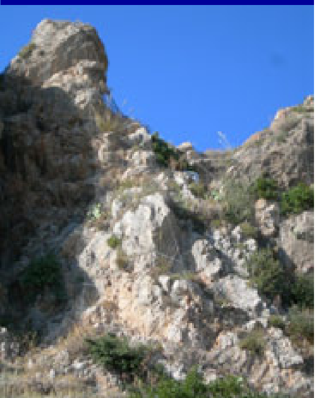

IN SITU CONSERVATION STRATEGY FOR THE ESTABLISHMENT OF GENETIC RESERVES OF CROP WILD RELATIVES (CWR) IN EUROPE: THE AEGRO PROJECT

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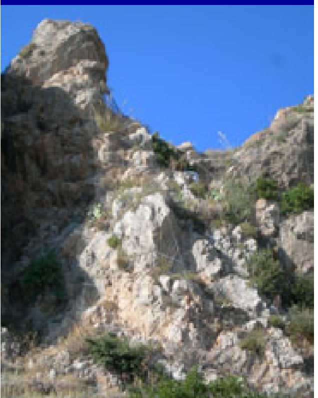
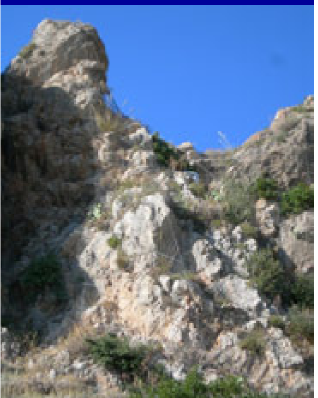


Crop wild relatives (CWR) are wild plants closely related to species of socio-economic importance

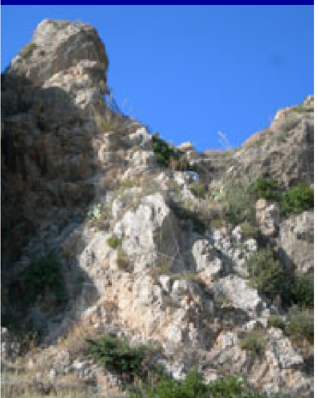
A landrace can be defined as a "dynamic population of a cultivated plant that has historical origin, distinct identity and lacks of formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming system".



According to the **Convention on Biological Diversity**

- ***ex situ*** conservation: the conservation of components of biological diversity **outside their natural habitat**
- ***in situ*** conservation: the **conservation of ecosystems and natural habitats and** the maintenance and recovery of **viable populations of species** in their natural surrounding and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties.

The term '**on-farm conservation**' is also used when referring to the conservation of landraces and CWR in an agricultural context.

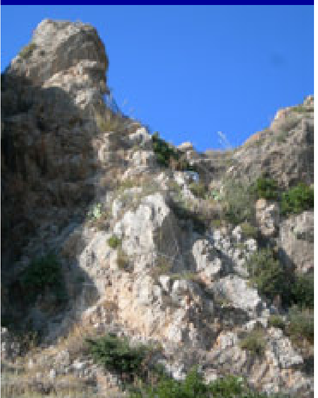


Why do we need to protect the CWR and landraces in their natural surroundings?

- **Agricultural activities** are nowadays based on the use of **highly performing F_1 hybrids** characterized by a narrow genetic base and landraces are slowly disappearing.

- **The survival of CWR *in situ*** is increasingly threatened by an increasing **loss of natural environments**.

  Main threats: **urbanization, forestation, fire, animal grazing, …**

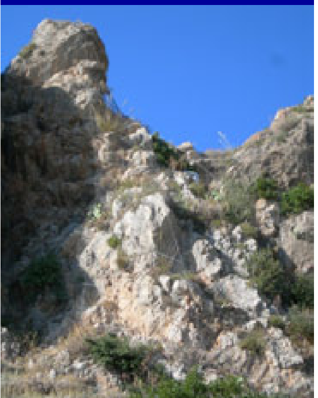


Why do we need to protect the CWR and landraces

in their natural surroundings?

Crop Wild Relatives are often not adapted to *ex situ* conservation and they will **often lose genetic variation during seeds reproduction cycles**.

Only a **fraction of the original genetic variation** will pass to the next generation.



How to ensure that CWR and landraces are better protected?

The genetic reserve conservation technique

CWR conservation approaches

Methodologies and con**servation techniques** are required to realize the *in situ* conservation strategy.

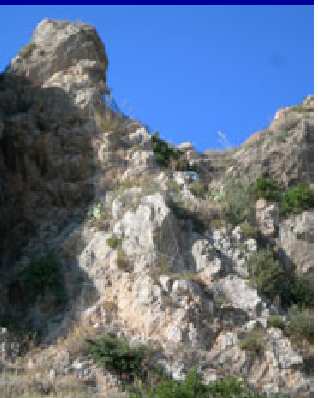
Genetic reserves are areas designated for the **active, long-term** in situ conservation of CWR populations where the primary consideration is to preserve their **genetic diversity**

Strategies for in situ conservation of CWR involve the **establishment of genetic reserves within protected sites.**

An Integrated European *In Situ* Management Work Plan:

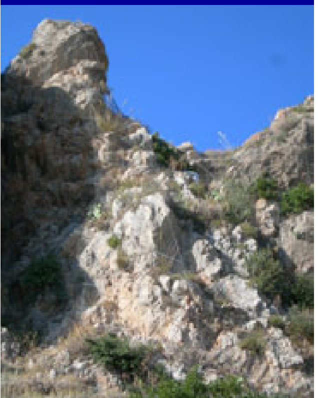
Implementing Genetic Reserve and On Farm Concepts (AEGRO)

AGRI GEN RES action 057



Appropriate in situ conservation methodologies for CWR were developed by the European Crop Wild Relative Diversity Assessment and Conservation Forum (**PGR Forum**) research project.

A group of the PGR Forum project team, together with experts of the working groups on **Avena, Beta, Brassica and Prunus** of the European Cooperative Programme for Plant Genetic Resources (ECPGR) in 2006, joined and continued to collaborate within AEGRO from 2007 to 2011.



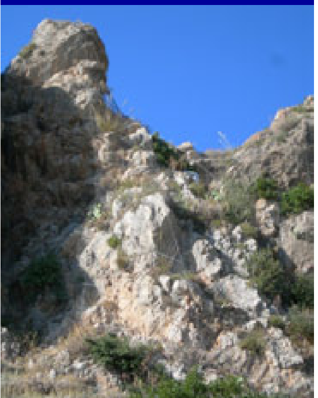
Objectives of the project

The main objectives of the AEGRO project for **CWR** were

- to improve the **conservation of CWR** and landrace diversity ***in situ*** and ***on-farm***,

- to enhance **methodologies** and **techniques**

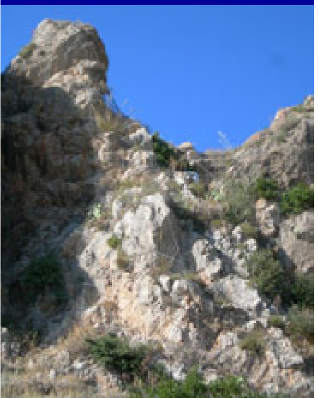
- to **reveal the constraints** impairing the application of the genetic reserve conservation technique in practice, as well as to **find solutions** to overcome such constraints



