDB for this crop (Bas, 2009) and in EURISCO, holds 935 accessions and including three species: *B. rapa*, *B. napus* and 542 accessions of *B. oleracea* L., which is the national collection resulting from collecting missions taken place between 1990 and 2005. 88 percent of collection resulted from accessions collected in farmers between 1990 and 1995, being 84% from the North and Central of Portugal.

To preserve and characterize Galician landraces of the *Brassica* genus, a germplasm bank was created at the Misión Biológica de Galicia (**MBG, CSIC**) at Pontevedra, Spain. This gene bank keeps a Brassica collection of local landraces adapted to Atlantic conditions, which have been collected from the northwestern Spain since the 1980s until the present and which passport data is in EURISCO. Currently, the collection comprises 507 accessions from three species including 250 accessions of *B. oleracea*.

As part of effective management of these collections, probable duplicate accessions are routinely identified by the curator, both through the examination of passport data and via field characterization.

A part of the Iberian collection was morpho-agronomic, molecular, and biochemical characterized (glucosinolates, phenolic compounds and minerals). However many aspects of the phylogeny within the species or cultivar groups and the classification of the different morphotypes need to be improved. In order to gain a better understanding of the genetic variability of the Iberian landraces, to look at genetic drift among accessions held in different locations, to improve the crop types classification and, the rationalization of germplasm collections by reducing the number of duplicate accessions the molecular markers can be useful. These goals are an important step for the application of MAA's model to European collection and to the AEGIS quality management

system "AQUAS" and to allow those genetic materials to be available for Food and Agriculture.

This proposal will contribute to the improvement of MAA's Brassica criteria workflow, and to the European Genebank Integrated System (AEGIS), making them available for breeding and research. Such material will be safely conserved under conditions that ensure genetic integrity and viability in the long term.

3. Background

The *Brassica* genus comprises a number of economically important species. Brassicas provide one of finest examples of convergent evolution in the horticultural forms of the European *Brassica oleracea* and Oriental *B. rapa*. *Arabidopsis thaliana* has achieved model system for molecular biological studies.

The *Brassicaceae* (*Cruciferae*) family are been studied by multiply scientific domains crossing nuclear knowing about Plant Genetic Resources, molecular characterization, phylogenies studies, ..., and studies at agriculture and food level. Since the 90s of last century the phylogenies studies were attained more relevance.

There is the multinational Brassica genome project since beginning the new millennium.

The ECPGR Vegetable Network and Brassica Working Group have been doing important service to Brassica PGR.

Simple sequence repeat (SSR; microsatellite) markers may provide a useful method for the characterization, conservation, and utilization of agricultural crop diversity. Identification and characterization of SSR markers have been done in different species of the *Brassica* genus. Microsatellite markers have been useful to study the genetic diversity of *Brassica*, to characterize and compare resynthesized rapeseed lines with spring rapeseed cultivars, and to distinguish between spring/winter types or genotypes. No studies have been conducted so far on the molecular characterization of *B. oleracea* germplasm from the Iberian collection. Hence, little is known about its genetic diversity as well as its relationship and about redundant accessions.

In the Bibliography point it will be presented some documents that support the proposal as background.

4. Main objective and specific objectives

Main Objective: Apply the MAA's selection criteria proposed for *B. rapa* to the Iberian of *B. oleracea* collection.

Specific objectives: i) **apply the MAA's selection criteria proposed for *B. rapa*** to the Iberian of *B. oleracea* collection; ii) **apply molecular markers** (ITS and SSR) to understanding of the genetic variability of the Iberian landraces, to look at genetic drift among accessions held in different locations, to improve the crop types classification and to eliminate the duplicates; iii) **propose the MAA's of Iberian collection.**

5. Materials and methods

Plant material: **BPGV collection** [542 accessions of *Brassica oleracea* L. (6 *B. oleracea* var. *capitata*; 107 accessions of *B. oleracea* var. *costata*; 395 accessions of *B. oleracea* var. *acephala*)]; **MBG collection** [250 accessions of *Brassica oleracea* L. (210 accessions of *B. oleracea* var. *acephala*; 2 accessions of *B. oleracea* var. *costata*; 38 accessions of *B. oleracea* var. *capitata*)].

Methodology: To apply *MAA's criteria selection* to *Brassica oleracea* accessions with germination above the minimum standard, determined by ISTA guidelines, and minimum 500 seeds as it is recommended in Quality management system for AEGIS doc, **and** carry out phylogeny study to MAA's defined by *B. rapa model* application with *IST chromosome region* and, genetic diversity by *microsatellite molecular markers*

The germination monitoring component will be a responsibility of BPGV and MBG teams. The application of the MAA's selection criteria will be a responsibility of: Centre for Genetic Resources (CGN) and Genetic Resources Unit of University of Warwick, with BPGV and MGB teams.

After applying the MAA's selection criteria, a specific group of accessions from the Iberian collection will be subject to phylogenetic and genetic diversity studies. The phylogeny studies will be a responsibility of BPGV and the University of Porto and genetic diversity by SSRs will be a responsibility of MBG partners.

Two technical meetings between all partners, will take place one at BPGV (Portugal) and the other at MBG (Spain).

6. Expected outputs

It will be expected a *scientific doc* presentation to *generalise the B. rapa model* to *B. oleracea* crop and/or a new model adapted to this crop with another workflow; to *get material* for the regeneration activity; *improvement* of different morphotypes classification and better knowledge of the Iberian landraces genetic variation; *quality standards* in the collection management procedures.

