In Progress Activities to Exploit the Mediterranean Wild and Cultivated Brassica Germplasm for Improving Human Health and Environmentally Friendly Farming

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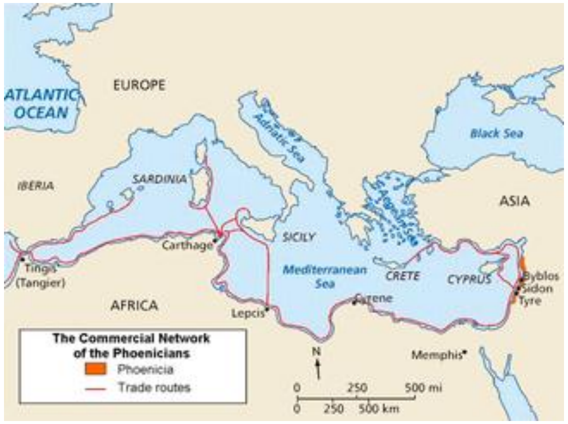
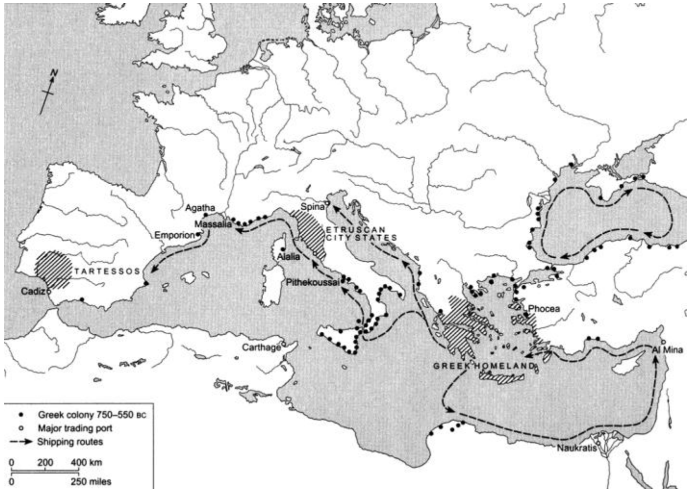
19th CRUCIFER GENETICS WORKSHOP AND BRASSICA 2014

Wuhan 3.30-4.2 2014

The history of the Mediterranean basin, traversed in turn by Phoenicians, Greeks, Carthaginians, Arabs, Normans, Spaniards and French, is reflected in its traditions and horticultural diversity



In this frame Sicily represented, such as some other Mediterranean islands, an important crossroad as is reflected by its food traditions and horticultural diversity



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As result of Sicilian ancient past and of its particular geographic location there are a great number of vegetables grown under different environmental, agronomical and social contexts including home gardens and peri-urban vegetable farms



In Sicily are also widespread several wild species, often representing crop wild relatives, which are either gathered or occasionally cultivated, that make up the richness of its agriculture genetic resources









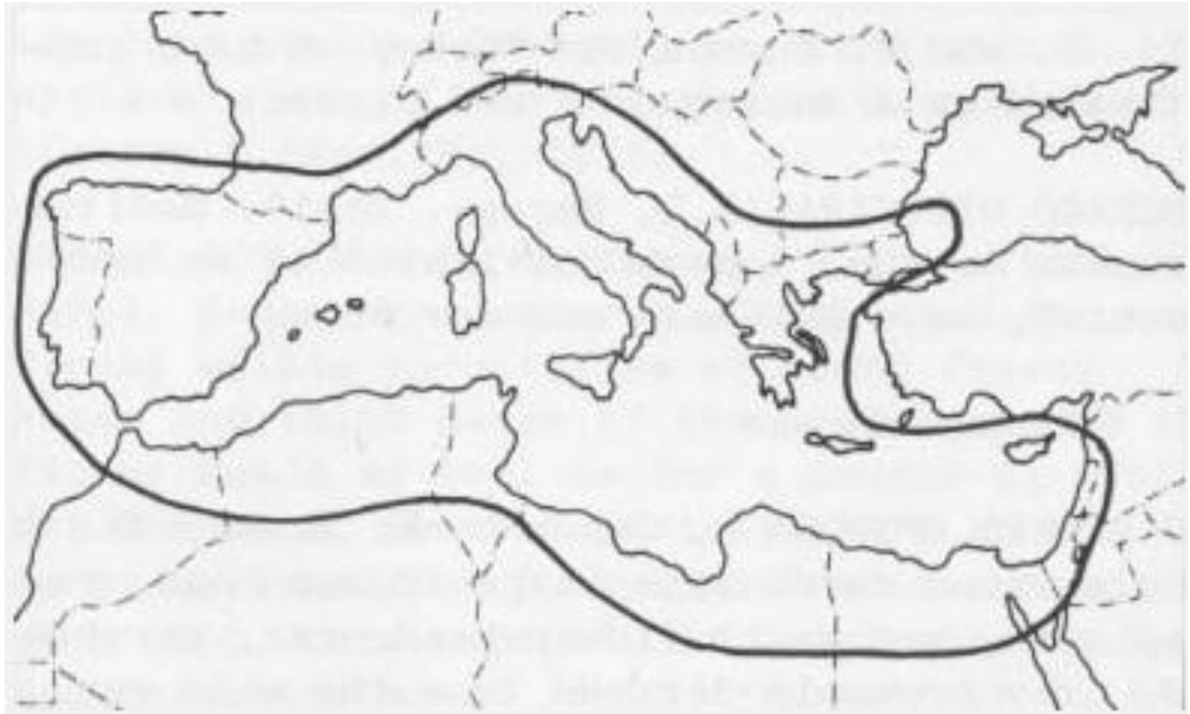

A rather particular contribution of Sicilian vegetable diversity is represented by varietal groups of Brassicas that are characterized by qualitative traits appreciated by local consumers. These include landraces of violet and green curded cauliflower, sprouting broccoli, red kohlrabi and leafy kale



Brassica vegetables stimulate great interest for:

- **Wide genetic diversity till available and not yet full exploited;**
- **Nutraceutical traits of the produce which support the attention of the international medical community;**
- **New perspectives for food industry to exploit the several landraces till now underutilized;**
- **Possibility to support environmentally friendly farming.**

New opportunities are offered by the new food technologies for exploiting traditional and new brassica phenotypes for new processes (IV and V gamma produce)

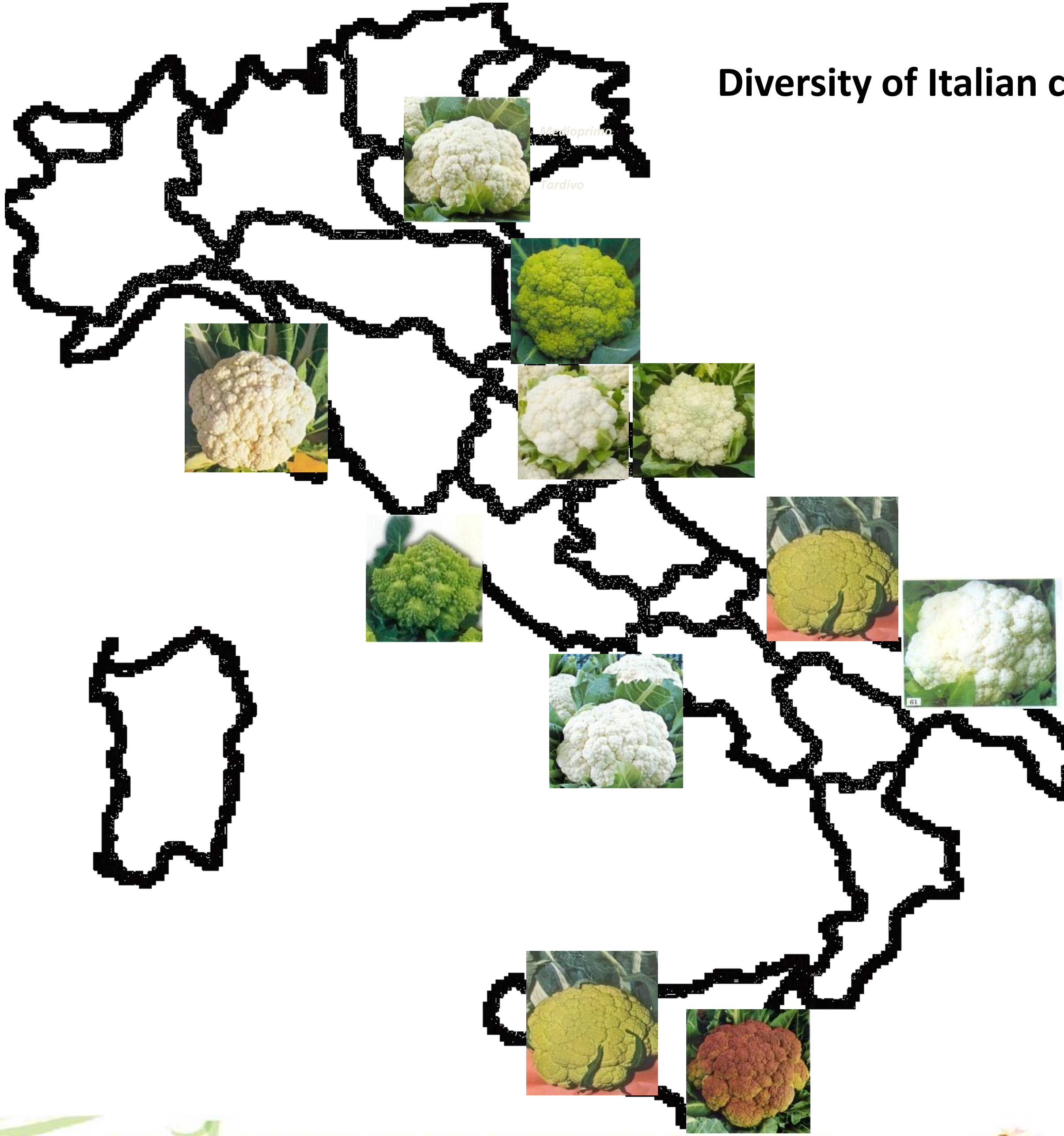


Several Authors showed molecular DNA evidences on the wide diversity of Mediterranean *Brassica* wild species in comparison to the *B. olearacea* wild type populations of the European Atlantic coasts indicating that domestication of *B. olearacea* cultigroups occurred in of the north-central and north-east areas of Mediterranean basin

The richness of *Brassica* wild relatives (n=9), is supported by perennial plants with woody stem, up to 1.5 m tall, large leaves and high glucosinolate content. The plants are often self-incompatible, with high tendency to mutations and freely inter-crossing among them and with *B. oleracea crops*.

The high anticancer proprieties have been individuated in some interspecific hybrids F1 between *B. oleracea* cultigroups and *Brassica* wild relatives allowed to obtain new cultivars with high levels of glucosinolates, such as glucoraphanin, a cancer fighting antioxidant which avoid DNA damage.

Diversity of Italian cauliflower LRs



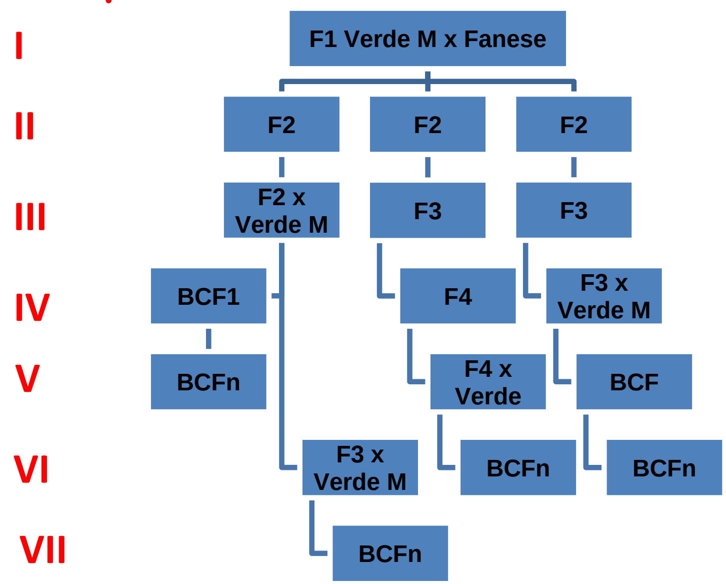





Main goals for Verde of Macerata Lrs improvement

- Improve morphological characters
- Amplify harvesting time
- Reduce physiological disorders
- Improve resistance against pest and diseases