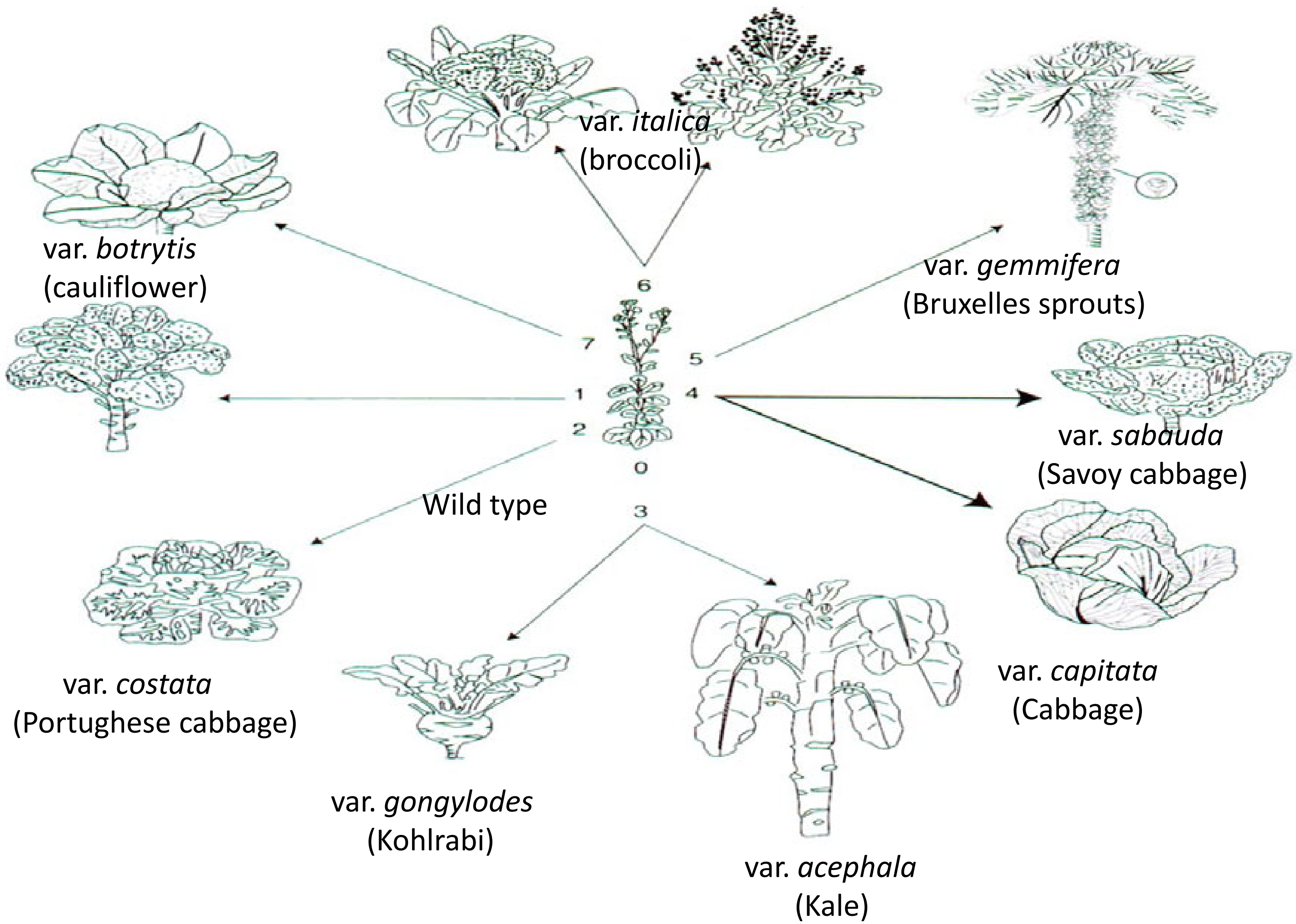
Objectives of the project

The AEGRO **landrace** workpackage focused Central Italy. It was known beforehand that local institutions and regional authorities had gathered landrace distribution data, which were used for the case study on landraces.

For the further development of *in situ* conservation management concepts for CWR, four crops (oat, sugar beet, brassica crop, and sweet cherry) were selected for crop-specific case studies.

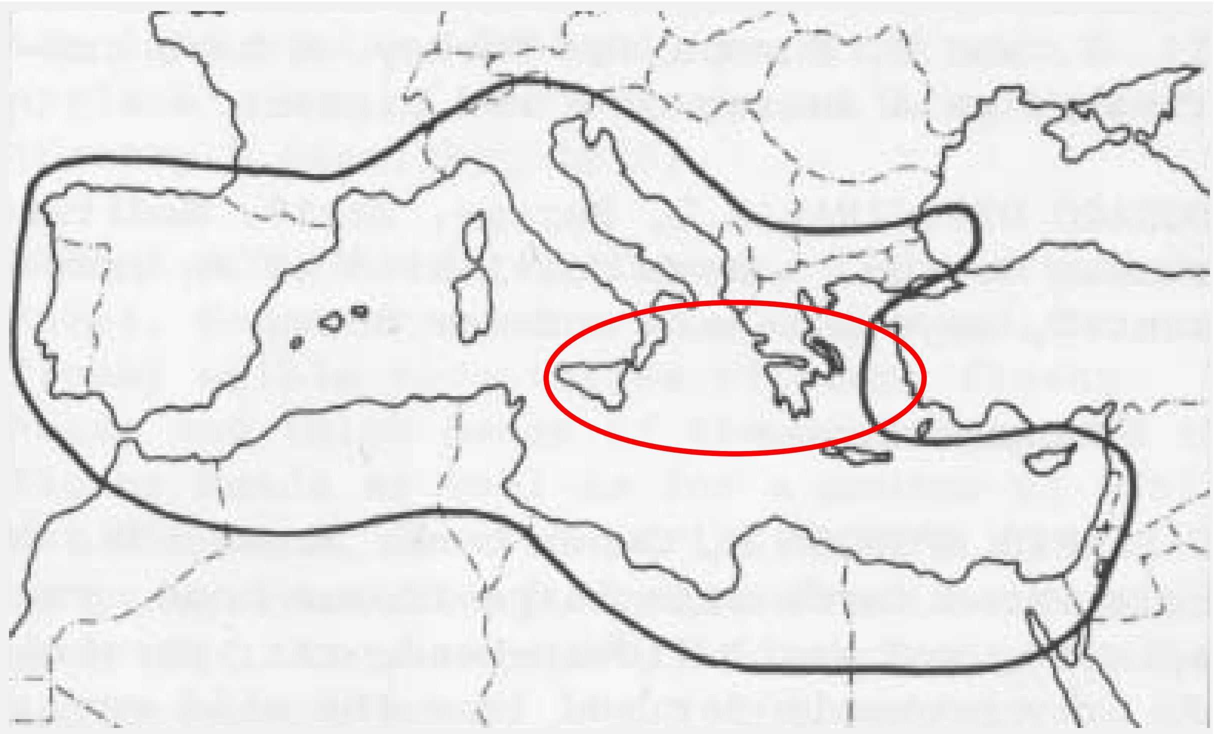


Evolution of the different *B. oleracea* crops from the wild relative



The interest in Brassica vegetable crops has recently grown due to the breeding programs carried out in several countries - mainly in Asia, Europe and the USA.

New opportunities are also offered by the food processing industry in exploiting traditional and new phenotypes for new products (IV and V gamma).



Recently several Authors showed molecular DNA evidences on the wide diversity between Mediterranean *Brassica* wild species and those from North Europe indicating that domestication of *B. olearacea* cultigroups occurred in of the central and central-eastern areas of Mediterranean basin (*Maggioni et al., 2010*)

Brassica wild relatives are perennial, with woody stems up to 1.5 m, large leaves and high glucosinolate content.

Genotypes are diploid (2n=18), often self-incompatible, with a high mutation tendency and cross pollinating.



Several Authors showed that in Sicily wild Brassicas (n=9) are fertile crossing with *B. oleracea* and they have bean monitored and studied for several purposes.



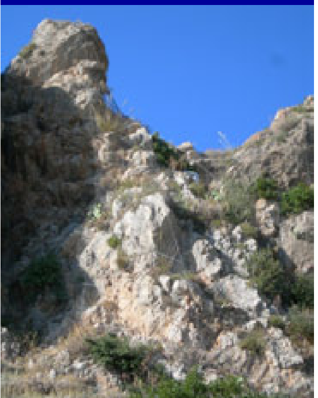
- *Brassica* wild relatives are valuable sources of genes for crop improvement. They have been widely used since more than one century to increase resistance/tolerance to biotic and abiotic stresses in several cvs.

- Recently they have been used to increase nutritional value in edible parts like enhancing proteins or vitamins content.



- **High anticancer proprieties were individuated in some Sicilian broccoli landraces.**

- **Crossing them with wild relatives allowed to obtain new cultivars with high levels of glucosinolates like glucoraphanin, a cancer fighting antioxidant which prevents DNA damages.**



WP 7: Case study on Brassica

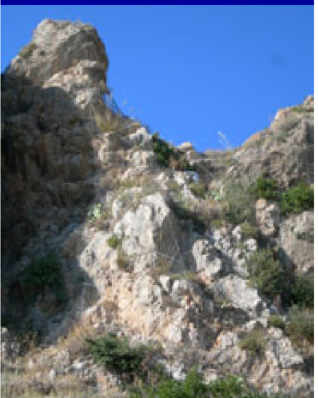
Main tasks

1) Collection of species and population distribution data in various information systems;

2) Priorization of species and populations;

3) Recommendation of sites suited to establish genetic reserves for Brassica in the EU;

4) Development of species specific guidelines for genetic reserves design, management and monitoring;



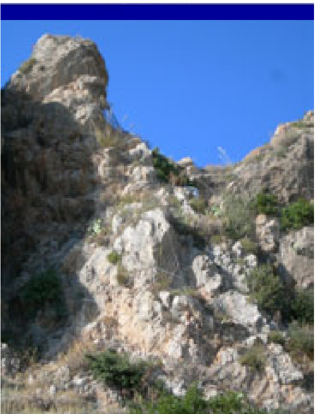
WP 7: Case study on Brassica

Main tasks

5) Establishment of a demographic and genetic baseline for a single Brassica genetic reserve;

6) Compilation of the national legal framework related to in situ management, annotation of the legal and national framework and derivation of a recommendations for a national strategy for in situ management;

7) Contribution to the establishment of an European integrated workplan for *in situ* management of crop wild relatives.



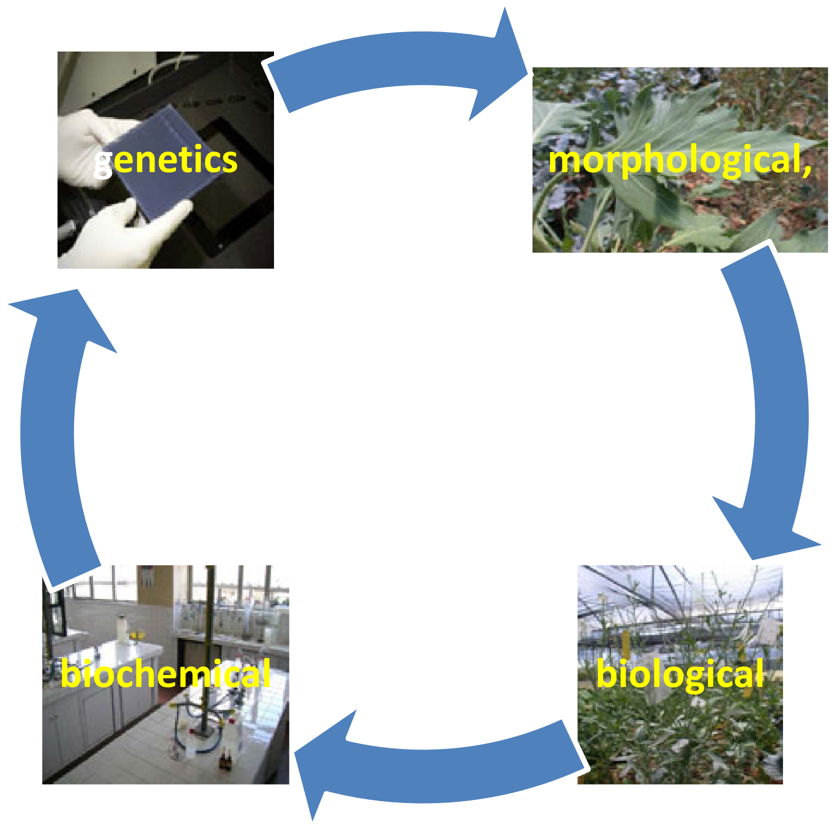
After a first step related to the

- identification
- collection
- monitoring

of wild Brassicas occurring in different sites

Priorities were pointed to their characterization and *in situ* conservation.

