PROJECT: BIO-MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF THE BRASSICA WORKING GROUP COLLECTION

Bioversity International Project Code 7207ECBEC001

Organization: Institute of Sustainable Agriculture – CSIC
Department of Plant Breeding
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Final Report

INTRODUCTION

During the meeting of the Brassica WG held in Olomouc (2007) it was decided to focus activities in the priority actions to the development of AEGIS (A European Genebank Integrated System). Later, during the ECPGR Vegetable Network meeting (2009) and the ECPGR Brassica working group meeting (2010), held respectively in Catania and in Linguaglossa, all the members proposed to point the attention on *B. rapa* which could be defined a multicrop species for its multiple utilisation and on wild Brassica species (n=9), that are distributed in the Mediterranean basin.

The general idea was to collect and/or set up an European core collection of about 20 accessions of wild Brassica species (n=9) and to characterize and evaluate them for bio-morphological traits, DNA and glucosinolate composition. In addition of this a collection of 40 accessions of *Brassica rapa* and 10 accessions belonging to other Brassica species received from different Genebanks should also be characterized and analyzed for glucosinolate composition. These activities have been carried out by DISPA (Department of Agriculture and Food Scienc dell' Universitá di Catania, Italy), CGN (Centre for Genetic Resources of Wageningen, The Netherlands), VIR (Vavilov Research Institute, St. Petersburg, Russia) and IAS (Institute of Sustainable Agriculture of Cordoba, CSIC.Spain).

The activities were mainly co-financed by specific projects which the members are carrying out and/or by the economical supports of breeders, research institutes/universities that are interested in regeneration, characterization and evaluation activities

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Description of the work

DISPA sown 26 accessions of wild Brassica species collected from several European gene banks, that were selected and provided by the Centre for Genetic Resources of Wageningen, The Netherlands (CGN). VIR sown 40 accessions of *B. rapa* and 10 accessions of *Brassica* spp received from different Genebanks. A sample of freeze-dried leaves from each accession was sent to the Institute of Sustainable Agriculture of Cordoba (CSIC-Spain) for glucosinolate analysis.

At the end of the project all data acquired will be inserted in the Bras-EDB.

Activities covered in the report

1) Analysis of glucosinolate content and composition of freeze-dried leaf samples from 26 accessions of wild Brassica species, provided by DISPA.
2) Analysis of glucosinolate content and composition of freeze-dried leaf samples from 40 accessions of *Brassica rapa* and 10 accessions of *Brassica* spp provided by VIR.
3) Provision of glucosinolate composition data to the Bras-EDB;
4) Provision of information and discussion about the obtained data to facilitate the preparation of a scientific publication on the results of this project, in collaboration with CGN, DISPA, VIR and Bioversity (to be completed after expiration of this agreement) .

MATERIAL AND METHODS

1. According with the agreement, we should analyse the glucosinolate content and composition of freeze-dried leaf samples from **26** accessions of wild Brassica species provided by DISPA.

On 06/07/2012 we received **26** different freeze-dried samples of wild Brassicas from Dr. Ferdinando Branca. We prepared an excel file with the information contained in the bag of each sample (see attached file “2012 2013 samples from DISPA), and we started the glucosinolate analysis of these samples by HPLC following the methods described in this section.

2. According with the agreement, we should analyse the glucosinolate content and composition of freeze-dried leaf samples from **40** accessions of *Brassica rapa* and **10** accessions of *Brassica* spp provided by VIR.

Brassica samples from Dra. Anna Artemyeva were sent via Germany on 29/08/2012, and arrived to Cordoba on 4/09/2012. We received **57** different samples of ground freeze-dried leaves of *Brassica rapa* and other Brassica species. We also received by email an excel file

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containing the description of the material received (see attached file “samples from VIR”). After checking the samples and the list we notice that samples 91 and 119 appeared in the list but not in the received material, and that we have received two bags of each of samples 92, 126 and 138. We communicate this fact to Anna, and we proceed to renumber the above mentioned duplicate bags as 92.1, 92.2, 126.1, 126.2, 138.1 and 138.2 to be differentiated and analysed separately, and we started the glucosinolate analysis of these samples by HPLC following the methods described in this section.