New variabilty in Romanesco: pure lines obtained

X

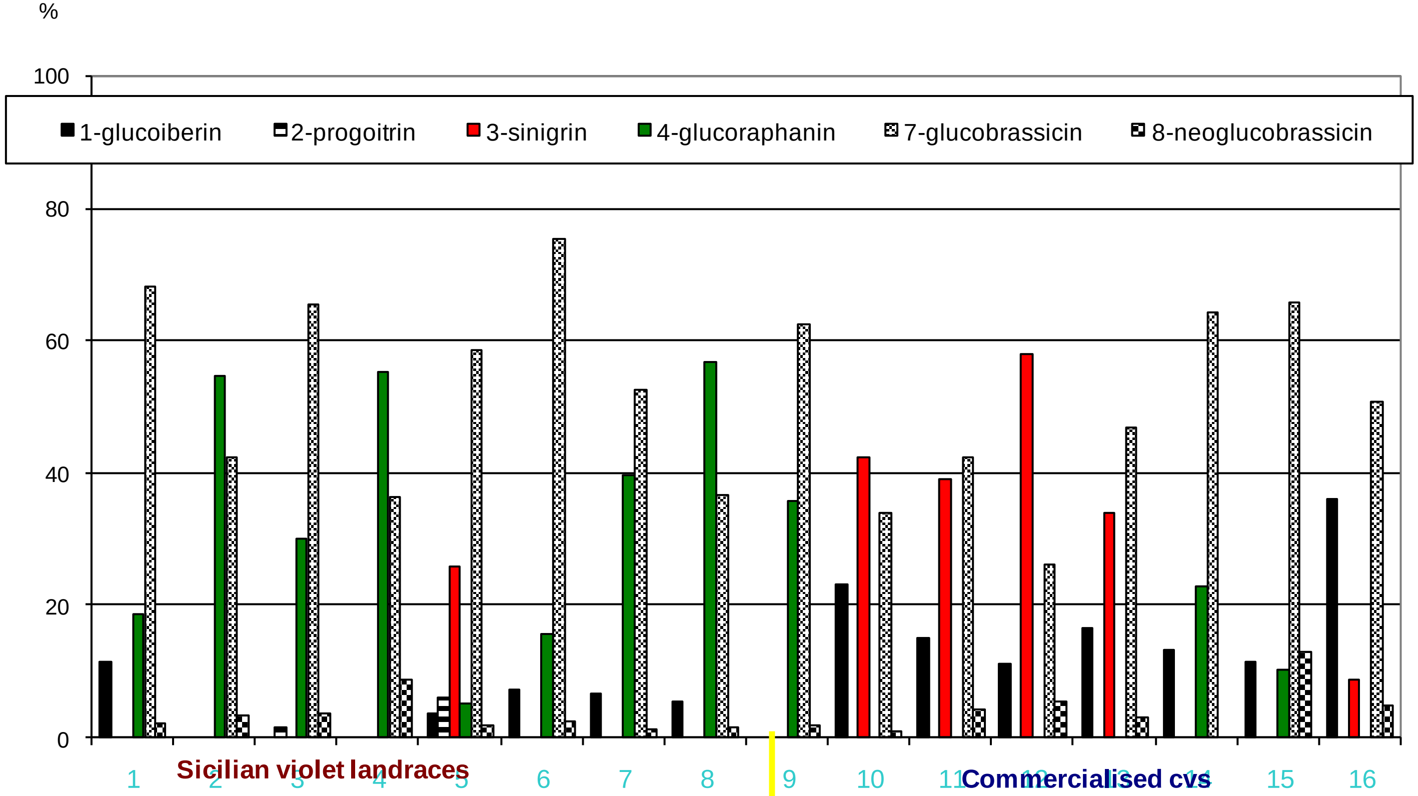
F1

Curd improved for Firmness, colour and density

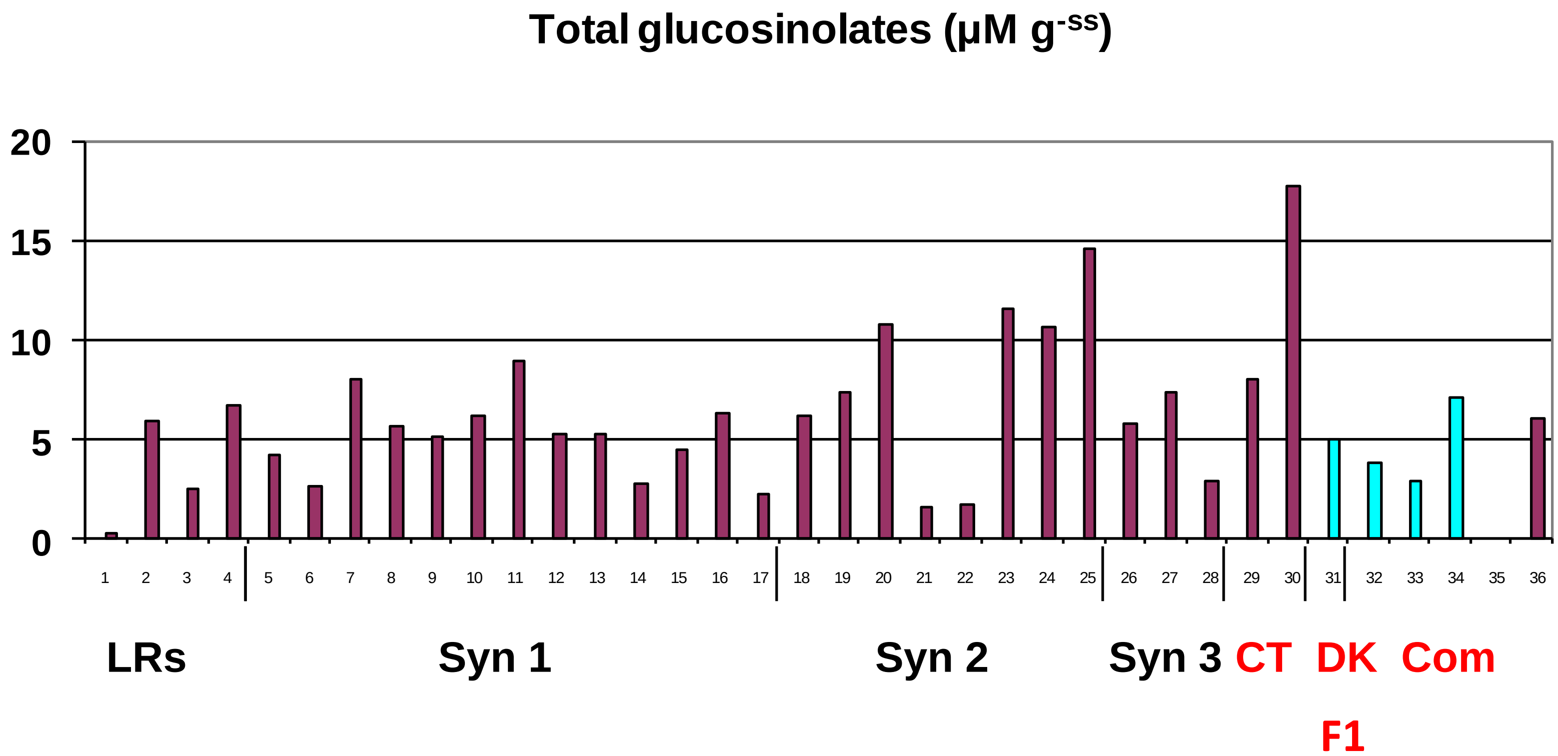




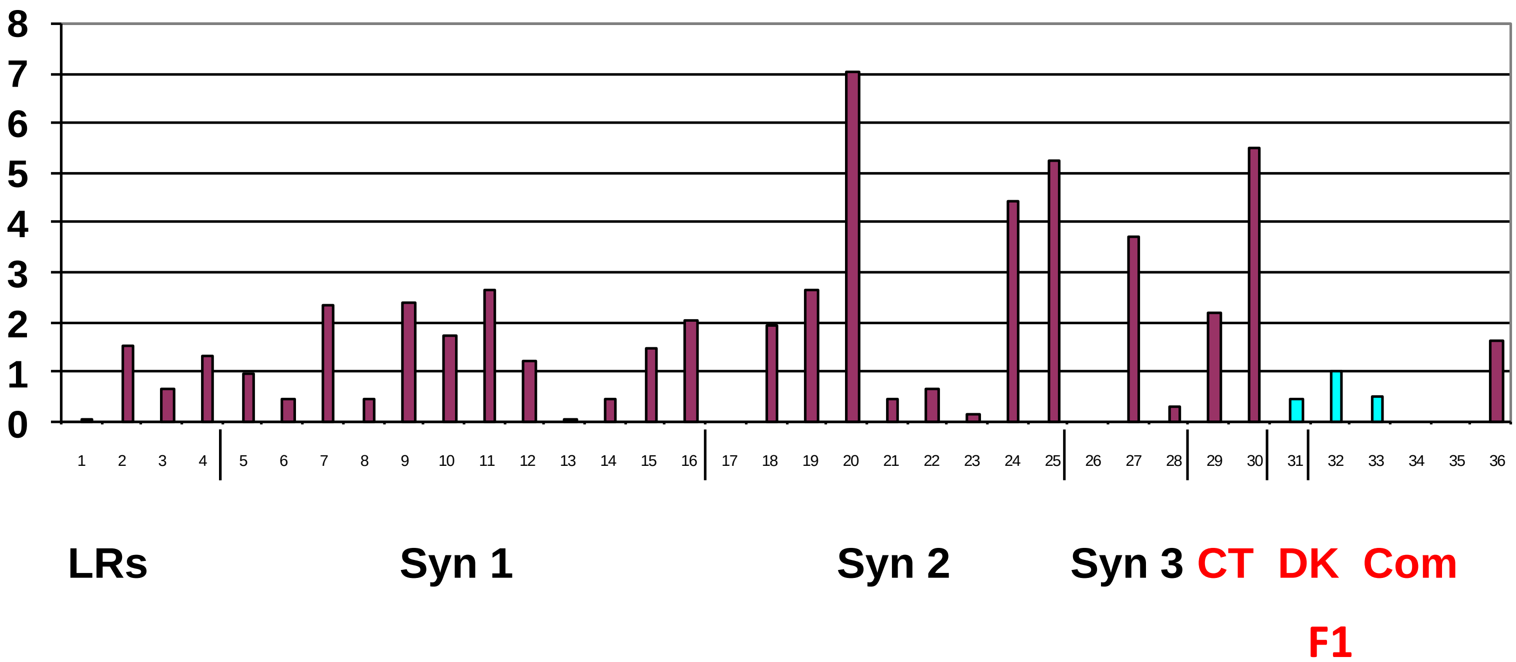
Glucosinolate profile of Sicilian violet landraces compared to commercialised white cvs

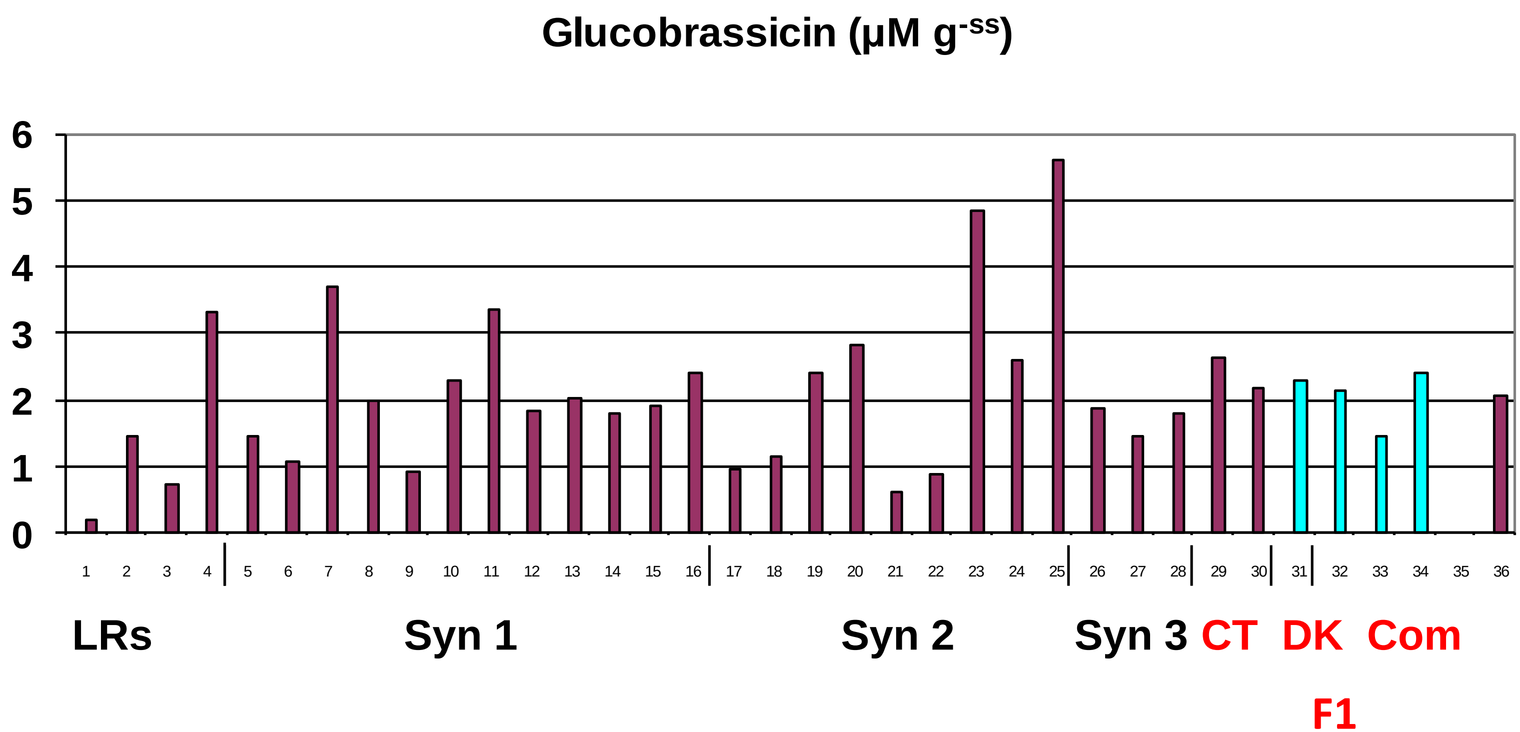


Branca F. et al., 2002. Phytochemistry, 59, 717-724.


Total glucosinolates (μM g-ss)
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
LRs
Syn 1
Syn 2
Syn 3
CT
DK
Com
F1

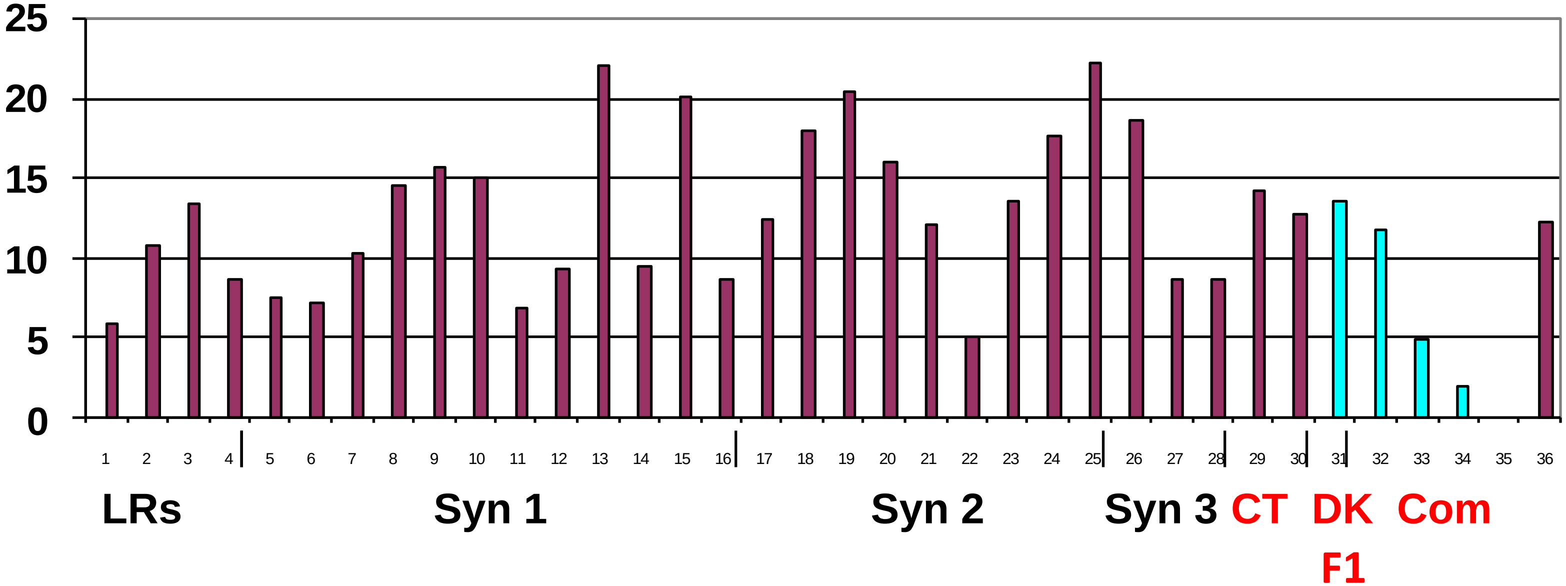
Glucoraphanin (µM g^{-ss})