Characterize the Brassica wild populations was the starting point for planning their protection and *in situ* conservation, and avoid duplication



In the frame of the Brassica case of study of AEGRO project, four wild Brassica species growing in Sicily have been analysed and characterised.

AEGRO

Brassica incana

Brassica macrocarpa

Brassica rupestris

Brassica villosa

After a first step related to the

- **identification**
- **collection**
- **monitoring**

of wild Brassicas occurring in different sites

Priorities were pointed to their *in situ* conservation.

AEGRO

For some accessions there was a high adaptability to environmental conditions

Among the Brassica wild relatives species priorities has been given on *Brassica macrocarpa* Guss.

This is an endemic wild Sicilian species, widespread on small islands where its populations grown on rocky cleaves and are endangered by human activity and animal grazing.



B. macrocarpa populations are still present in Favignana and Marettimo while recently disappeared in Levanzo.

Althought nature protection is a large concept our case of study related to Sicilian _Brassica_ wild relatives deals with their protection and with their _in situ_ conservation.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- **Many International Institutions involved in nature protection listed these species in the "IUCN Red List of Threatened Species", which provides taxonomic, conservation status and distribution information on taxa that are facing a high risk of global extinction.**

- **Since ten years the Sicilian Brassica wild species represent a priority for the Brassica working group of the European Cooperative Programme on Genetic Resource (ECP/GR) managed by Biodiversity International.**



The aim of the genetic reserve proposed is the conservation of the whole existing gene pool in both islands in view to maintain all the genetic variability.

AEGRO

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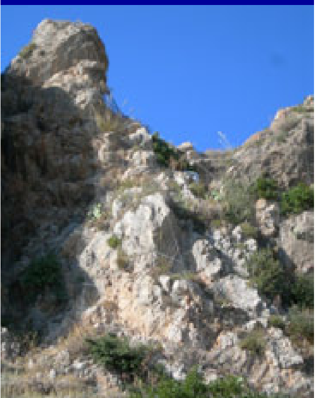
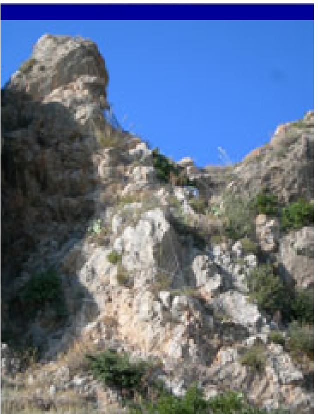


Species	Origin
Brassica incana	Agnone Bagni
Brassica incana	Lago Albano
Brassica incana	Sortino
Brassica incana	Agnone Bagni
Brassica macrocarpa	Favignana
Brassica montana	Monte Conero
Brassica rupestris	Roccella Valdemone
Brassica rupestris	Stilo
Brassica rupestris	Ragusa Ibla
Brassica villosa	Pizzo Telegrafo
Brassica villosa	Marianopoli

AEGRO




DA *B. montana* (Monte Conero)
DC
B. Incana
(Lago Albano)
BB
B. macrocarpa
(Favignana)
BX
B. villosa
(Marianopoli)
BU 15 *B. rupestris* (Stilo)
BU 5 *B. rupestris* (Roccella Valdemone)
BY 5 e BN *B. incana* (Agnone Bagni)
BW *B. incana* (Sortino)
BU 9 *B. rupestris* (Ragusa Ibla)

BY 5 – *Brassica incana* (Agnone Bagni)

BB – *Brassica macrocarpa* (Favignana)




BU 5 – *Brassica rupestris* (Roccella Valdemone)

BL – *Brassica villosa* (Pizzo Telegrafo)

DA – *Brassica montana* (Monte Conero)

AEGRO

DC – *Brassica incana*

(Lago Albano)

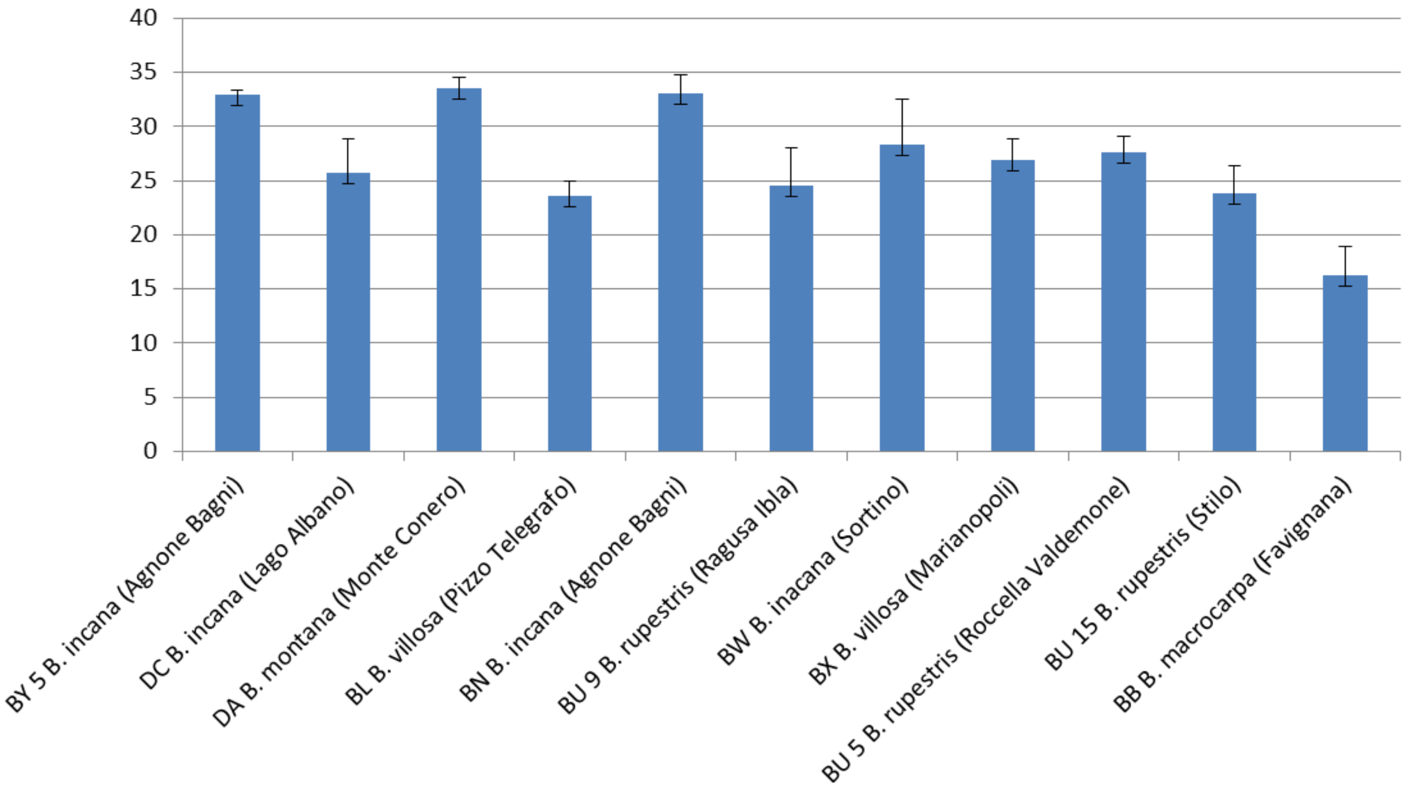
Leave samples has been frozen at -80°C, liophylized and powdered for successive analysis:

Glucosinolates	-Official method, -ISO 9167-1 (1992)
Ascorbic acid	-Titration, Tillmans et al. (1932)
Total phenols	Folin reaction, Singleton (1965)
Total carotenoids	Spectrophotometer, Mayenfield et al. (1986)
Antioxidant capacity	DPPH and peroxyl radical quenching

AEGRO

Contents of antioxidant compounds

Total polyphenols (mg g $^{-1}$ dw)