The glucosinolate composition of the samples was obtained by following the official reference method of the European Community. For each sample, 100 mg dry wt was weighed and a two step glucosinolate extraction was carried out in a water bath at 75 ºC to inactivate myrosinase according with our experience in the analysis of glucosinolate content in freeze-dried samples of Brassica leaves (Font et al., 2005). The sample was heated for 10+10 min in 2.5 + 2,5 ml 70% aqueous methanol and 2 micromol of glucotropaolin was added as internal standard. The combined glucosinolate extracts was pipetted onto the top of an ion-exchange column containing 1ml Sephadex DEAE-A25. Desulphation was carried out with purified sulphatase (E.C. 3.1.6.1, type H-1 from Helix pomatia) solution. Desulphated glucosinolates were eluted with Milli-Q(Millipore) ultra-pure water and analysed with a Model 600 HPLC instrument (Waters) at a wavelength of 229 nm. Separation was carried out by using a Lichrospher 100 RP-18 in Lichrocart 125-4 (Merck). HPLC solvents and gradient were according to the ISO protocol (ISO 9167-1, 1992). The HPLC chromatogram was compared to the desulpho-glucosinolate profile of three certified reference materials recommended by U.E. and ISO (CRMs 366, 190 and 367) (Wathelet et al., 1991). The amount of each individual glucosinolate present in the sample was calculated by mean of the internal standard, and expressed as micromol/ g dry wt. The total glucosinolate content was computed as the sum of all the individual glucosinolates present in the sample. Data were corrected for UV response factors for different types of glucosinolates (ISO 9167-1; EEC 1992). Each sample was analyzed in duplicate.

RESULTS AND DISCUSSION

A) Glucosinolate content of wild Brassicas from DISPA (attached file, 2013 Table 1)

The accessions analyzed shown a wide range of variation in total glucosinolate content: from 0,48 micromol/g dw in *B. bourgeaui* from Spain (BRA 2998 I Rip) to 52,48 micromol/g dw in *B. balearica* also from Spain.

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We have also found different patterns of glucosinolates within and between the species analyzed: *B. fruticolosa* from ITA has low content in total glucosinolates (9,22 micromol/g dw) and glucobrassicanapin is the predominant glucosinolate, whereas *B. fruticolosa* from Spain has high content in total glucosinolates (27,28 micromol/g dw) and gluconapin as predominant glucosinolate (Fig. 1).