Glucobrassicin (µM g-ss)
0
1
2
3
4
5
6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
LRs
Syn 1
Syn 2
Syn 3
CT
DK
Com
F1

Total polyphenols (mg g^{-ss})

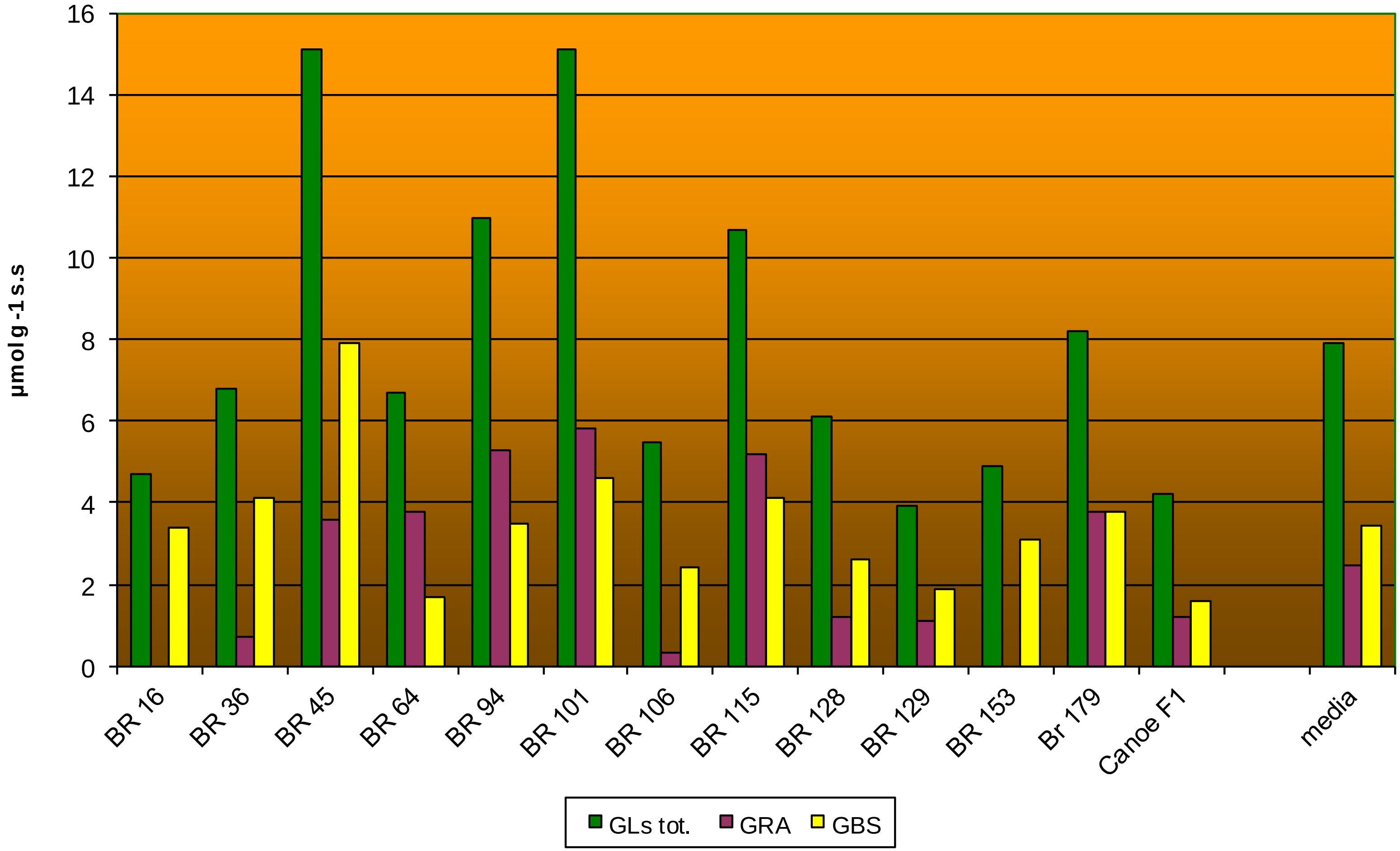


Diversity of Italian broccoli LRs

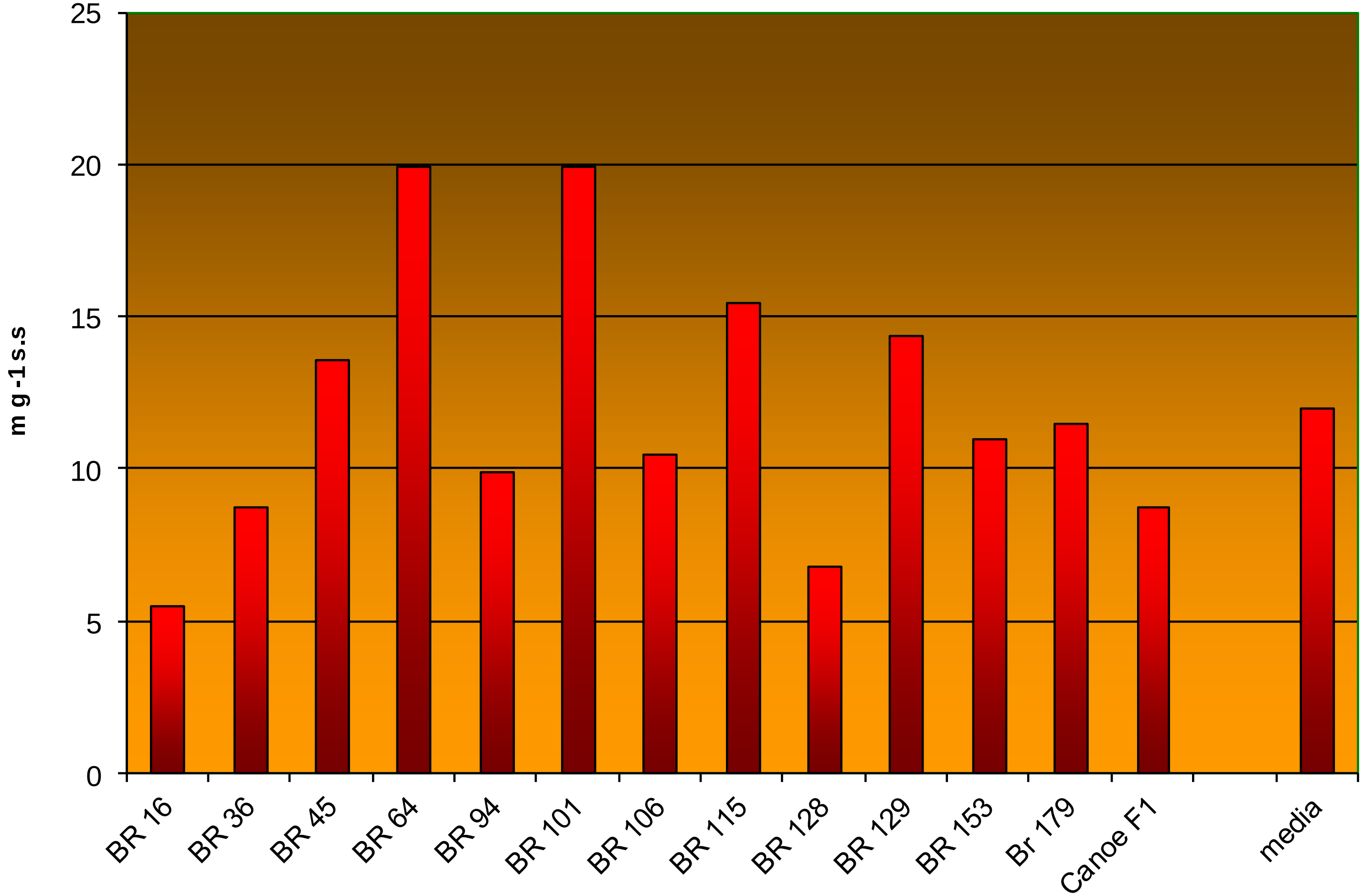


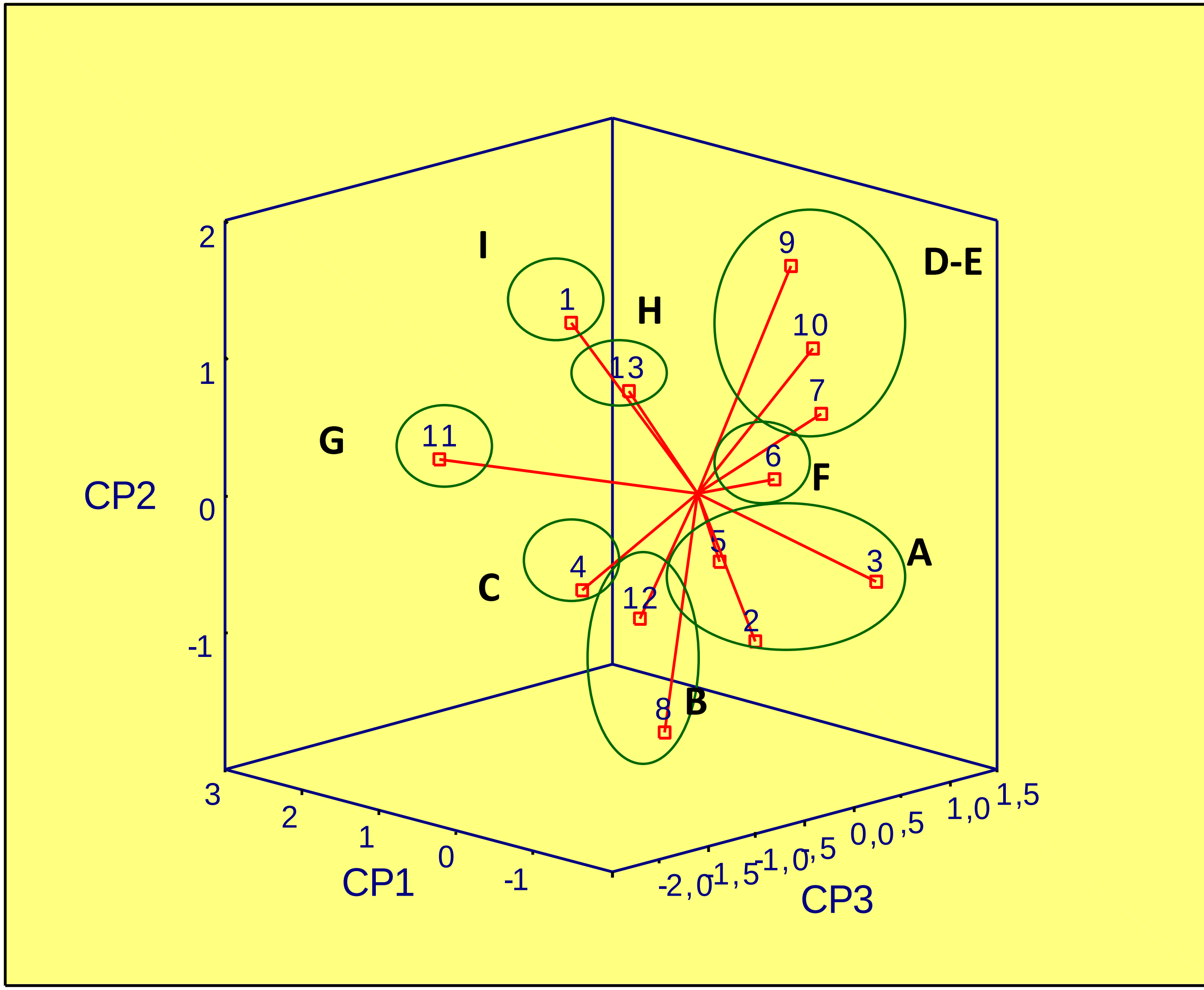



Total glucosinolates

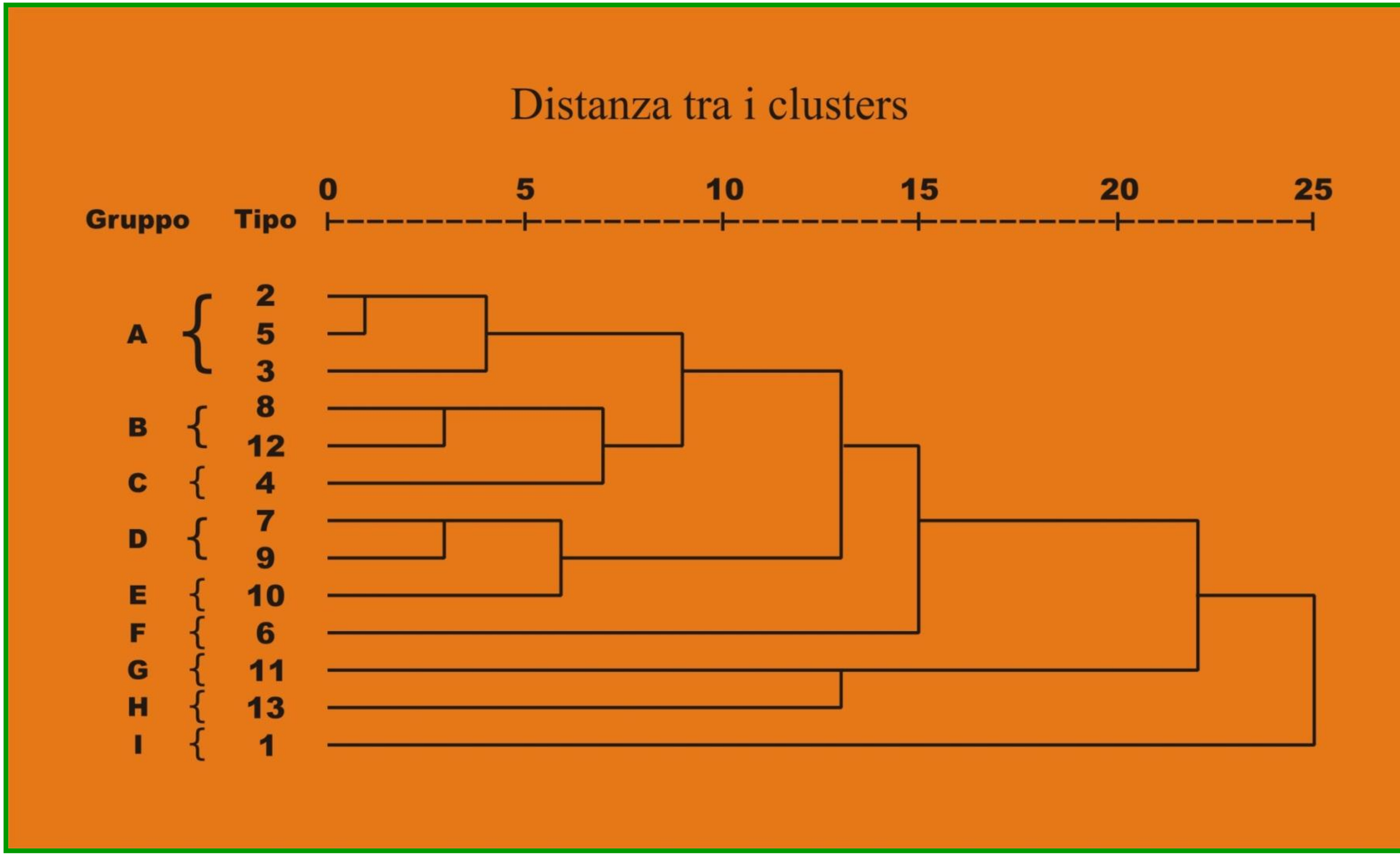


Total poliphenols





I
H
D-E
G
F
A
C
B
1
2
3
4
5
6
7
8
9
10
11
12
13
CP2
2
1
0
-1
CP1
3
2
1
0
-1
CP3
-2,0
-1,5
-1,0
-0,5
0,0
0,5
1,0
1,5



Distanza tra i clusters
0
5
10
15
20
25
Gruppo
Tipo
A
2
5
3
B
8
12
C
4
D
7
9
E
10
F
6
G
11
H
13
I
1