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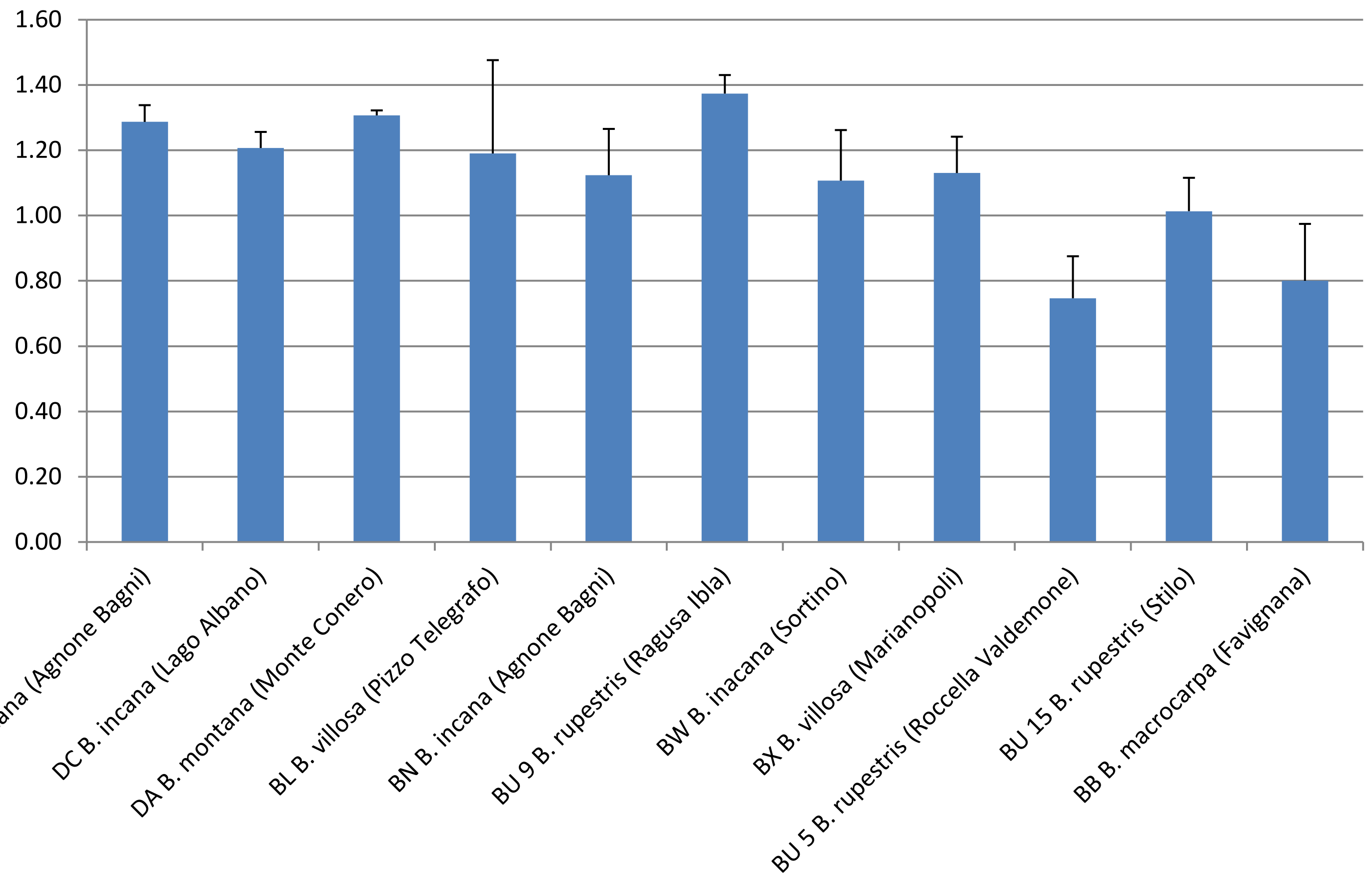
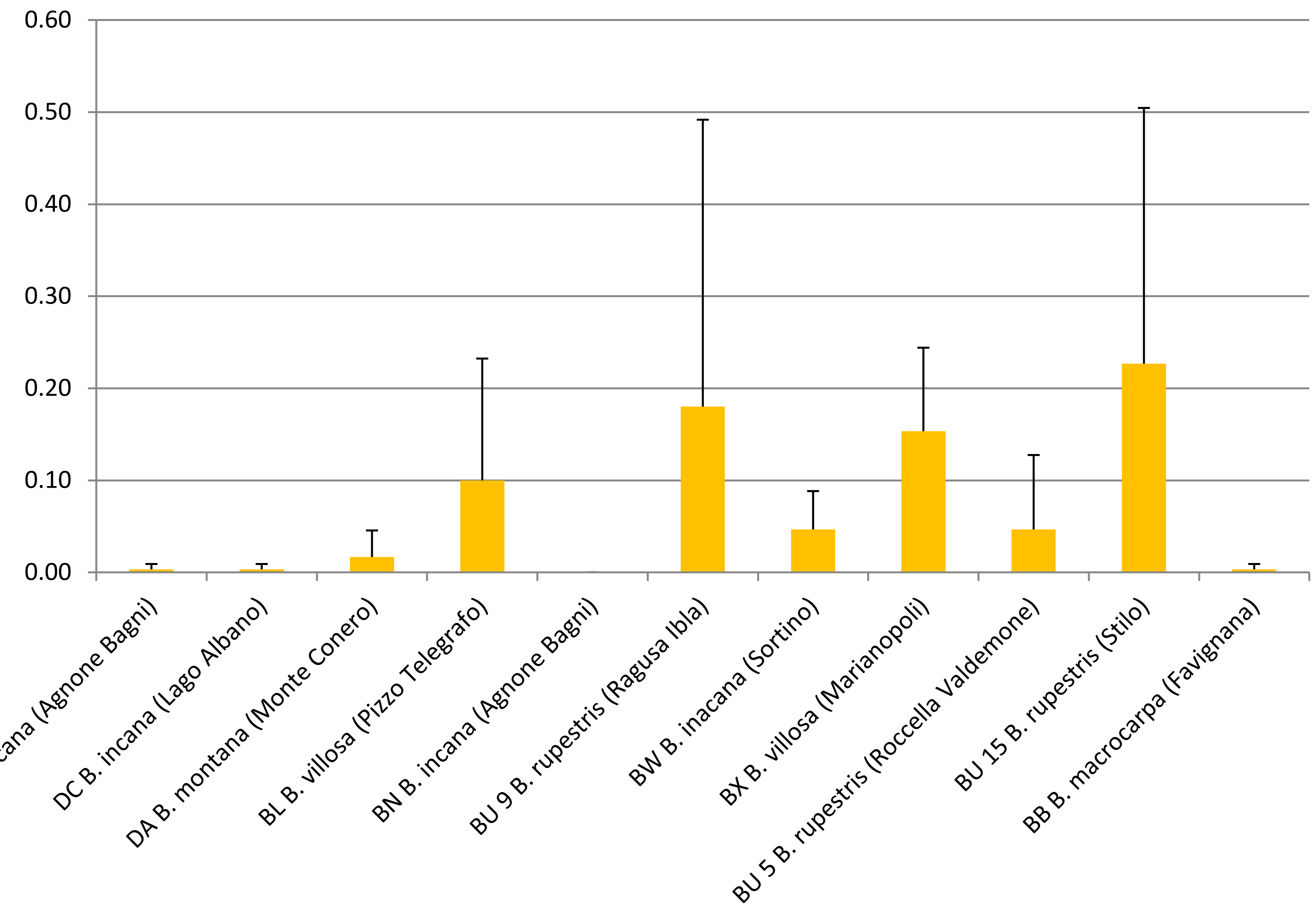
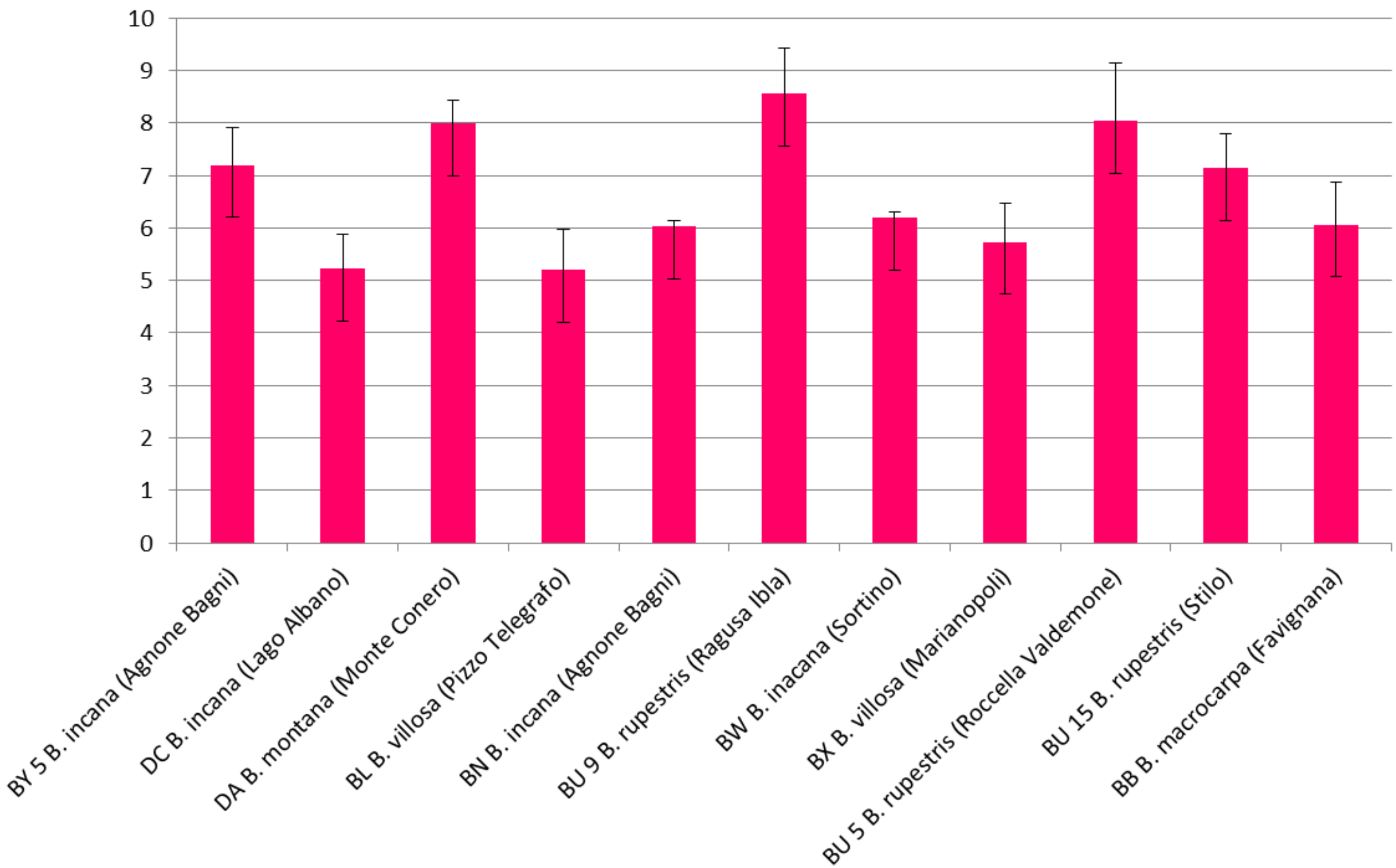
Total carotenoids (mg/g d.w.)