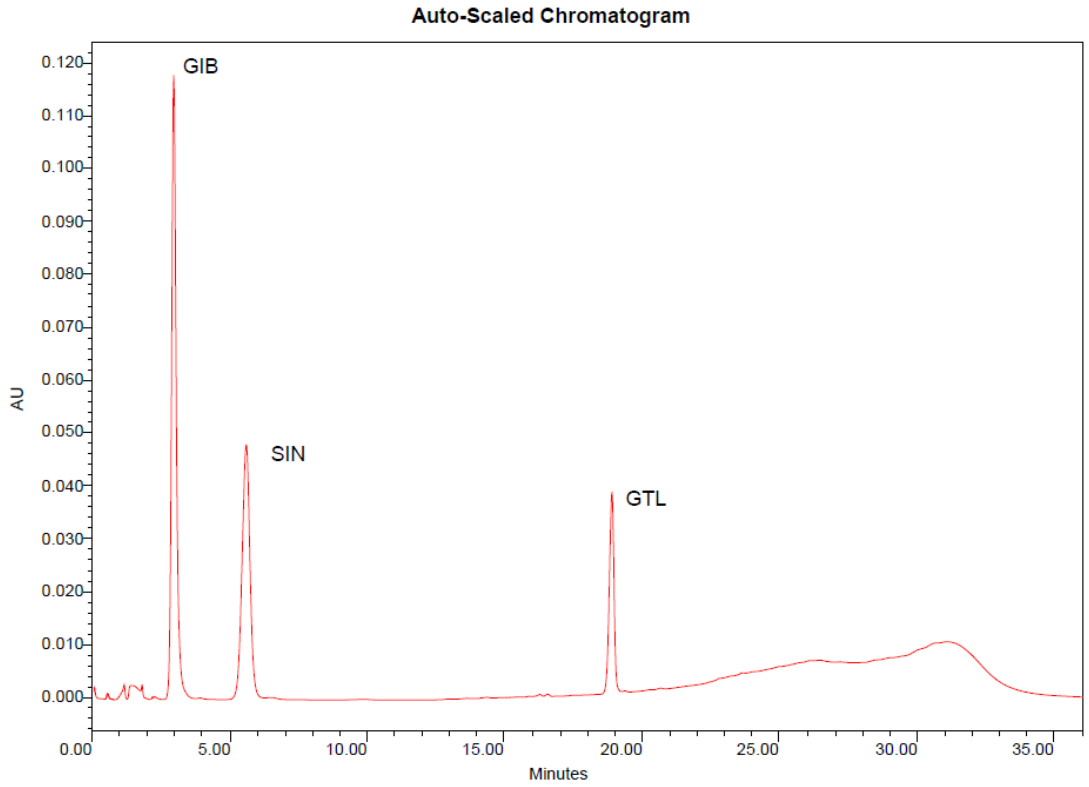

Fig. 1

Glucoiberin is the principal glucosinolate in *B. balearica*, *B. montana* and *B depranensis*. On the contrary, sinigrin is the predominant glucosinolate in *B. macrocarpa*. These species are good candidates to the genetic study of the pathway of sinigrin synthesis by genes located in the B genome.

Interestingly, we have found very high glucoraphanin content in two *B. rupestris* accessions (BRA 2945 and BRA 1896). This glucosinolate is also present in some accessions of *B. incana* and *B. villosa*. The above mentioned accession BRA 2945 also contains an important amount of glucoerucin. These accessions should be more deeply studied to discriminate both the genetics and the environmental effects on the content in these glucosinolates with recognized “anticancer” properties and preventive effects on DNA damages.

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B) Glucosinolate content of *Brassica rapa* and others Brassicas from VIR (attached file, 2013 Table 2)

We have also found a wide range of variation in total glucosinolate content: from 0,37 micromol/g dw in broccoletto (K 7884) to 53,30 micromol/g in turnip (K 9394).

All the accessions belonging to *B. napus* show a similar glucosiolate pattern, with progoitrin as the predominant glucosinolate, (with the exception of accession HRIGRU 2397, that contains high gluconapin content). On the other hand, the high sinigrin content and the lack of progoitrin of the accession CR 2301 (number 108) is in agreement with the presence of *B. carinata* or *B. jun*cea plants, but not with the presence of *B. napus* plants in this accession.

The predominant glucosinolates in *B. oleracea* accessions analyzed are gluconapin, glucobrassicanapin and neoglucobrassicin.

These glucosinolates are also present in the *B. rapa* accessions analyzed, although they have lower neoglucobrassicin content that *B. oleracea* accessions (Fig.2).