Kale diversity in Europe


Stilo Bivongi Latassa –IT B (CL)


Caltavuturo Romano -IT C (CL)


Couve Galega –PT A (CL)


Couve Todos os Dias –PT B (CL)


Artvin –TR A (CL)


Kocaeli –TR B (CL)


Veteran –DE (COM)


Hoj Amager Tofto - DK A (COM)


Lav Opretvoksende Lavo-DK B (COM)


Cavolo di Toscana nero Sgaravatti –IT (COM)


Garna Red F1 –SE (COM)


Wild Brassica oleracea –ES (WT)


Wild Brassica oleracea –FR (WT)


Wild Brassica oleracea –UK (WT)


Choux de Jalhay –BE (CL)


Kale Bih - BIH (CL)


Berza –ES (CL)


Fo 44-30 Chou Fourrager Cavalier-FR (CL)

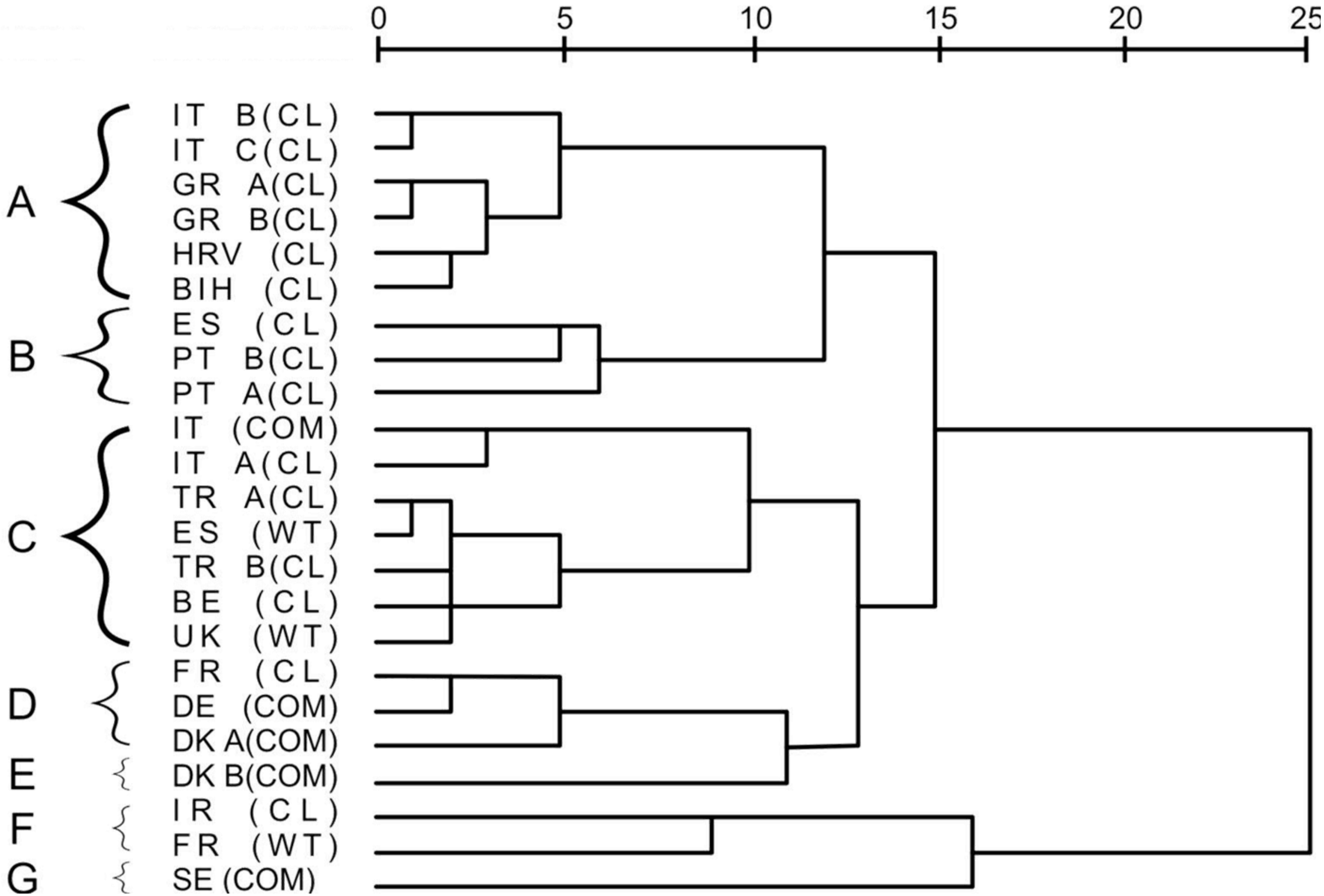

HL 207/07 –GR A (CL)


LKK 038/07 –GR B ((CL)


Istria, Kale-Croatia –HRV (CL)


Cut & Come Again –IR ((CL)


Cavolo nero vecchio –IT A (CL)



0
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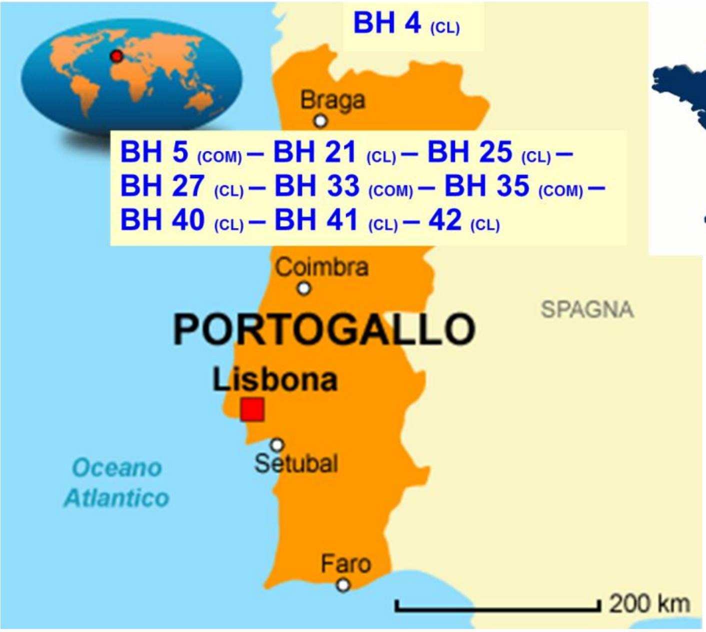
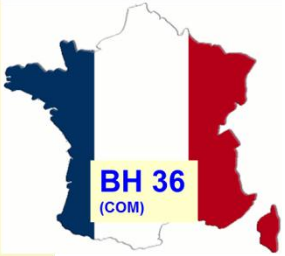
10
15
20
25
A
IT B(CL)
IT C(CL)
GR A(CL)
GR B(CL)
HRV (CL)
BIH (CL)
B
ES (CL)
PT B(CL)
PT A(CL)
C
IT (COM)
IT A(CL)
TR A(CL)
ES (WT)
TR B(CL)
BE (CL)
UK (WT)
D
FR (CL)
DE (COM)
DK A(COM)
E
DK B(COM)
F
IR (CL)
FR (WT)
G
SE (COM)

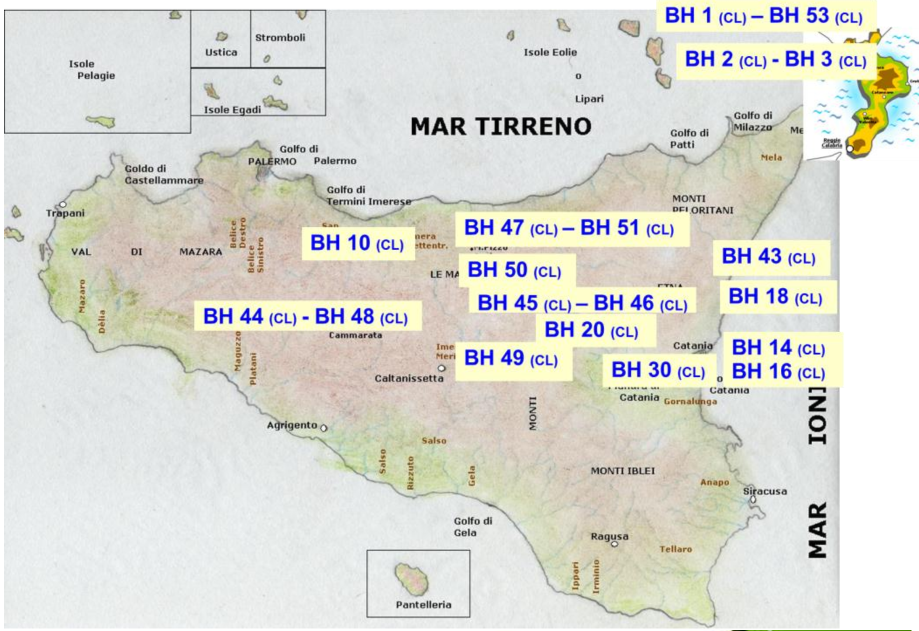


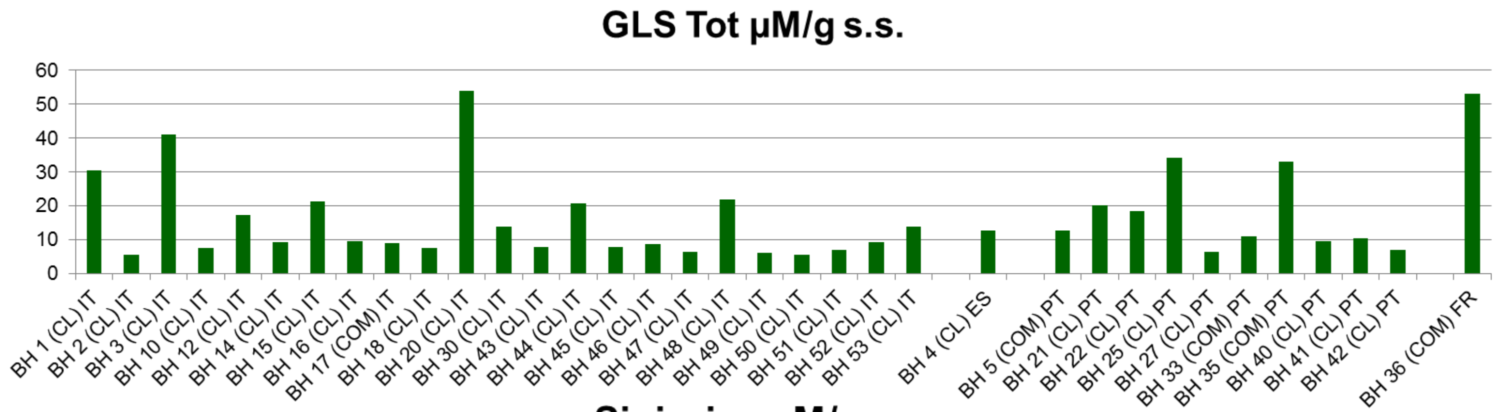


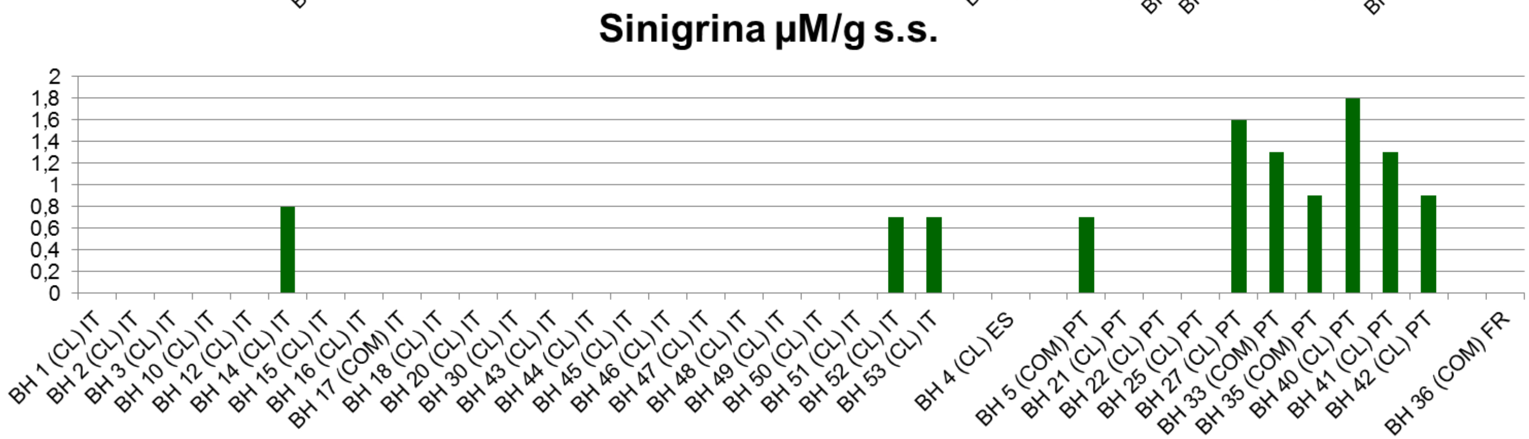


Diversity for antioxidant compounds of Italian and Iberian LRs of kale





GLS Tot μM/g s.s.
60
50
40
30
20
10
0
BH 1 (CL) IT
BH 2 (CL) IT
BH 3 (CL) IT
BH 10 (CL) IT
BH 12 (CL) IT
BH 14 (CL) IT
BH 15 (CL) IT
BH 16 (CL) IT
BH 17 (COM) IT
BH 18 (CL) IT
BH 20 (CL) IT
BH 30 (CL) IT
BH 43 (CL) IT
BH 44 (CL) IT
BH 45 (CL) IT
BH 46 (CL) IT
BH 47 (CL) IT
BH 48 (CL) IT
BH 49 (CL) IT
BH 50 (CL) IT
BH 51 (CL) IT
BH 52 (CL) IT
BH 53 (CL) IT
BH 4 (CL) ES
BH 5 (COM) PT
BH 21 (CL) PT
BH 22 (CL) PT
BH 25 (CL) PT
BH 27 (CL) PT
BH 33 (COM) PT
BH 35 (COM) PT
BH 40 (CL) PT
BH 41 (CL) PT
BH 42 (CL) PT
BH 36 (COM) FR