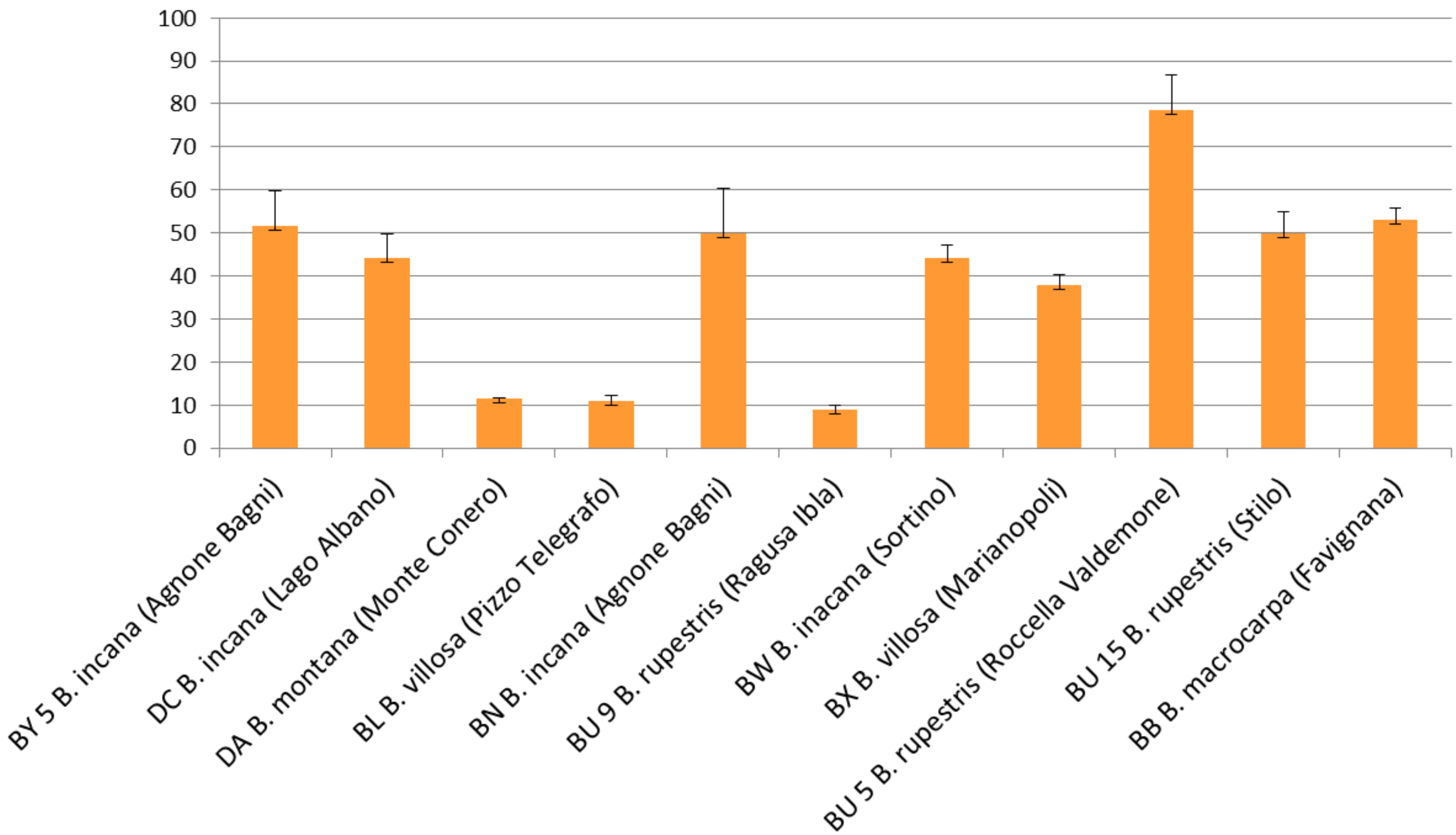
Anthocyanin content (mg/g d.w.)



Ascorbic acid (mg/g d.w.)

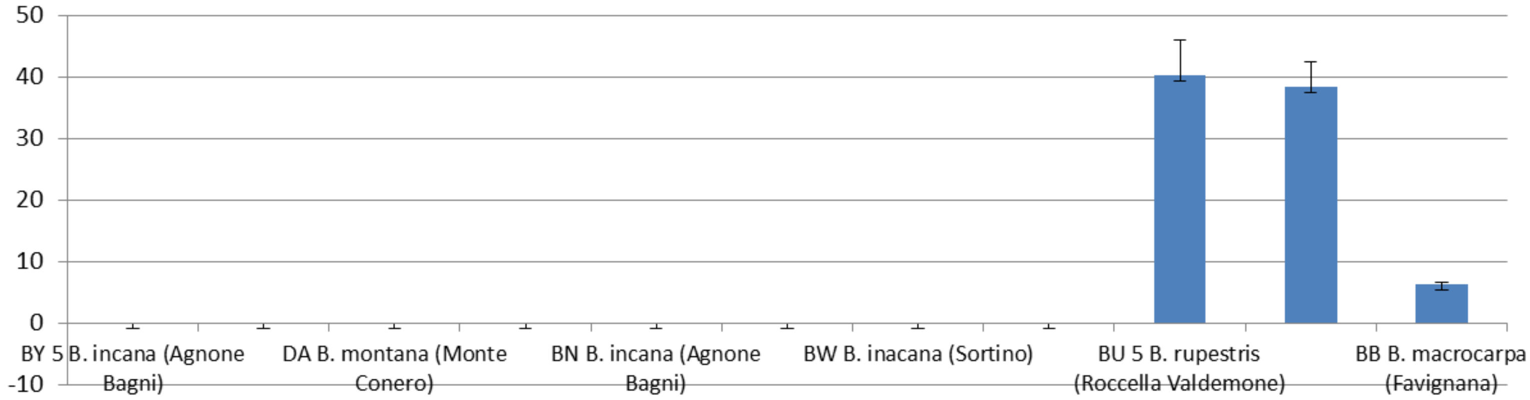


Glucosinolate Total ($\mu M/g$ $^{d.w.}$)

