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# Characterization of a Spanish *Brassica oleracea* collection by using molecular and biochemical markers

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ABSTRACT

*Brassica oleracea* landraces are widely grown in northwestern Spain but also in other regions of the Spanish territory like the Canary Islands, where environmental conditions are very different from the peninsular ones. A collection of *B. oleracea* accessions from different Spanish locations is kept at the germplasm bank of *Misión Biológica de Galicia* (MBG, Spain). Considering that *B. oleracea* is the most diverse species within the *Brassica* genus, the study of these accessions is crucial to ensure the availability for breeding programs as a genetic variability source. In order to assess the genetic and biochemical diversity across this collection, we used simple sequence repeats (SSR) as molecular markers and the glucosinolate content and profile as a chemical marker. We concluded that there are significant genetic differences among accessions and that they are grouped by the same geographical provenance. Furthermore, different glucosinolate profiles were observed between peninsular and insular accessions, thus supporting the results obtained in the genetic analysis. Moreover, we identified accessions with a high GSL content as potential candidates to be used in breeding programs in order to obtain GSL-rich accessions.



## 1. Introduction

Genetic diversity can be defined as the total number of genetic characteristics of a species, which allows it to evolve and adapt to new environments and natural selection pressures (McCouch et al., 2012). Therefore, the availability of diverse genetic sources at a local level is essential to cope with emerging threats, which pose as a danger to genetically-homogeneous commercial crops (Chaudhary, 2013). The major purpose of a germplasm bank is to safeguard this genetic variation so that even if it is lost from original environments, it will remain accessible to plant biologists or breeders. However, a germplasm bank has not the sole purpose of storing genetic resources, but should also provide information about them. Thus, proper germplasm bank management requires continuous classification and characterization of preserved accessions in order to know the level of genetic, morphological and biochemical variability of the collection and to eliminate redundant accessions (Bhullar et al., 2010).

*Brassica oleracea* is the most diverse species within the *Brassica* genus, being important for human nutrition, with multiple cultivars that can be classified by the morphology of their edible structures. This vegetable species includes crops like cauliflower, broccoli, or kales, among others (Parkin et al., 2014). Its origin is located on the coast of northwestern Europe, but nowadays, this species is extensively cultivated in Europe and Central Asia and it is an important source of vegetables in many countries (Gomez Campo and Prakash, 1999).

In Galicia, northwestern Spain, different *B. oleracea* varieties can be found, especially cabbage and kale crops. *Misión Biológica de Galicia* (MBG, CSIC, Spain) has been collecting *B. oleracea* varieties from this region since the 1980s, which allowed the creation of a *B. oleracea* germplasm bank (Cartea et al., 2008). The gathering was also spread to Galicia's neighboring areas, where *B. oleracea* crops are important too (Fig. 1). In recent years, the gathering was extended to the Canary Islands, where orography and environmental conditions are different from the Iberian Peninsula ones. The presence of these varieties in the Canary Islands may be explained by the fact that there have been migrants from northwestern Spain since the 16th century. These varieties have been adapted to the insular conditions, without mixing with other peninsular varieties, and they have not been the object of a systematic gathering so far. Several

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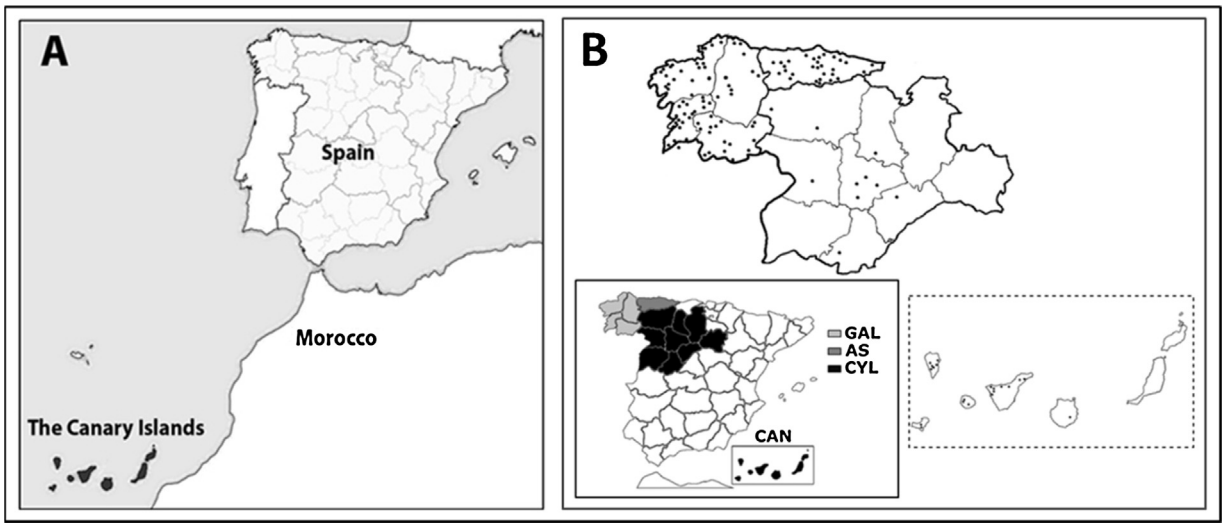