7. Benefits and impact

The output will consist in a feasibility study on how the of *B. rapa model* will apply to the *B. oleracea* accessions, for a better establishment and operation of

AEGIS. Furthermore more knowledge will be carry out about the Iberian Collection and the European *Brassica oleracea* variability.

8. Innovation

The proposal will add knowledge about Iberian *Brassica oleracea* landraces collection in the European *Brassica oleracea* collection. Enlargement of *B. rapa* model to *B. oleracea* and/or a new model adapted to this crop with other workflow.

9. Application of results

For the Iberian collection management we will apply the AEGIS quality management system, making these genetic materials available for Food and Agriculture. This genetic material will be a part of the AEGIS collection, with registration of the MAA's accessions, safely preserved under conditions that ensure genetic integrity and viability in long term conditions.

9. Workplan

The activities are planned for twelve months beginning in March 2011.

BPGV, Instituto Nacional de Recursos Biológicos, Braga, Portugal

Main activities	Timeline (months)											
	M	A	M	J	J	A	S	O	N	D	J	F
A1: Germination monitoring	■	■	■	■								
A2: Quantity of sample available	■	■	■	■								
A3: MAA's criteria selection			■	■	■	■						
A4: ITS chromosome region methodology application					■	■	■	■	■	■		
A6: Meetings		■									■	■
A7: Scientific doc elaboration									■		■	■

University of Porto, Porto, Portugal

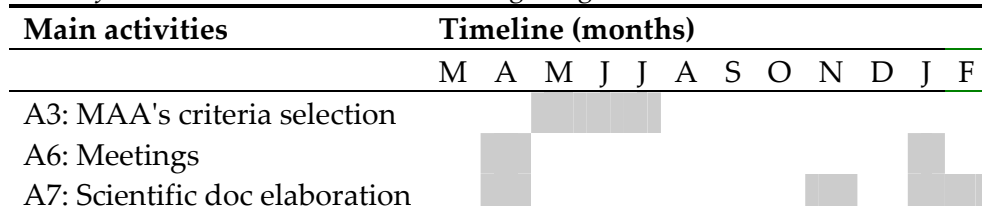
Main activities	Timeline (months)											
	M	A	M	J	J	A	S	O	N	D	J	F
A4: ITS chromosome region methodology application					■	■	■	■	■	■		
A6: Meetings		■									■	■
A7: Scientific doc elaboration									■		■	■

Misión Biológica de Galicia, Pontevedra, Spain

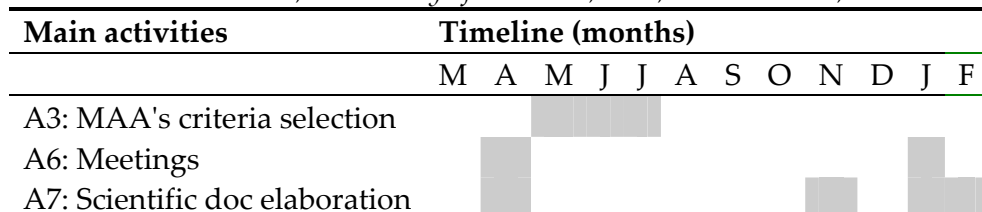
Main activities	Timeline (months)											
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A1: Germination monitoring	■	■	■	■								
A2: Quantity of sample available	■	■	■	■								



Centre for Genetic Resources (CGN), Wageningen, the Netherlands



Genetic Resources Unit, University of Warwick, HRI, Wellesbourne, United Kingdom



10. Budget

BPGV, Braga, Portugal

▪ Travel: non	
▪ Meetings:	500,00€
▪ Equipment: Laboratory consumables	2 000,00€
▪ Supply/Services	450,00€
▪ Total	2 950,00€

University of Porto, Porto, Portugal

▪ Travel: non	
▪ Meetings: Technical meetings	500,00€
▪ Equipment: Laboratory consumables	1 500,00€
▪ Supply/Services	750,00€
▪ Total	2 750,00€

Misión Biológica de Galicia, CSIC, Pontevedra, Spain

▪ Travel	
▪ Meetings: Technical meetings	500,00€
▪ Equipment: Laboratory consumables	2 200,00€
▪ Supply/Services	550,00€

▪ Total	3 250,00€
Centre for Genetic Resources (CGN)	
▪ Staff time	1 000,00€
▪ Travel: non	
▪ Meetings: Technical meetings	750,00€
▪ Supply/ Services: non	
▪ Total	1 750,00€
Genetic Resources Unit of Warwick, HRI.	
▪ Staff time	1 000,00€
▪ Travel: non	
▪ Meetings: Technical meetings	750,00€
▪ Supply/ Services: non	
▪ Total	1 750,00€
Total	
▪ Staff time	2 000,00€
▪ Travel: non	
▪ Meetings	3 000,00€
▪ Equipment: Laboratory consumables	5 700,00€
▪ Supply/ Services	1 750,00€
▪ Total	12 450,00€

11. Contributions foreseen by applicant.

The applicants will give the working times for coordination and development of the tasks considered in the project, except *Centre for Genetic Resources (CGN)* and *Genetic Resources Unit of Warwick*, as an input in kind, together with energy costs, working places, germination tests and the use of laboratory devices.

12. Bibliography

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