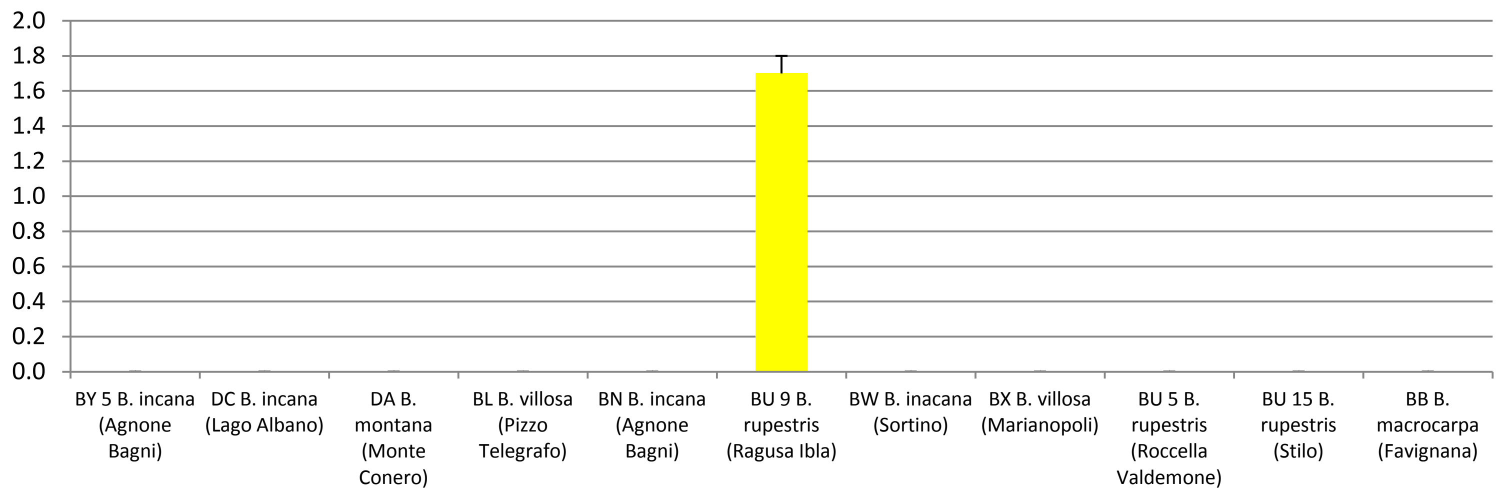


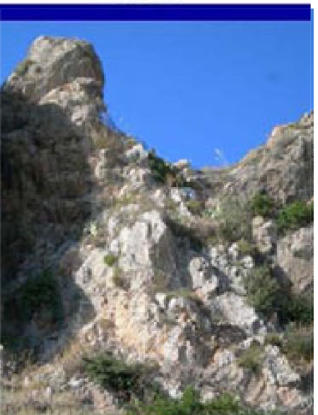
Aliphatic

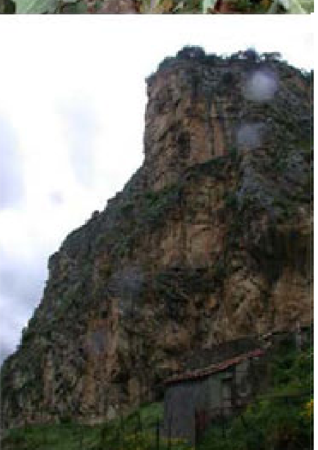
Glucoiberin (μM/g d.w.)



Progoitrin (μM/g d.w.)

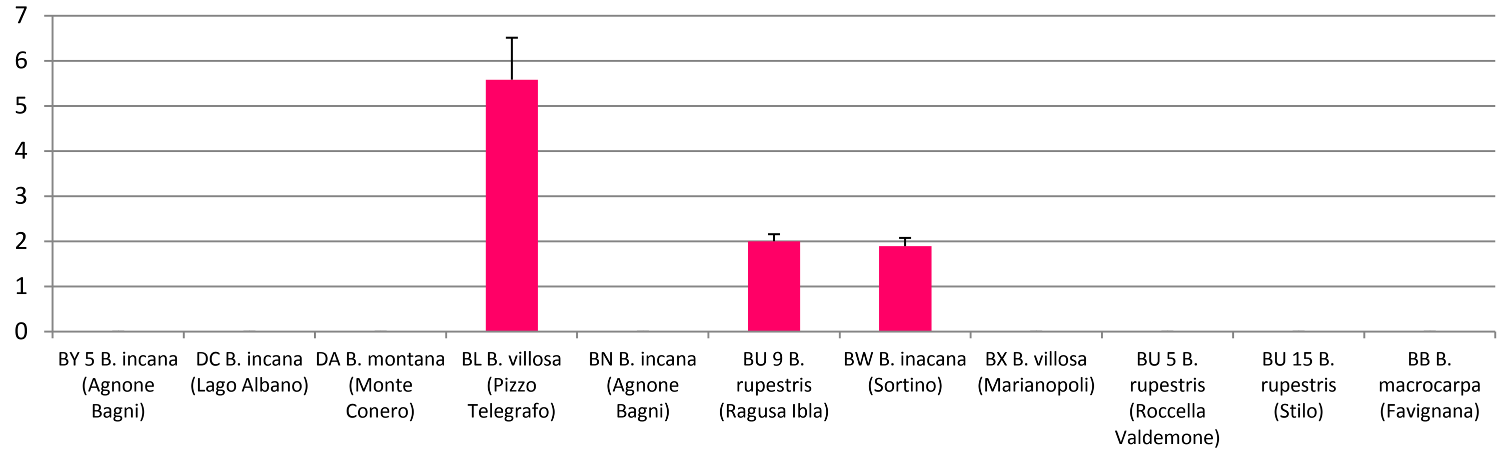




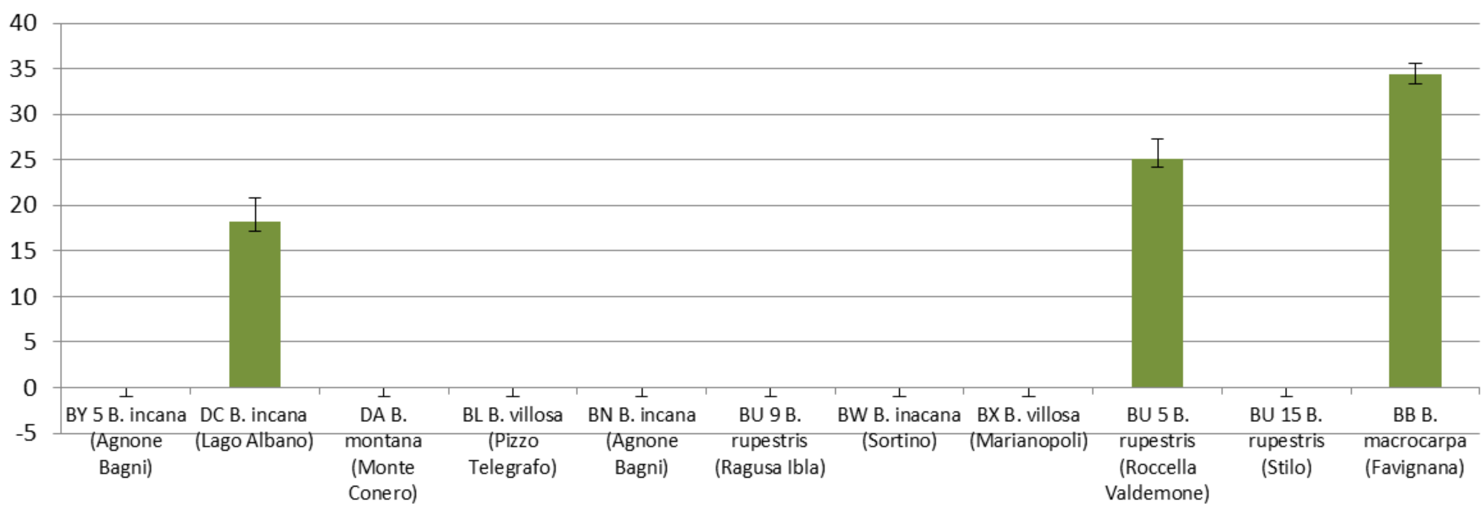


Aliphatic

Glucoraphanin (µM/g d.w.)

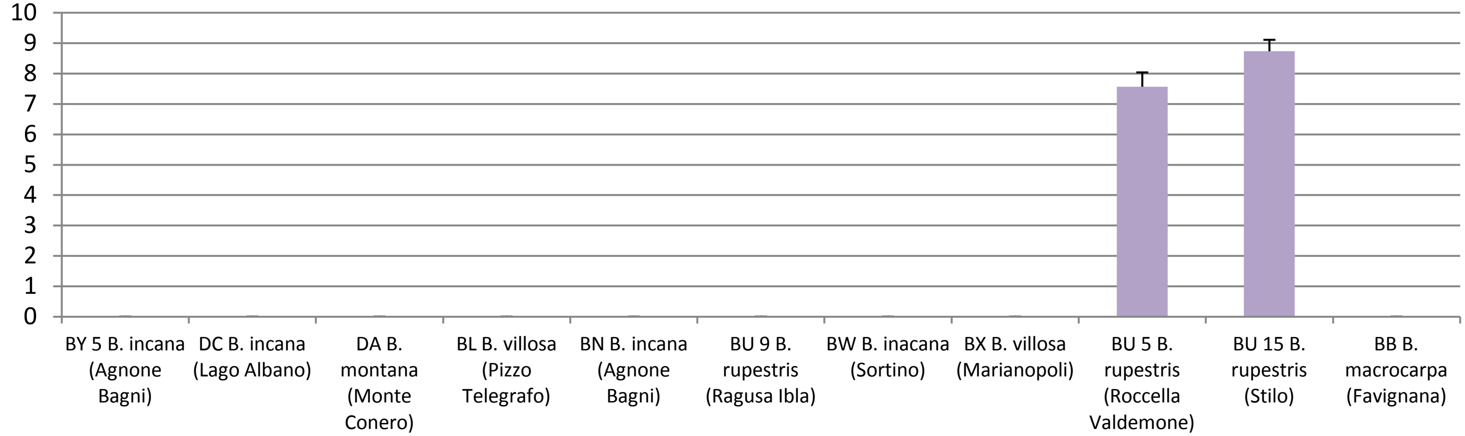


Sinigrin (µM/g d.w.)