Sinigrina μM/g s.s.
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
BH 1 (CL) IT
BH 2 (CL) IT
BH 3 (CL) IT
BH 10 (CL) IT
BH 12 (CL) IT
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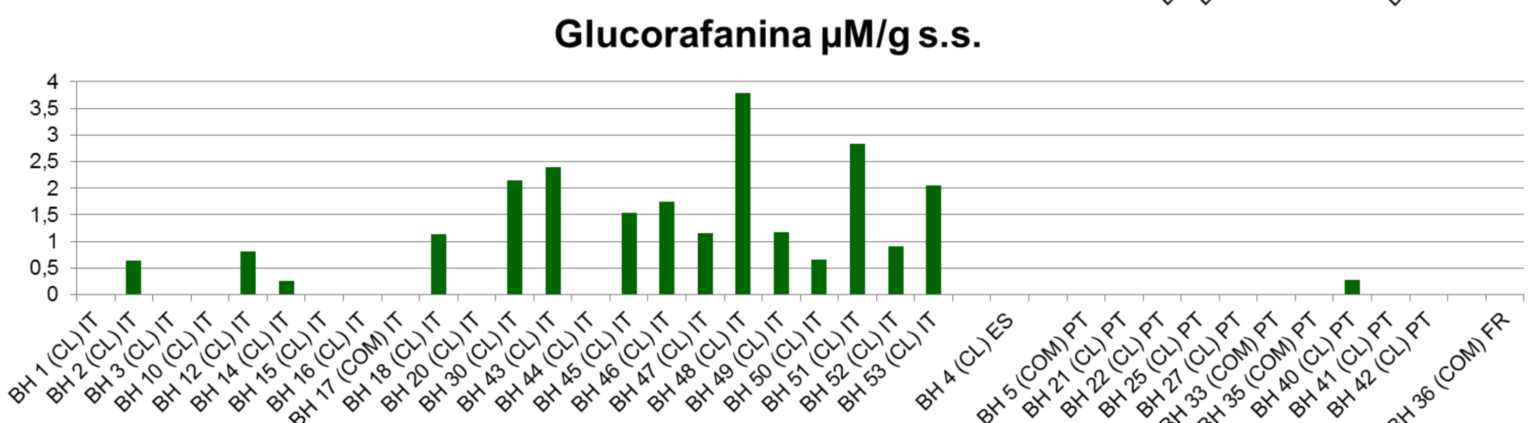
BH 48 (CL) IT
BH 49 (CL) IT
BH 50 (CL) IT
BH 51 (CL) IT
BH 52 (CL) IT
BH 53 (CL) IT
BH 4 (CL) ES
BH 5 (COM) PT
BH 21 (CL) PT
BH 22 (CL) PT
BH 25 (CL) PT
BH 27 (CL) PT
BH 33 (COM) PT
BH 35 (COM) PT
BH 40 (CL) PT
BH 41 (CL) PT
BH 42 (CL) PT
BH 36 (COM) FR



Glucorafanina μM/g s.s.
4
3,5
3
2,5
2
1,5
1
0,5
0
BH 1 (CL) IT
BH 2 (CL) IT
BH 3 (CL) IT
BH 10 (CL) IT
BH 12 (CL) IT
BH 14 (CL) IT
BH 15 (CL) IT
BH 16 (CL) IT
BH 17 (COM) IT
BH 18 (CL) IT
BH 20 (CL) IT
BH 30 (CL) IT
BH 43 (CL) IT
BH 44 (CL) IT
BH 45 (CL) IT
BH 46 (CL) IT
BH 47 (CL) IT
BH 48 (CL) IT
BH 49 (CL) IT
BH 50 (CL) IT
BH 51 (CL) IT
BH 52 (CL) IT
BH 53 (CL) IT
BH 4 (CL) ES
BH 5 (COM) PT
BH 21 (CL) PT
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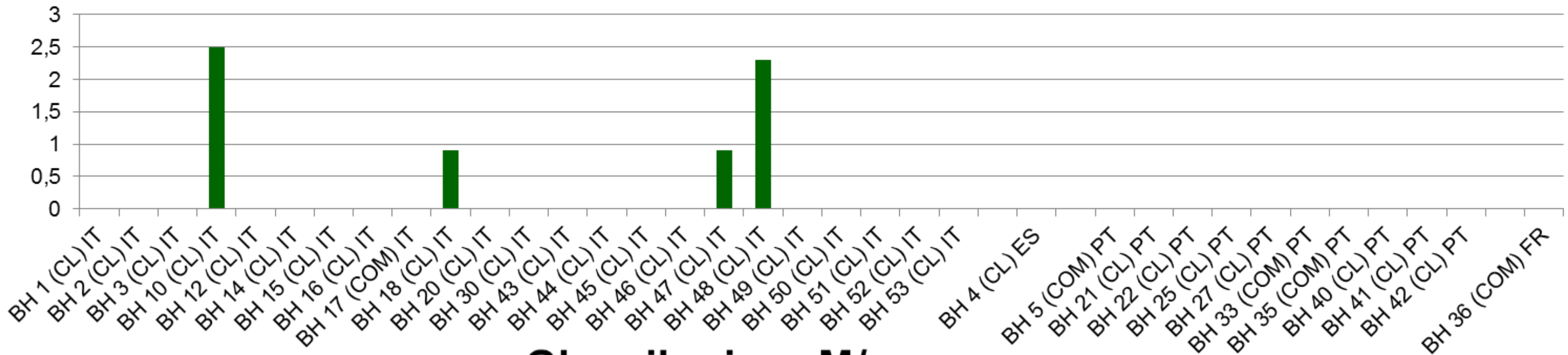
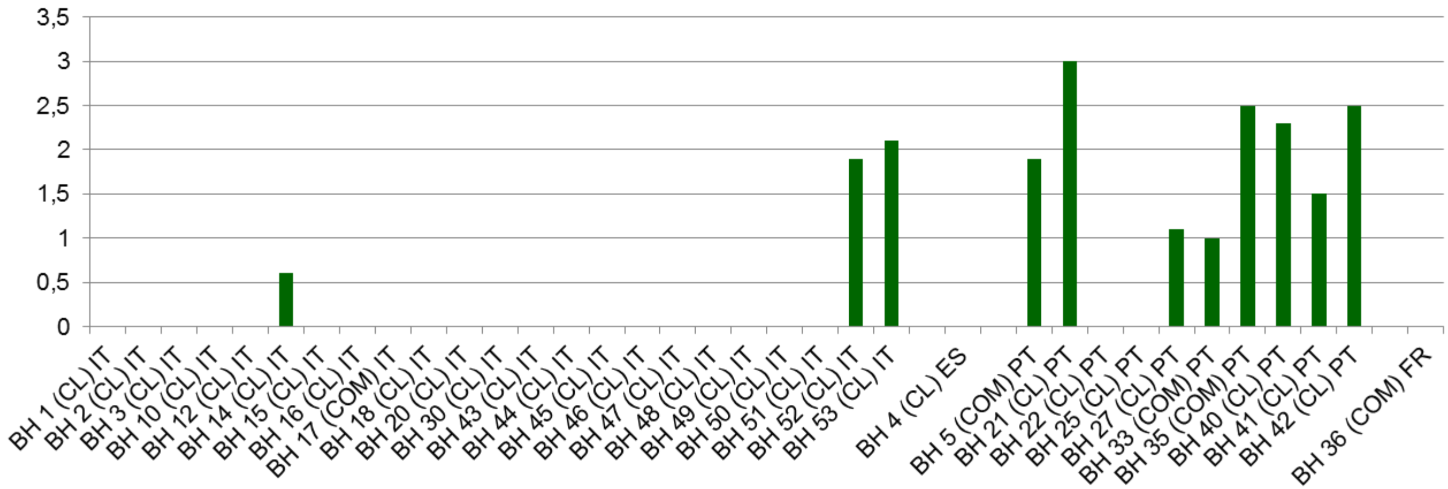
BH 25 (CL) PT
BH 27 (CL) PT
BH 33 (COM) PT
BH 35 (COM) PT
BH 40 (CL) PT
BH 41 (CL) PT
BH 42 (CL) PT
BH 36 (COM) FR

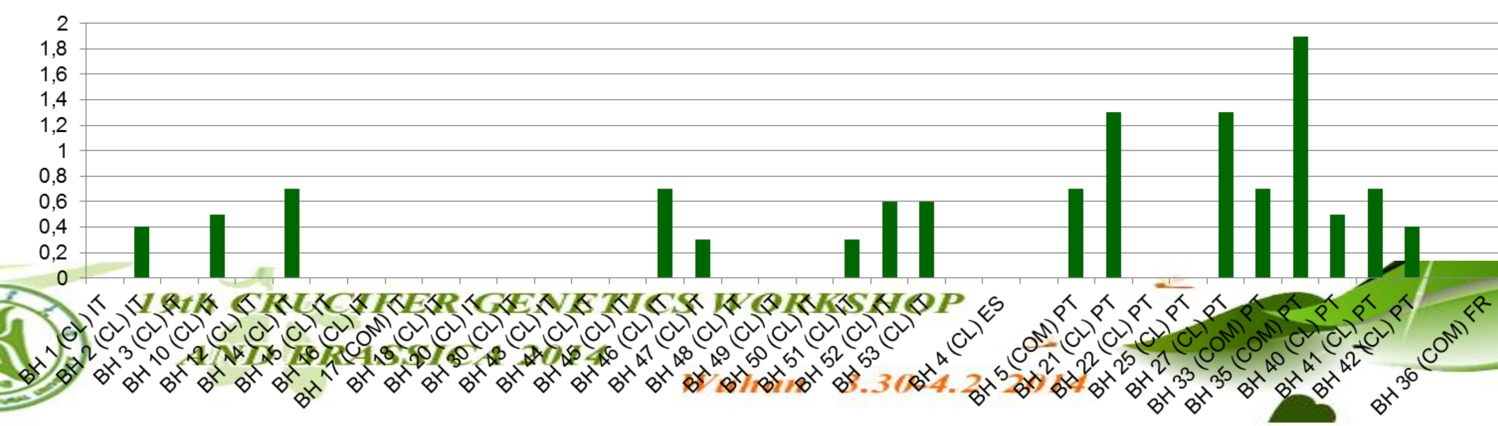
Progoitrina µM/g s.s.

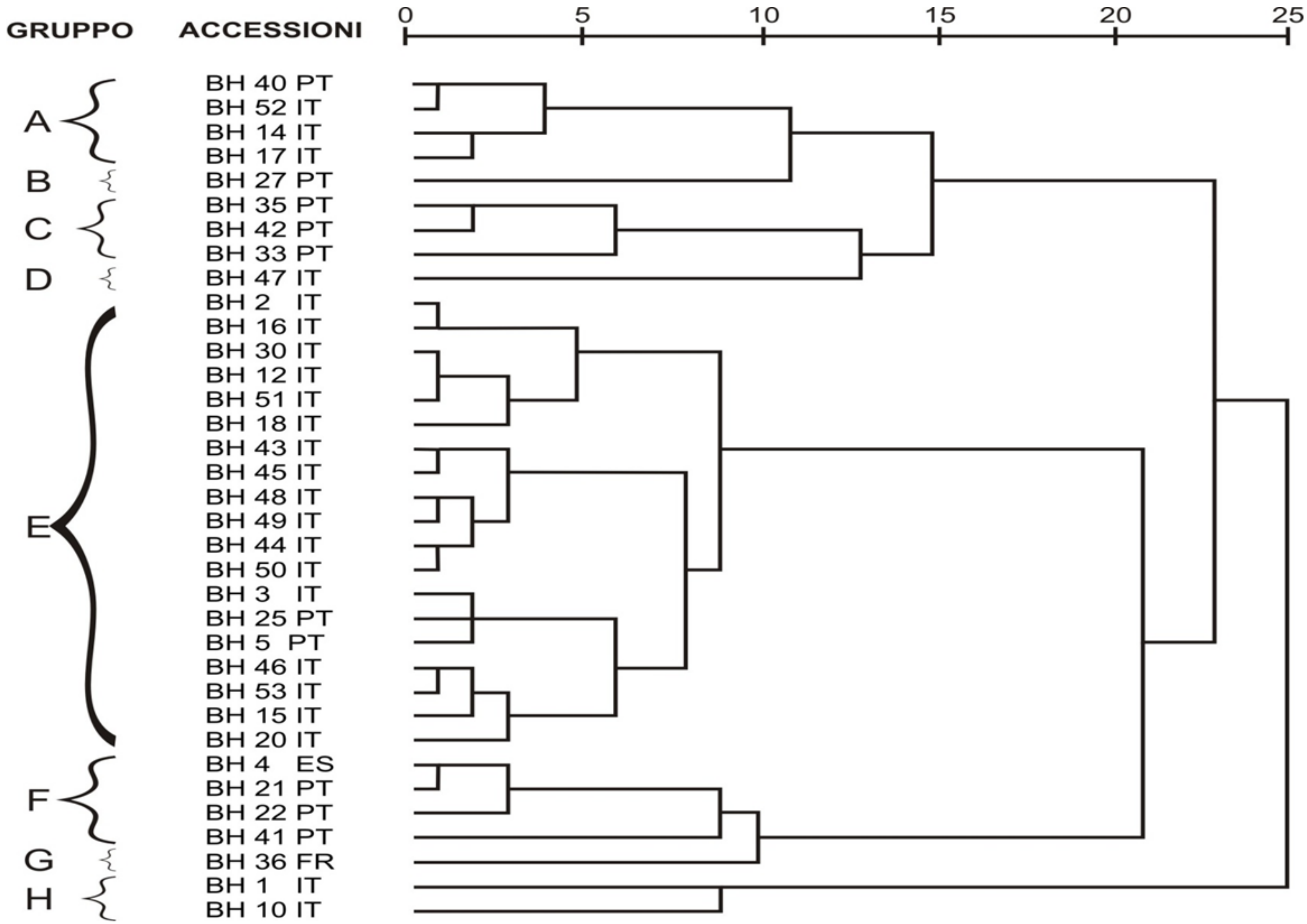


Glucoiberina µM/g s.s.