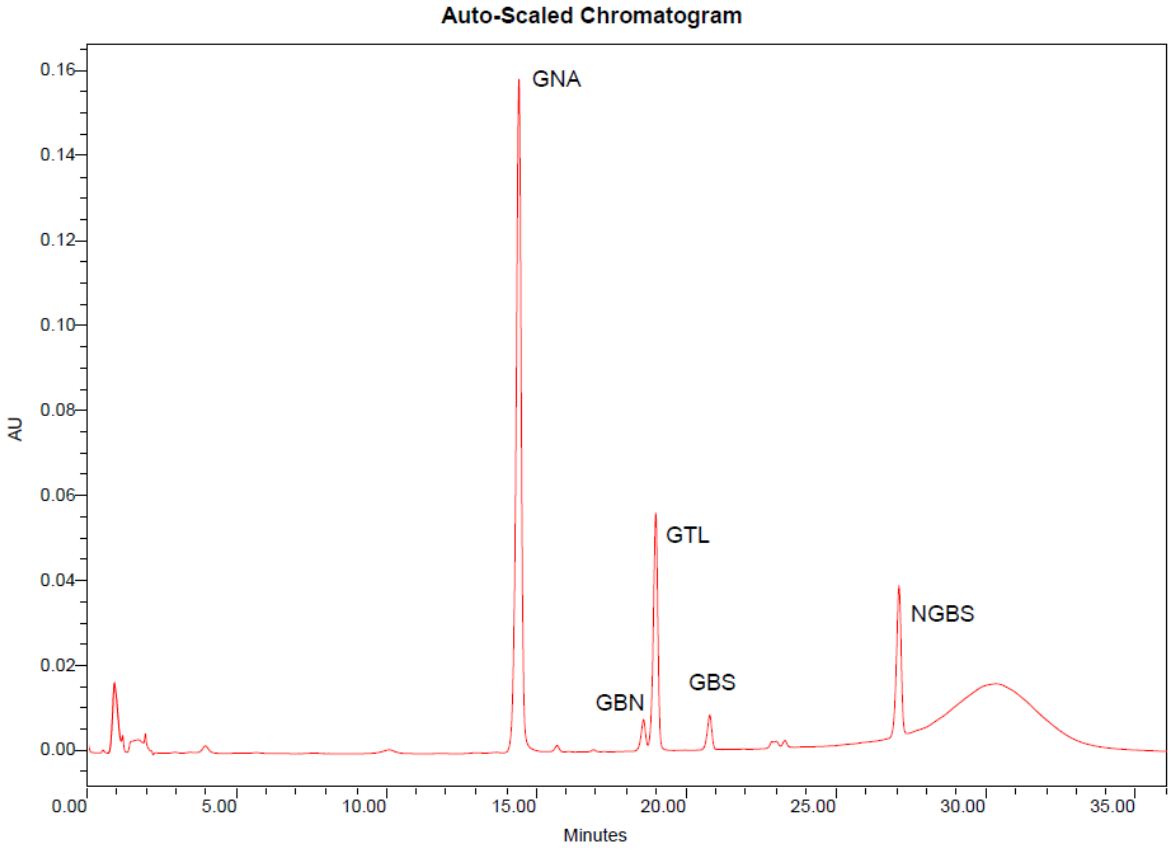

Fig. 2

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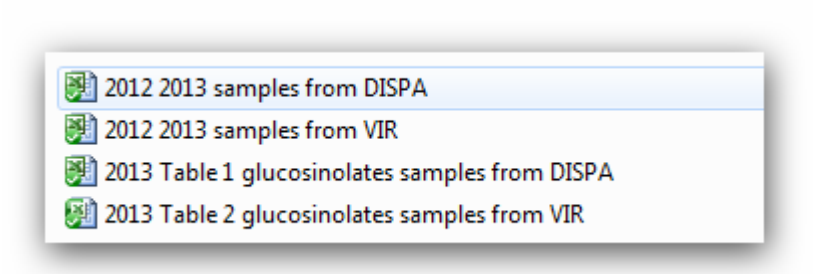
Finally, in all the *B. juncea* accessions analyzed, sinigrin and gluconapin are the predominant glucosinolates. These finding confirm that B genome is linked to the presence of important amounts of sinigrin (like in *B. nigra, B. carinata* and *B. juncea*).

DISSEMINATION OF RESULTS

On 06/11/2012 we sent to Dr. Branca the data of the glucosinolate analysis of samples, both from DISPA and VIR, obtained until that moment. We also sent to Dr. Branca some comments about these preliminary results. This information was included in the poster "In Progress activities of the Brassica working group of the European Cooperative Programme for Plant Genetic Resources (ECPGR)" that was shown at the 6th ISHS International Symposium on Brassicas and 18th Crucifer Genetics Workshop, held at Catania, Italy, 12-16 November 2012. These results have been published in Acta Horticulturae (Branca et al., 2013).

ATTACHMENTS



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