**Fig. 1.** A) Location of Spain and the Canary Islands in a political map; B) Distribution of the accessions analyzed in northwestern Spain and the Canary Islands. Dots represent the locations where accessions were collected GAL: Galicia; CAN: The Canary Islands; CYL: Castilla y León; AS: Asturias.

investigations aiming to characterize the morphological diversity of *B. oleracea* accessions have been published over the last years (Cartea et al., 2002; Vilar et al., 2007). However, the *B. oleracea* collection from MBG has not been characterized yet and the acquisition of new accessions requires a continuous characterization effort in order to minimize the number of germplasm bank's accessions but without losing genetic variability (Padilla et al., 2007).

Characterization based on molecular markers reflects the level of genetic variation existing among genotypes at the DNA level. Among the different molecular markers available, simple sequence repeats (SSR) are one of the most widely used for germplasm characterization (Baraket et al., 2011). These markers have a plethora of interesting features; their low cost, easy use and high degree of polymorphism should be highlighted (Van Inghelandt et al., 2010).

Glucosinolates (GSL) are a class of secondary metabolites ubiquitous in all *Brassica* species and they have a role in the plant defense against biotic and abiotic stresses, among other interesting properties (Traka and Mithen, 2009). The adaptation of *B. oleracea* crops to different climatic conditions and biotic stresses may cause a change in the GSL profile. The distribution of these compounds is diverse and varies among species and among crops from the same species. Thus, the GSL profile is an important chemotaxonomic criterion for classification within the *Brassicaceae* family (Heaney and Fenwick, 1980). Although several *Brassica* characterization studies have been made by using GSL as chemical markers (Cartea et al., 2008; Velasco et al., 2008), the whole *B. oleracea* collection from MBG germplasm bank has not been characterized yet.

Consequently, the objectives of the present work are (i) to assess the genetic structure and diversity present in *B. oleracea* accessions from MBG germplasm bank by using simple sequence repeats (SSR); and (ii) to determine the diversity of GSL content and profile in leaves of *B. oleracea* crops from different geographic origins.

## 2. Material and methods

### 2.1. Plant material and growth conditions

A collection of 191 *B. oleracea* accessions from different geographic origins was selected for performing genetic and biochemical analysis (Fig. 1). One hundred and sixty of them were used for SSR analysis and 108 were used for GSL analysis (Supplemental Table 1), being 77 of them common to both studies. The discrepancy between the number of accessions employed in SSR and GSL analysis is caused by field and seed availability.

Most of the accessions came from MBG germplasm bank, while others were requested from alternative germplasm centers in order to obtain the maximum genetic diversity. *B. oleracea* accessions selected in this study include the most important crops for these regions, i.e. kales, cabbages, tronchuda cabbages, and a putatively wild *Brassica* species (*B. bourgeaui*) from the Canary Islands. The latter is used in some areas as a vegetable crop and therefore, it may have hybridized with local varieties. Including *B. bourgeaui* in this study could mean a further step towards the understanding of the origin of this species.

For the molecular analysis, 20 individuals from each accession were sown in seedbeds in the greenhouse. Forty days later, the third leaf of each individual was collected for DNA extraction. In order to determine the individual and total GSL content of *B. oleracea* crops, accessions were sown in seedbeds in the greenhouse and were transplanted into the field in spring in 2013, at the three or four-leaves stage. Then, they were evaluated in a completely randomized design with three replicates. All trials were performed at MBG (Pontevedra, Spain. 42° 24′20" N, 8°38′24" W).

### 2.2. DNA extraction and SSR analysis

The DNA of each individual was extracted by following Liu and Whittier's method (1994). The concentration of each sample was measured spectrophotometrically and adjusted to 50 ng/μl. In order to account for intrapopulation variability, two bulks were made per accession by mixing DNA from 10 individuals. Each bulk was screened with a set of 12 SSR (Table 1).

A multiplex PCR analysis was performed for each bulk. This strategy allows the simultaneous amplification of several SSR in the same reaction by using labeled primers, which increases the amount of information generated per assay and reduces consumable and labor costs (Hayden et al., 2008). Amplifications were performed with a PTC-100 TM Thermal Cycler (MJ Research, Watertown, MA, USA). Amplified DNA products were separated by means of a capillary electrophoresis system (CEQ 8000, Beckman, Coulter). Amplified bands were scored as presence (1) or absence (0) across accessions. In addition, the total number of present alleles was calculated per SSR and accession.