Gluconasturtina µM/g s.s.





GRUPPO
ACCESSIONI
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15
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25
A
B
C
D
E
F
G
H
BH 40 PT
BH 52 IT
BH 14 IT
BH 17 IT
BH 27 PT
BH 35 PT
BH 42 PT
BH 33 PT
BH 47 IT
BH 2 IT
BH 16 IT
BH 30 IT
BH 12 IT
BH 51 IT
BH 18 IT
BH 43 IT
BH 45 IT
BH 48 IT
BH 49 IT
BH 44 IT
BH 50 IT
BH 3 IT
BH 25 PT
BH 5 PT
BH 46 IT
BH 53 IT
BH 15 IT
BH 20 IT
BH 4 ES
BH 21 PT
BH 22 PT
BH 41 PT
BH 36 FR
BH 1 IT
BH 10 IT

Brassica incana

Brassica macrocarpa

Brassica rupestris

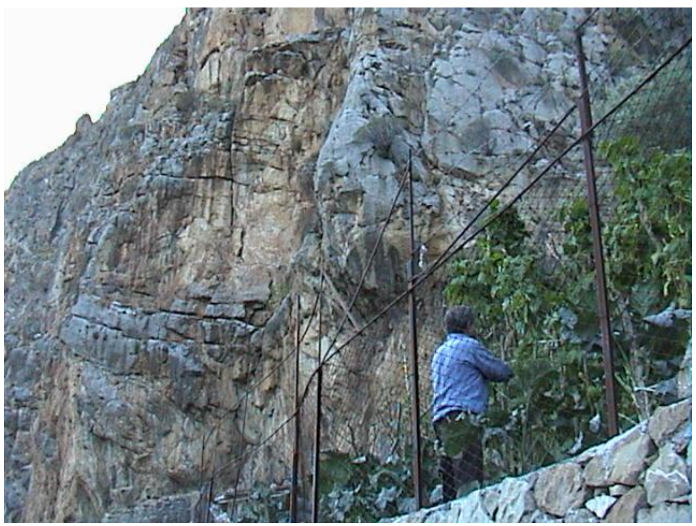
Brassica villosa









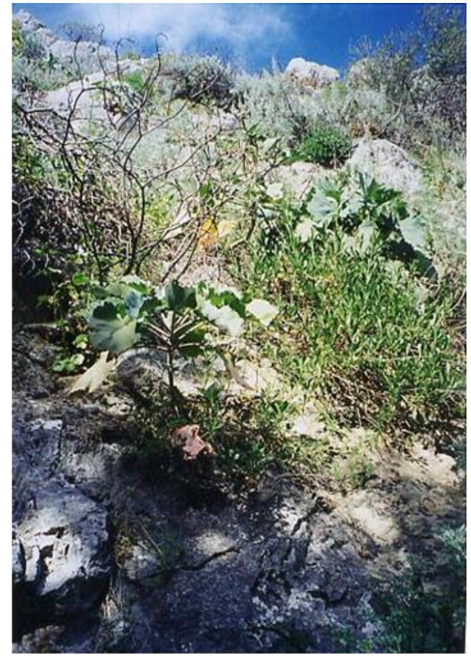












AEGRO


N
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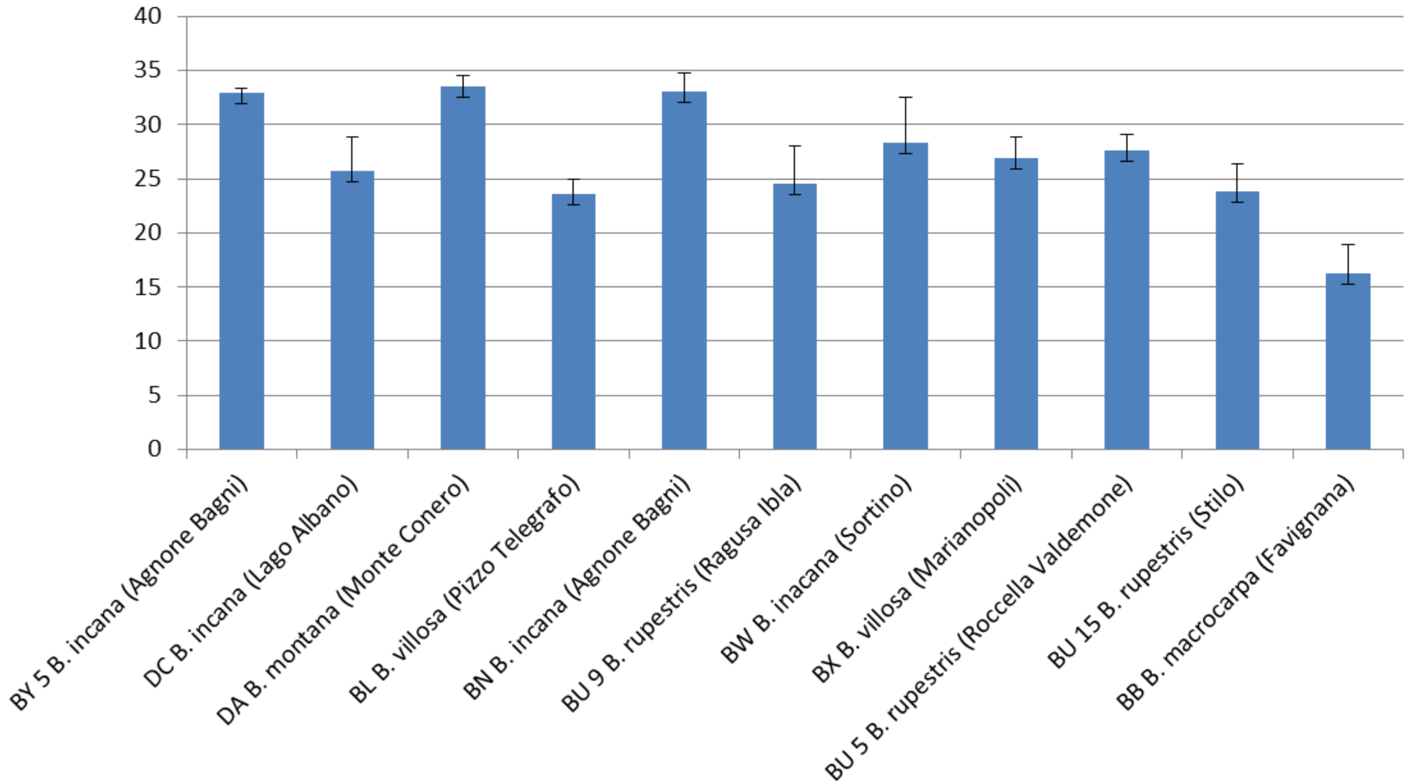
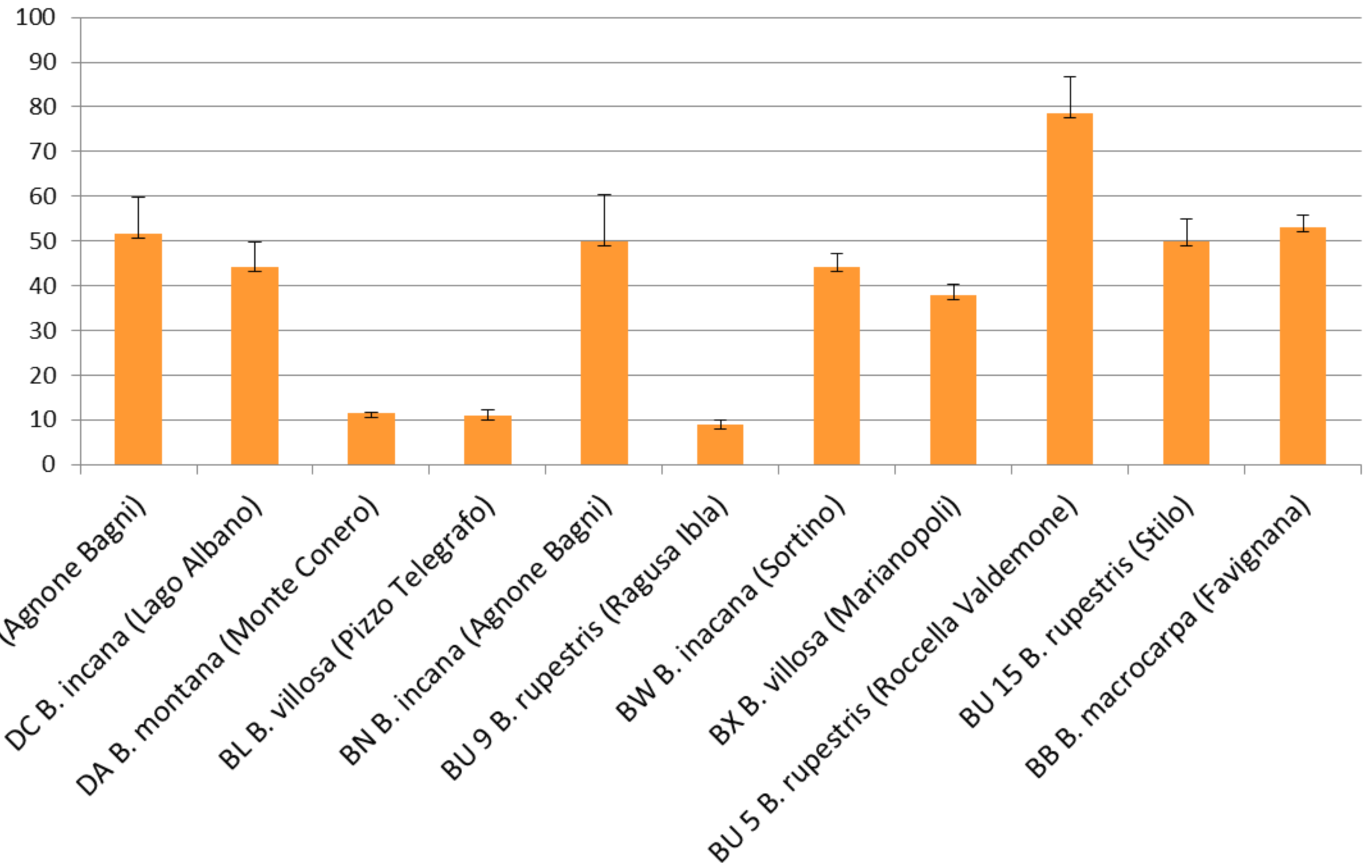
AEGRO

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

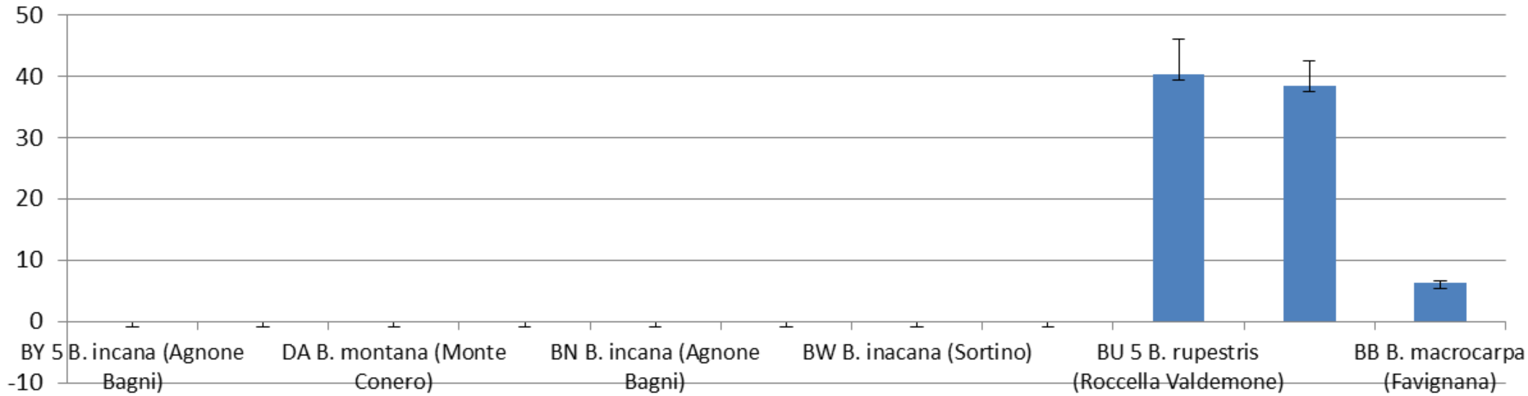


Glucosinolate Total (μM/g d.w.)

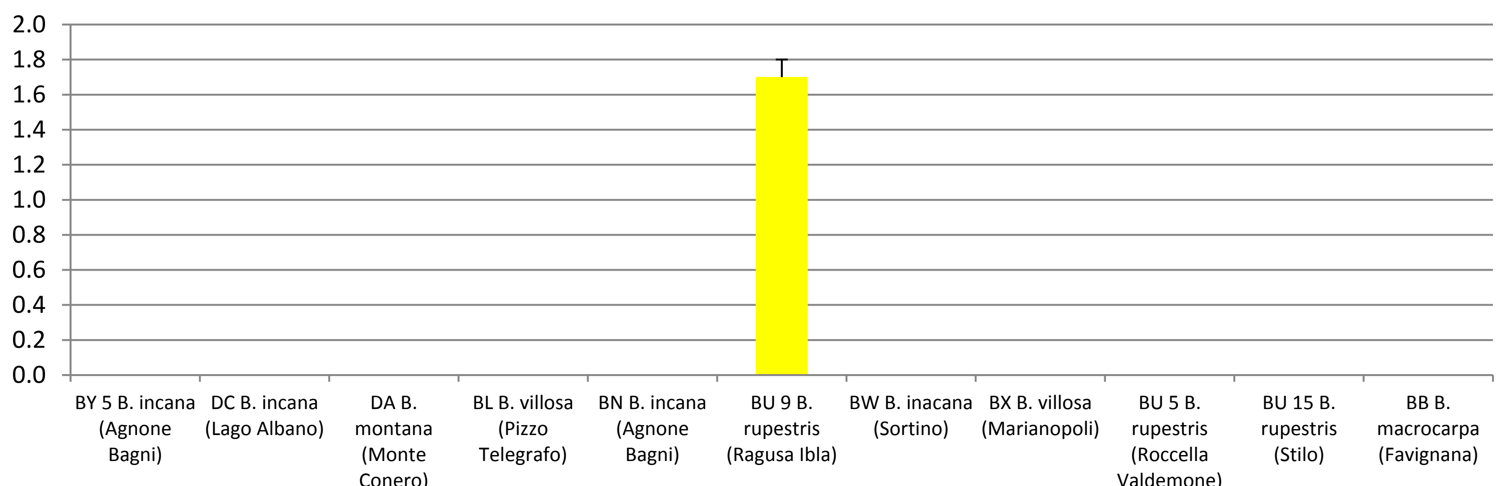


Aliphatic

Glucoiberin (μM/g d.w.)