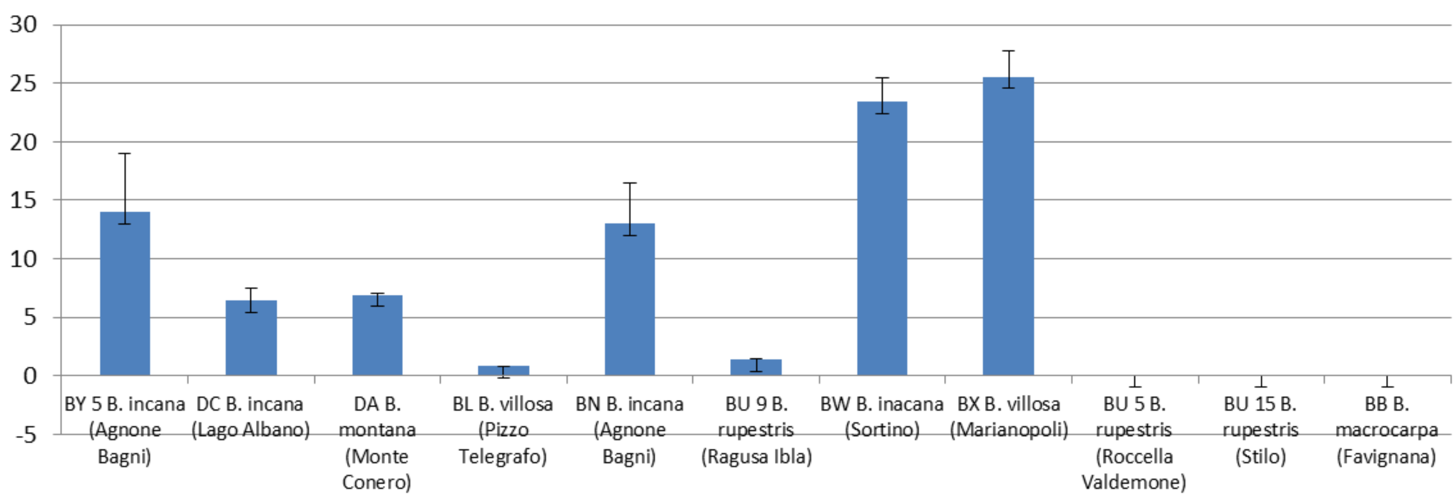


Aliphatic

Sinalbin ($\mu M/g$ d.w.)

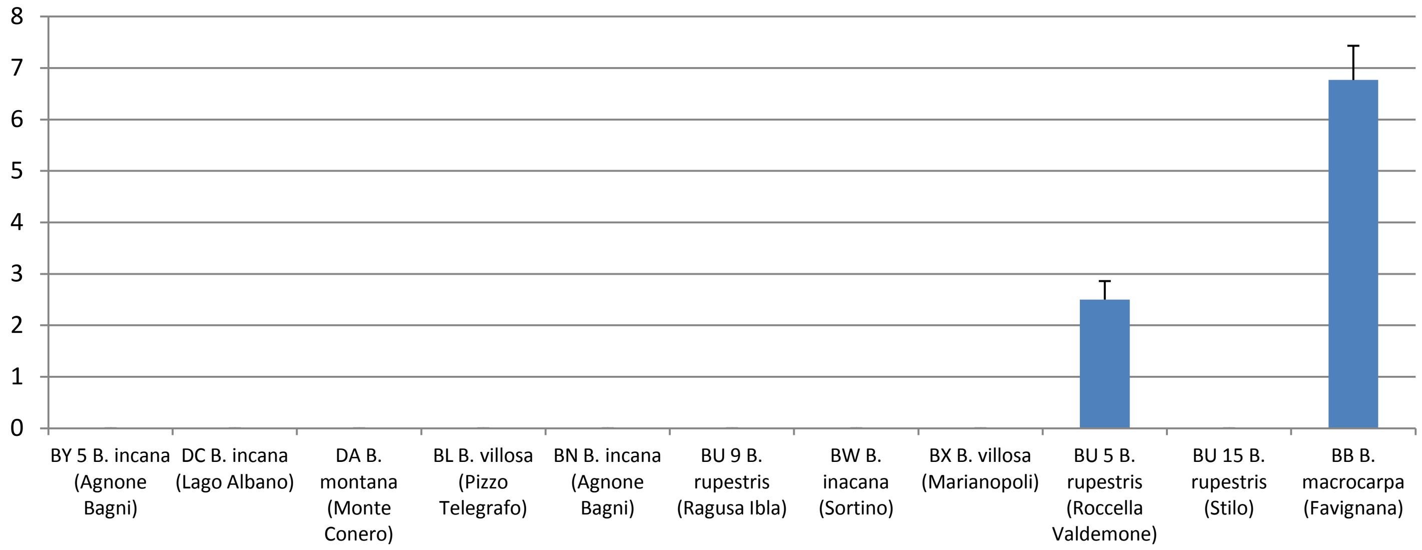


Gluconapin ($\mu M/g$ d.w.)



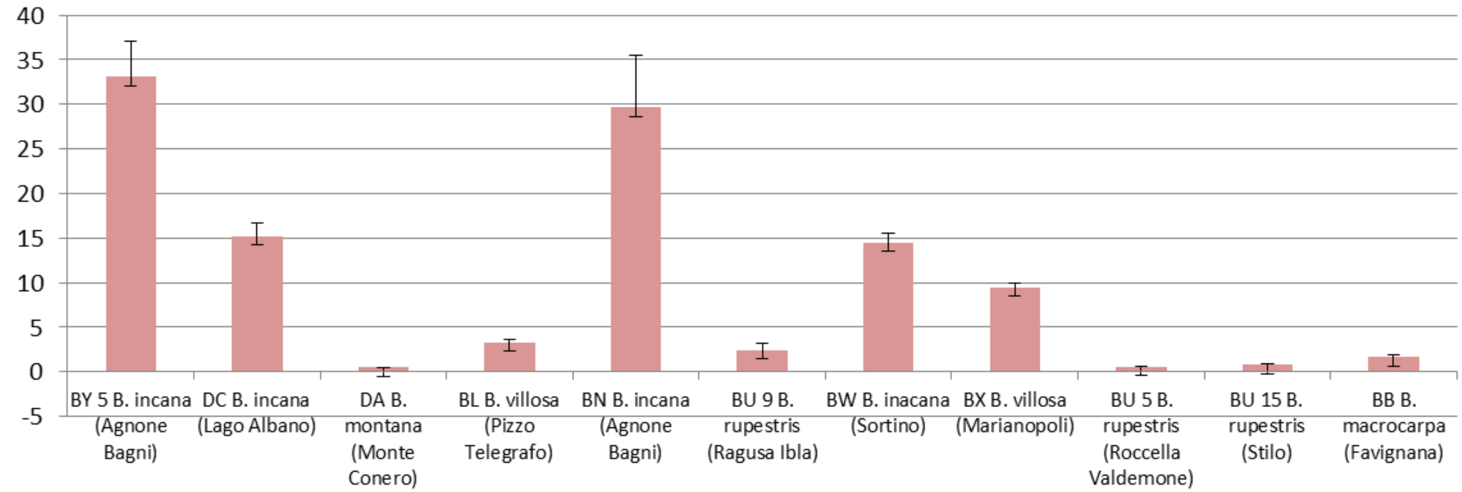
Aliphatic

Glucoiberverin (μM/g d.w.)

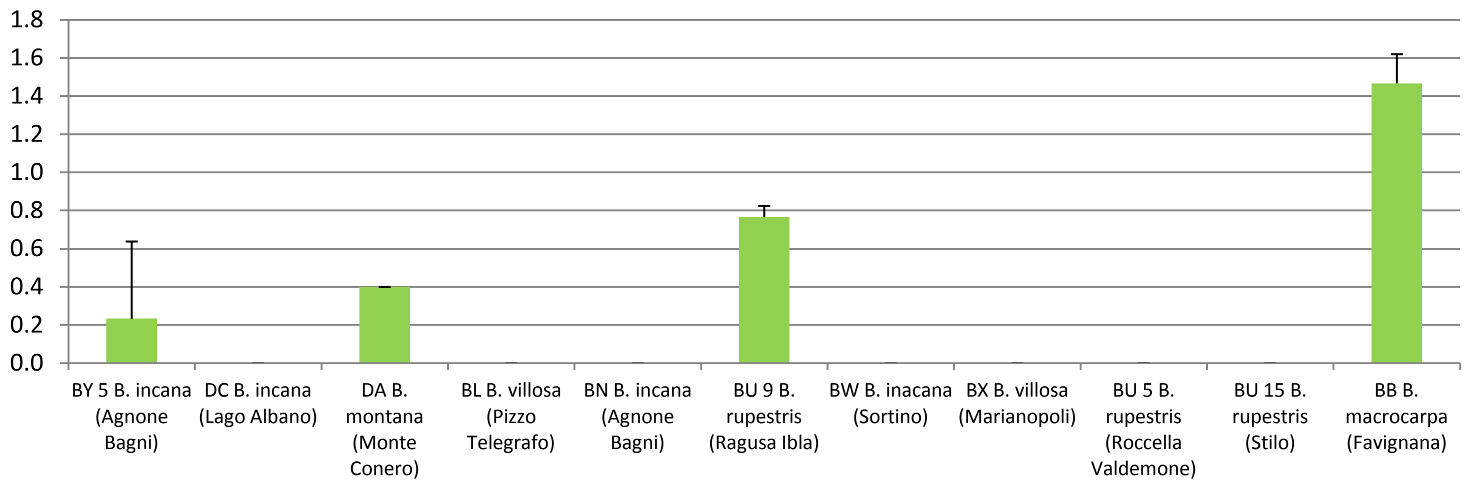


Indolic

Glucobrassicin ($\mu M/g$ d.w.)