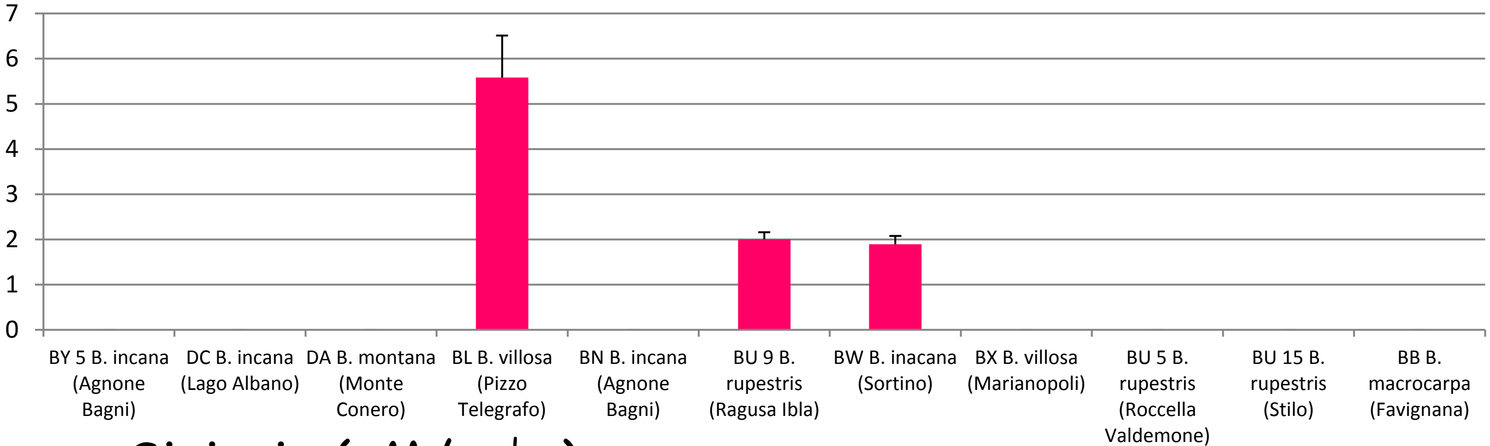


Progoitrin (μM/g d.w.)

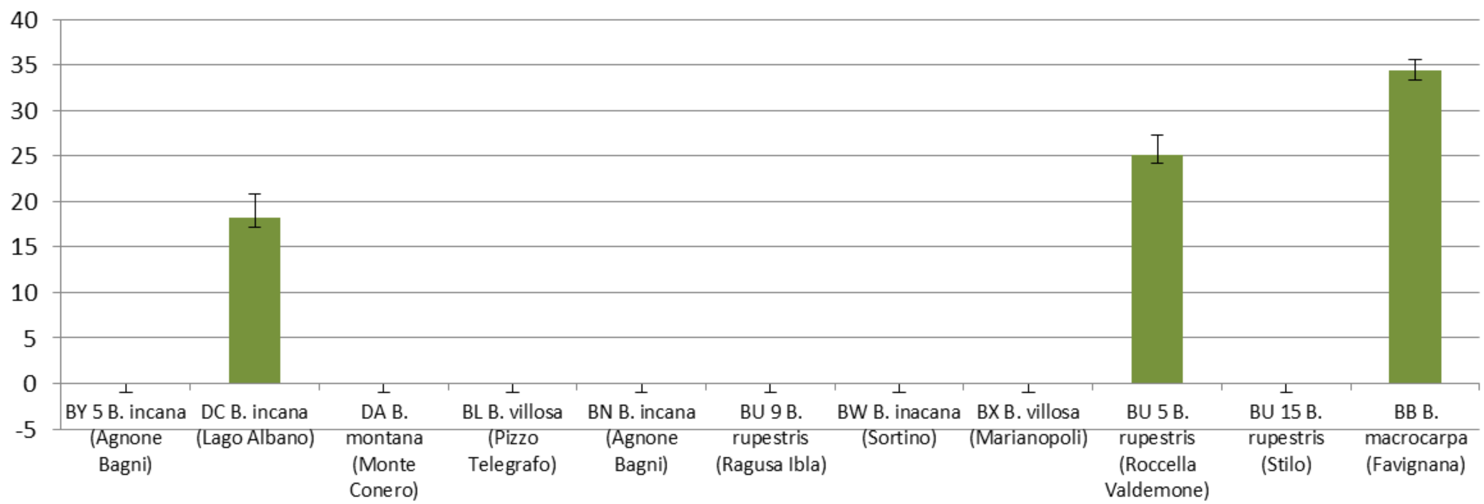


Aliphatic

Glucoraphanin (μM/g d.w.)

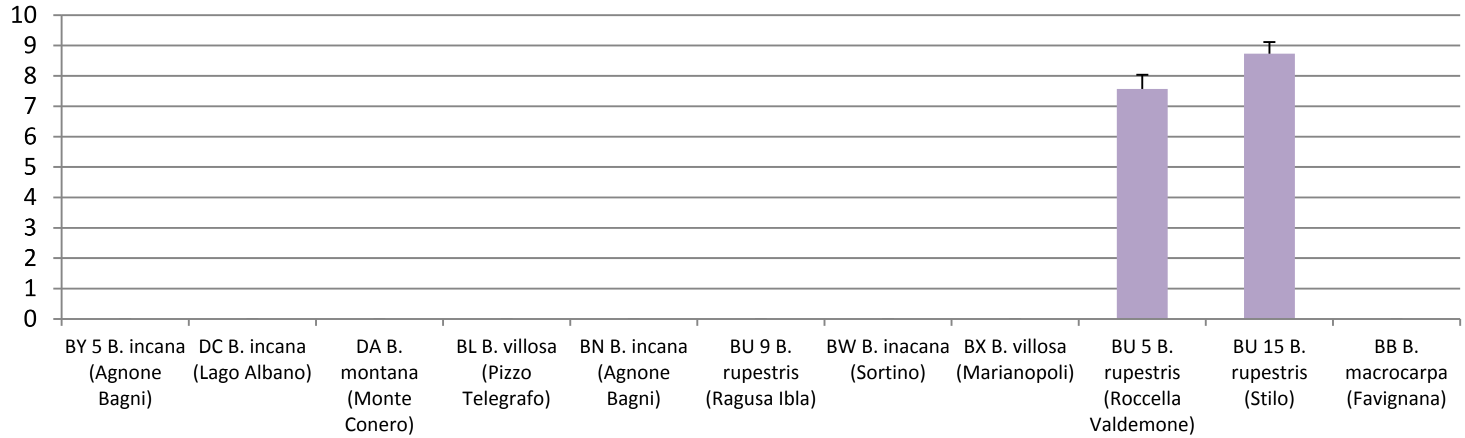


Sinigrin (μM/g d.w.)

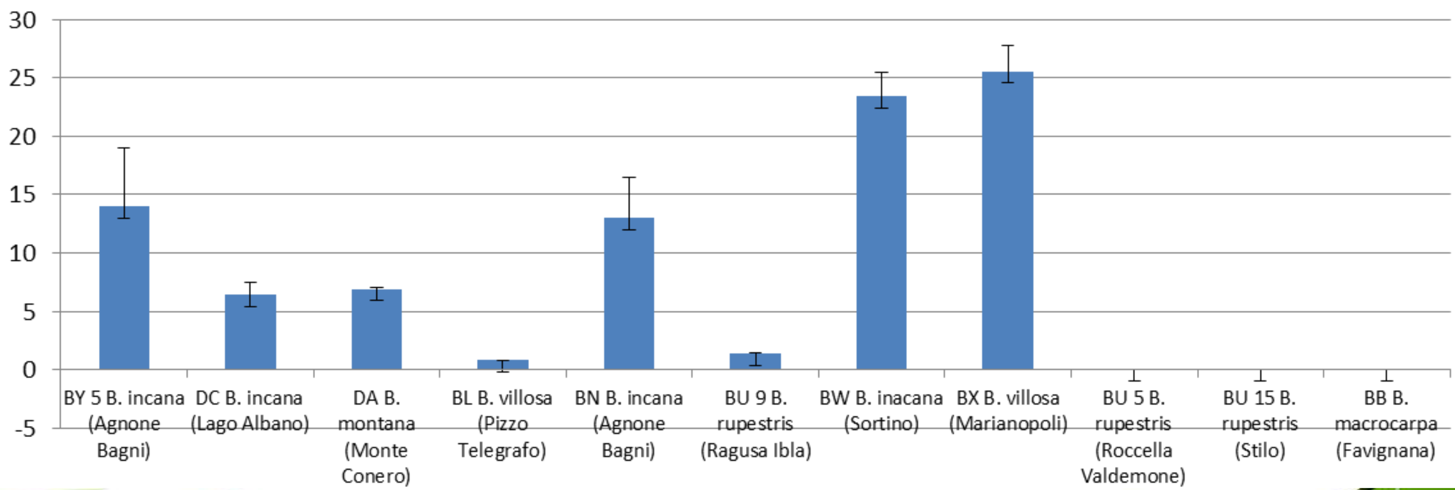


Aliphatic

Sinalbin (μM/g d.w.)

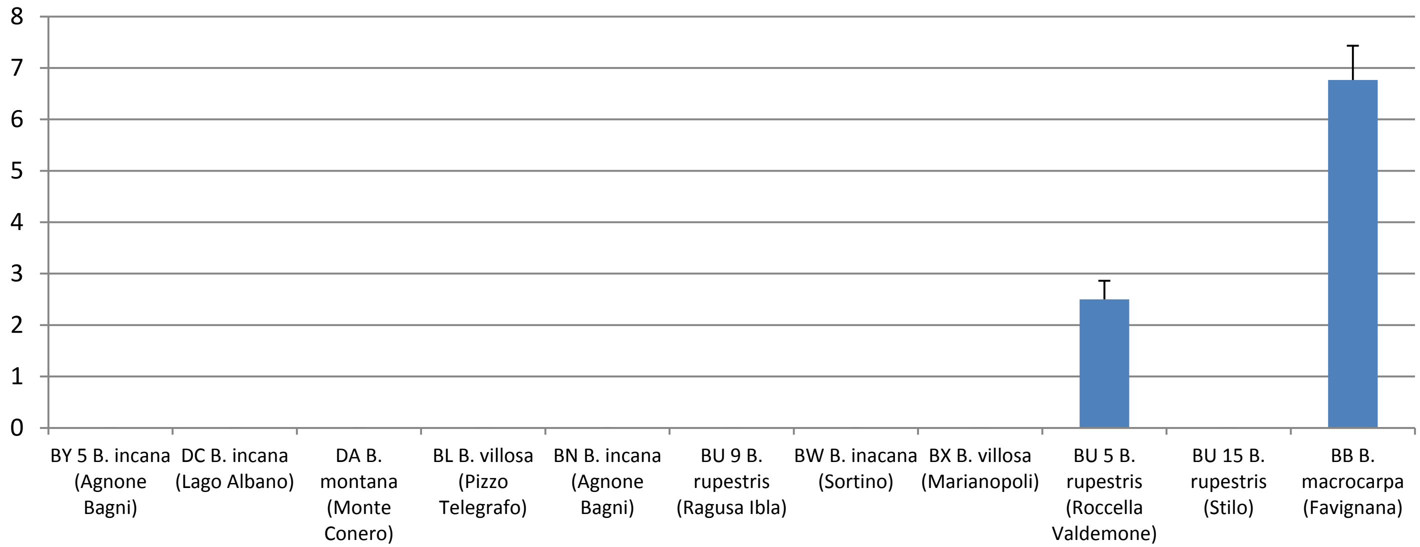


Gluconapin (μM/g d.w.)



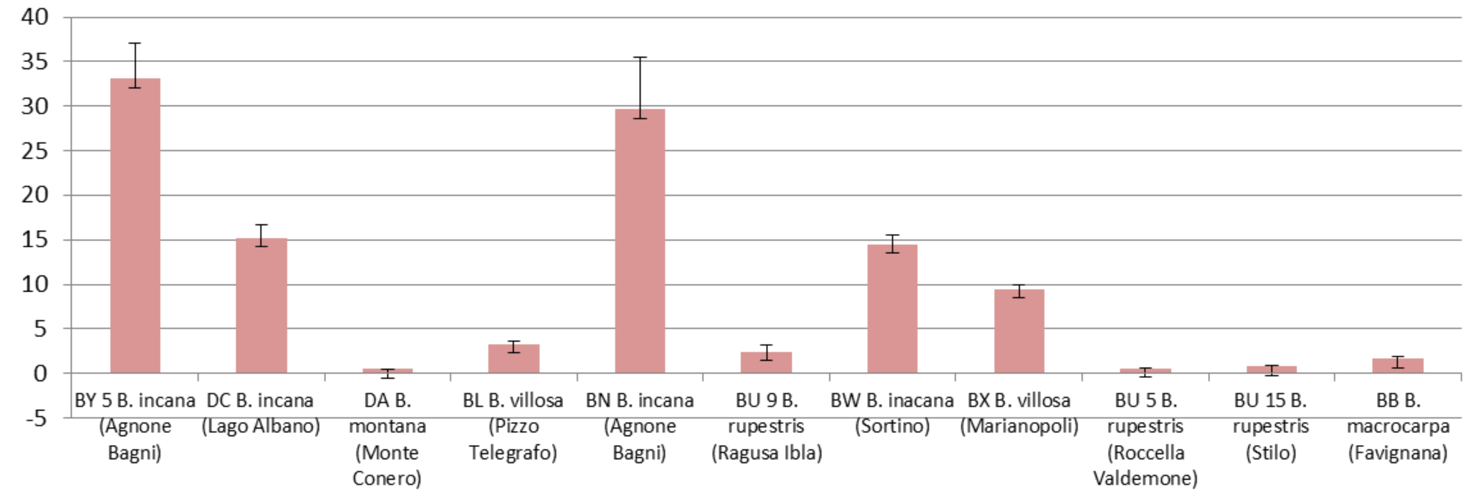
Aliphatic

Glucoiberverin (μM/g d.w.)