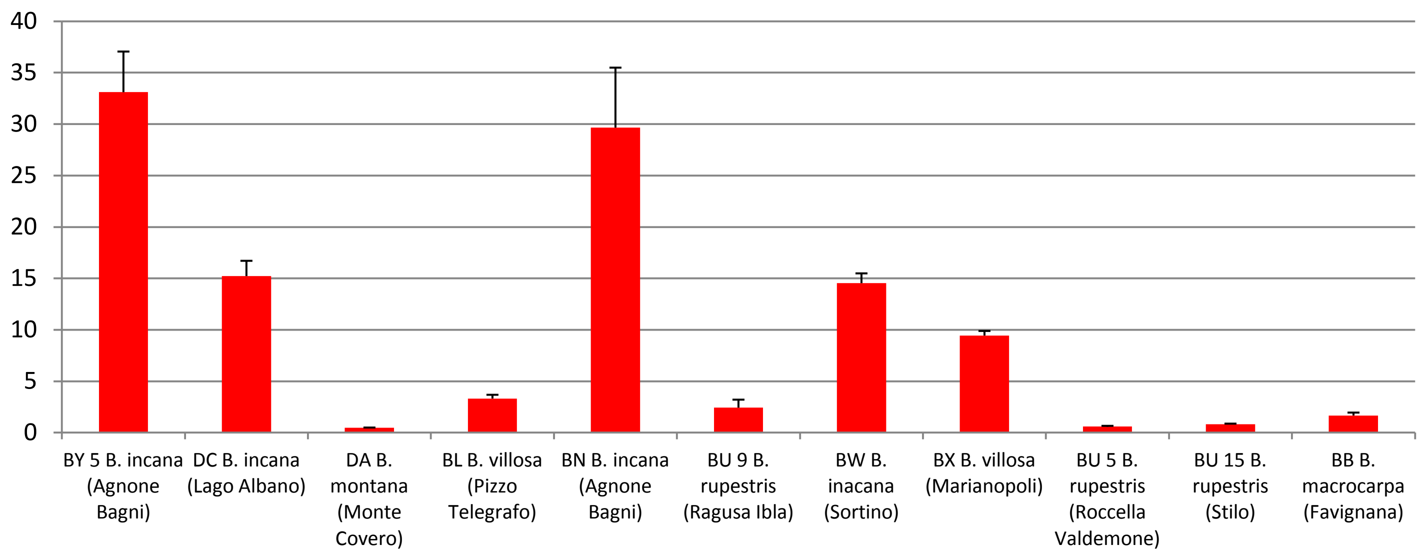


Neo-Glucobrassicin ($\mu M/g$ d.w.)



Aromatic

Gluconasturtiin ($\mu M/g^{d.w.}$)



Measurement of antioxidant capacity

Species	Origin	DPPH	LIPOX-CROCIN
		(mmol AsA/100g dw)	(mmol TROLOX/100 g dw)
Brassica incana	Agnone Bagni	39,7 ± 1,0	19,3 ± 1,8
Brassica incana	Lago Albano	10,1 ± 0,4	10,7 ± 1,6
Brassica incana	Sortino	14,9 ± 0,4	8,3 ± 1,0
Brassica incana	Agnone Bagni	22,1 ± 3,6	8,6 ± 0,7
Brassica macrocarpa	Favignana	3,6 ± 0,1	2,3 ± 0,3
Brassica montana	Monte Conero	69,9 ± 12,5	17,6 ± 3,5
Brassica rupestris	Ragusa Ibla	13,0 ± 0,4	11,2 ± 3,5
Brassica rupestris	Roccella Valdemone	23,1 ± 0,4	3,6 ± 0,8
Brassica rupestris	Stilo	37,7 ± 1,3	9,2 ± 1,8
Brassica villosa	Marianopoli	15,0 ± 0,4	8,8 ± 0,6
Brassica villosa	Pizzo Telegrafo	15,1 ± 0,1	8,1 ± 0,6



DPPH vs CROCIN	0.705	*
DPPH vs ATH	-0.043	ns
DPPH vs TPC	0.648	*
DPPH vs AsA	0.506	ns
DPPH vs CAR	0.323	ns
DPPH vs GLS	-0.193	ns
CROCIN vs ATH	-0.103	ns
CROCIN vs TPC	0.687	*
CROCIN vs AsA	0.293	ns
CROCIN vs CAR	0.811	**
CROCIN vs GLS	-0.420	ns

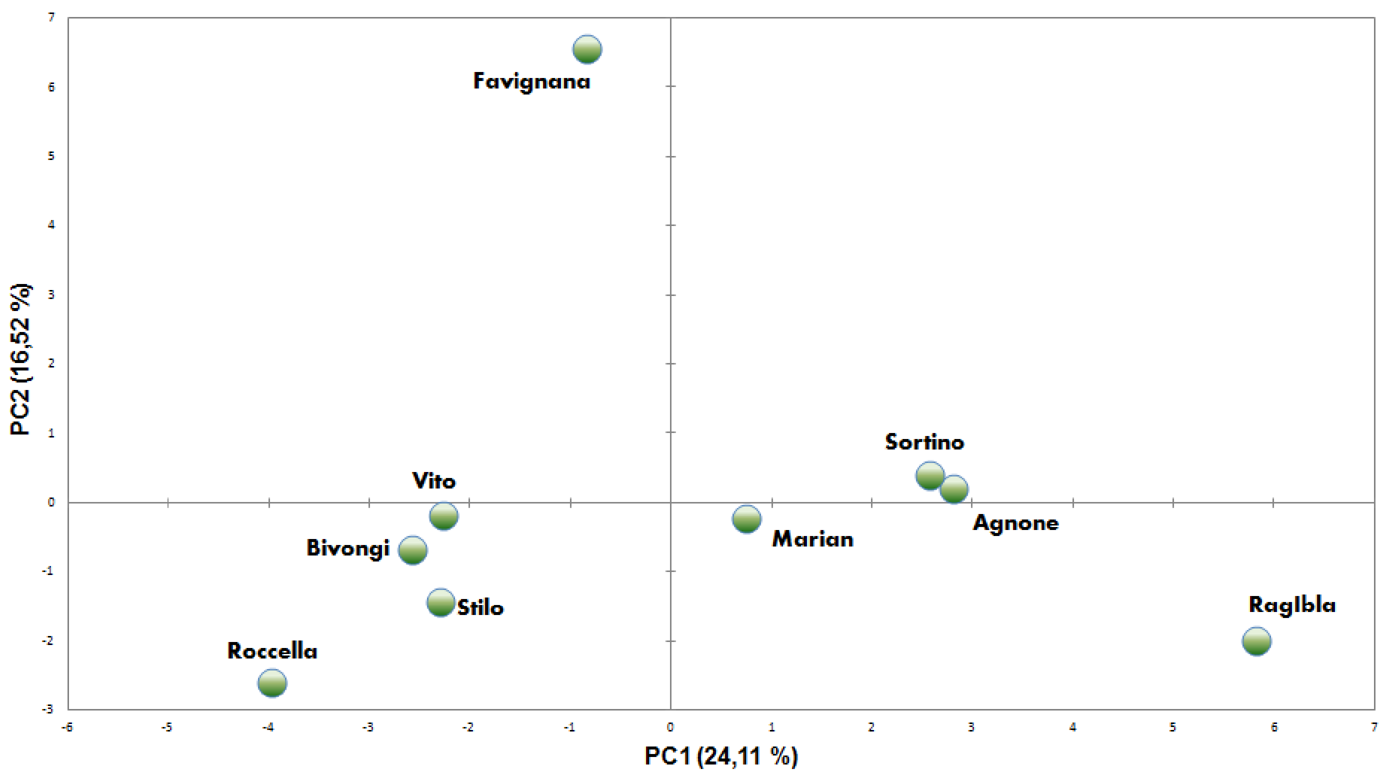
Correlation coefficients (rxy) obtained by simple linear regression analysis between antioxidant indexes and chemical parameters.

*: $p<0.05$; **: $p<0.01$; ns: $p>0.05$

Table 7.1. Wild Brassica occurrences collected and studied by DOFATA and their origin

Accession number	Species	Origin
UNICT 3253	B. macrocarpa	Favignana
UNICT 3270	B. rupestris	Stilo
UNICT 3410	B. rupestris	Pazzano
UNICT 3405	B. rupestris	Roccella Valdemone
UNICT 3458	B. rupestris	Ragusa
UNICT 3512	B. incana	Augusta
UNICT 3944	B. villosa	Marianopoli
UNICT 4158	B. incana	Sortino

Principal components analysis (axes PC1 and PC2: 40,62 %)



Conclusions

B. incana, B. rupestris and *B. villosa*, already occur in protected areas and managers were contacted in order to sensibilize them on the genetic relevance of these crop wild relatives and to individuate specific actions to protect their integrity.

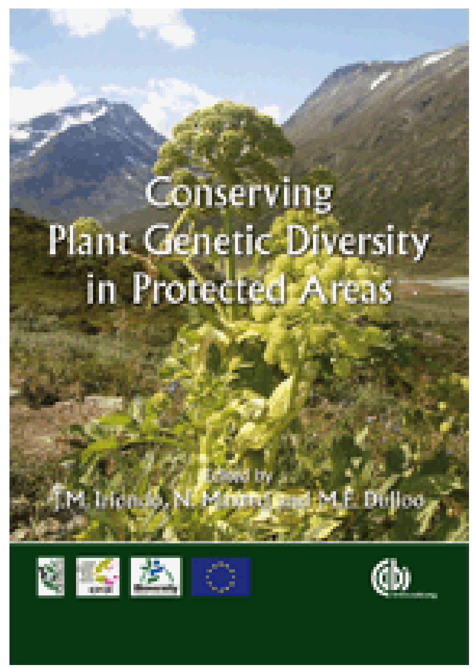
Conclusions

- DISPA cooperates with the Centro Universitario per la Tutela e la Gestione degli Ambienti Naturali ed Agricoli (CUTGANA - University of Catania) which already manages seven natural reserves in Sicily and offers its expertizes to set up Brassica genetic reserves in Sicily.

- CUTGANA manages now about 430 Ha of inland reserves and about 1000 Ha of marine reserves.

Conclusions

The results obtained in the frame of AEGRO project represent a good starting point for the establishment of genetic reserves in Sicily for Brassica



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