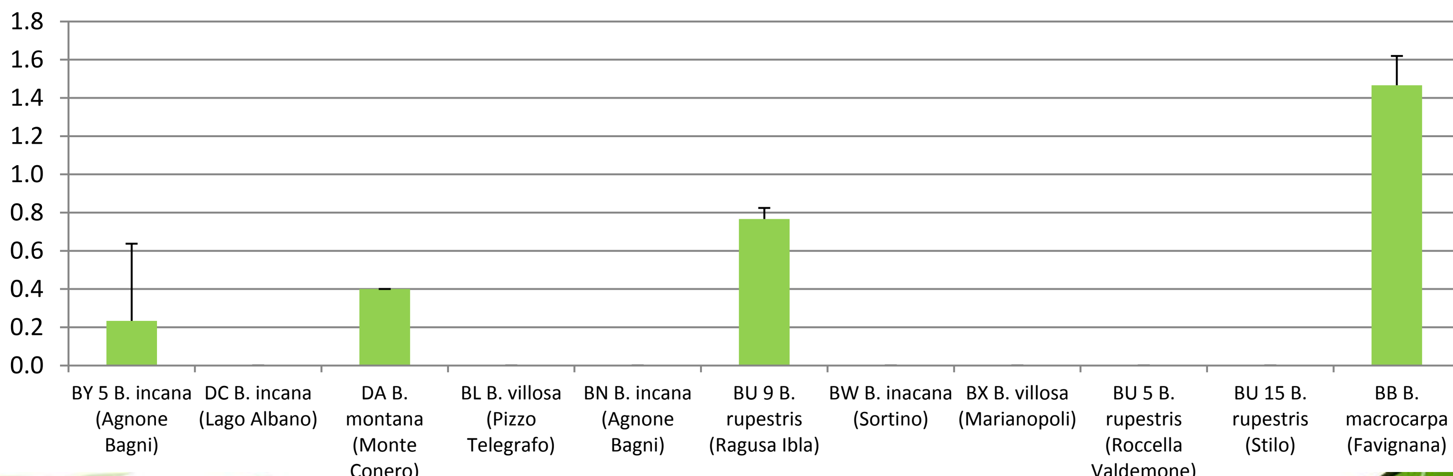


Indolic

Glucobrassicin (µM/g d.w.)

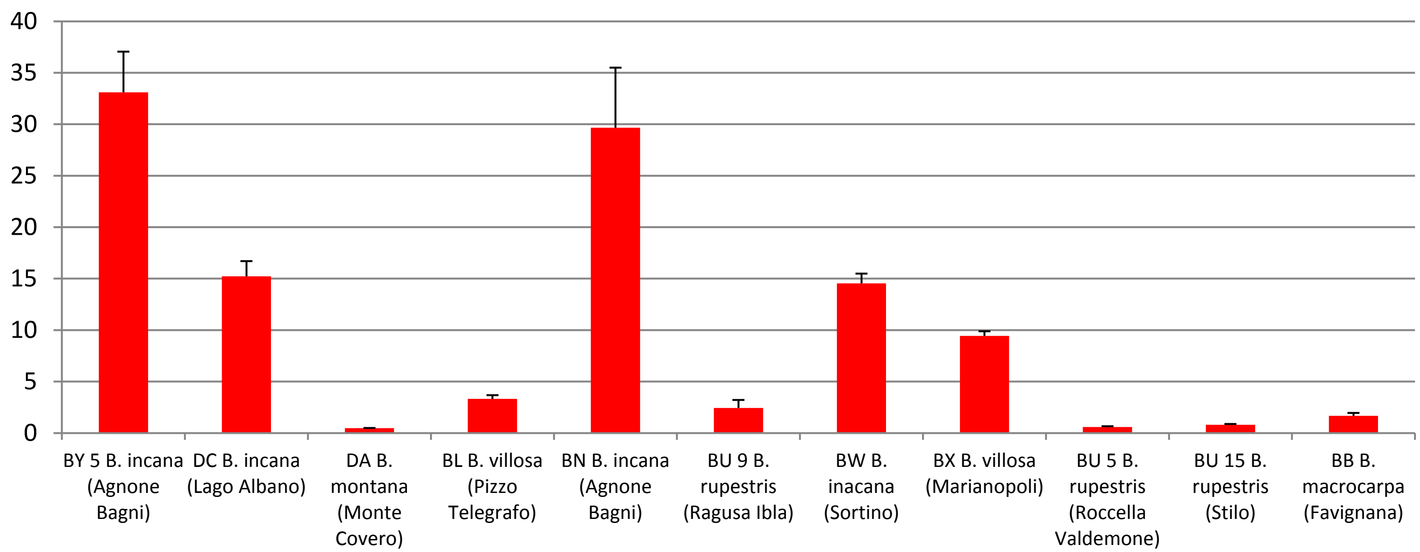


Neo-Glucobrassicin (µM/g d.w.)



Aromatic

Gluconasturtiin (μM/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

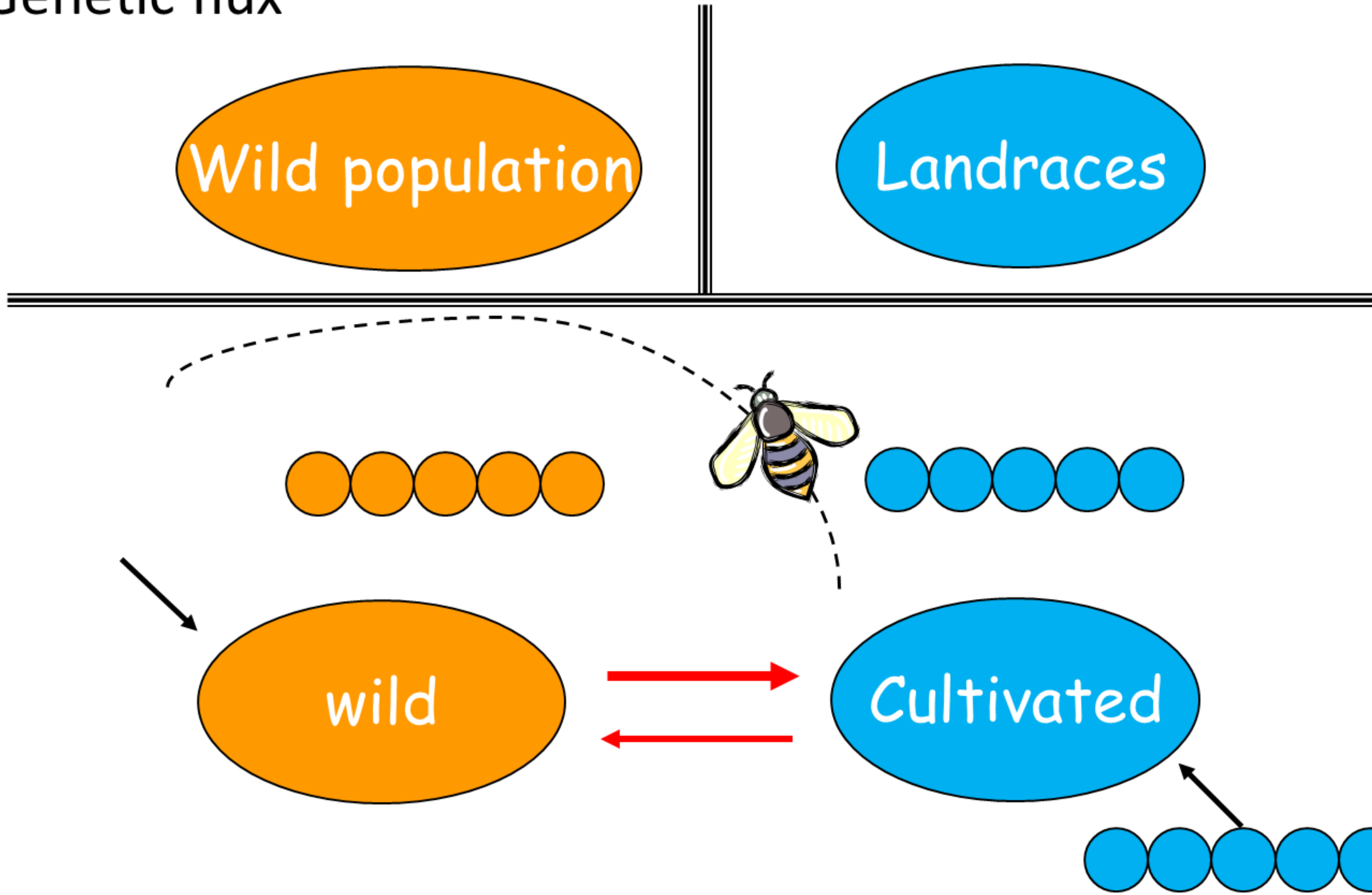
DPPH vs CROCIN	0.705	*
DPPH vs ATH	-0.043	ns
DPPH vs TPC	0.648	*
DPPH vs AsA	0.506	ns
DPPH vs CAR	0.323	ns
DPPH vs GLS	-0.193	ns
CROCIN vs ATH	-0.103	ns
CROCIN vs TPC	0.687	*
CROCIN vs AsA	0.293	ns
CROCIN vs CAR	0.811	**
CROCIN vs GLS	-0.420	ns

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

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

Genetic flux between wild species and landraces and their role for crop differentiation

Genetic flux



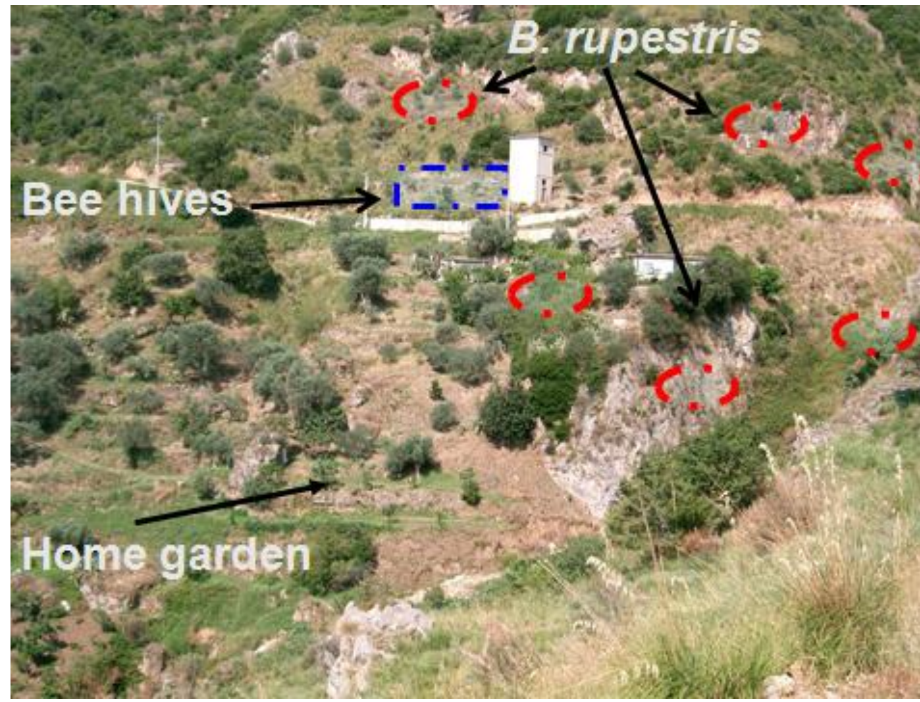





Signals of Inter-crossing between Leafy Kale Landraces and *Brassica rupestris* in South Italy

Maggioni et al, 2013, Acta Hort. 1005[1]

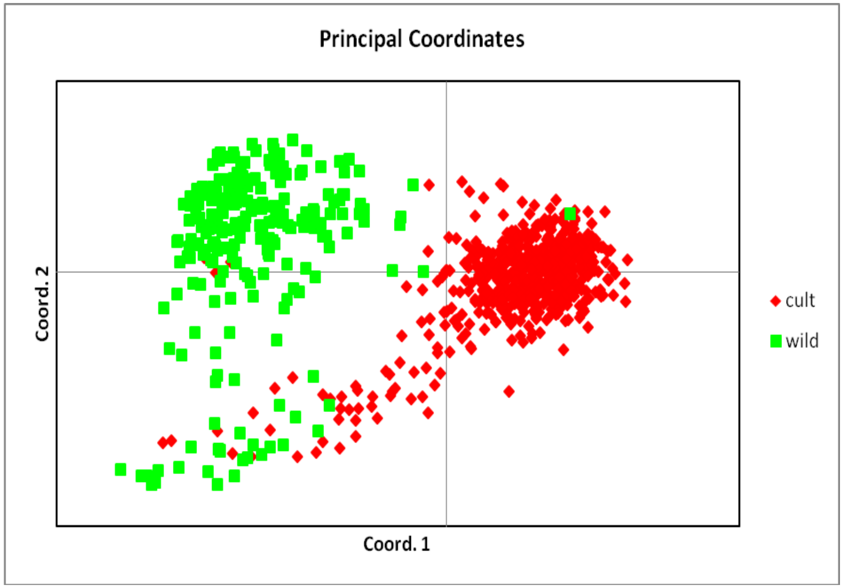
During two years we collected leafy kale landraces from 6 home gardens and 5 wild populations of *B. rupestris* in Calabria



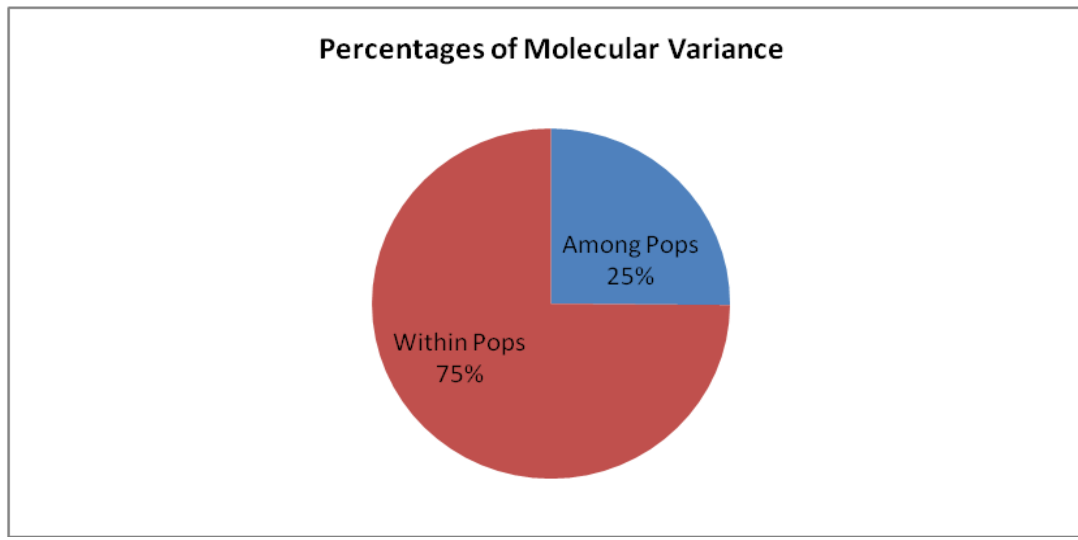
Leafy kale from 17 home gardens and 4 wild populations in Sicily.

A putative hybrid population was collected in Sicily.

A total of 889 individuals were analyzed by AFLPs



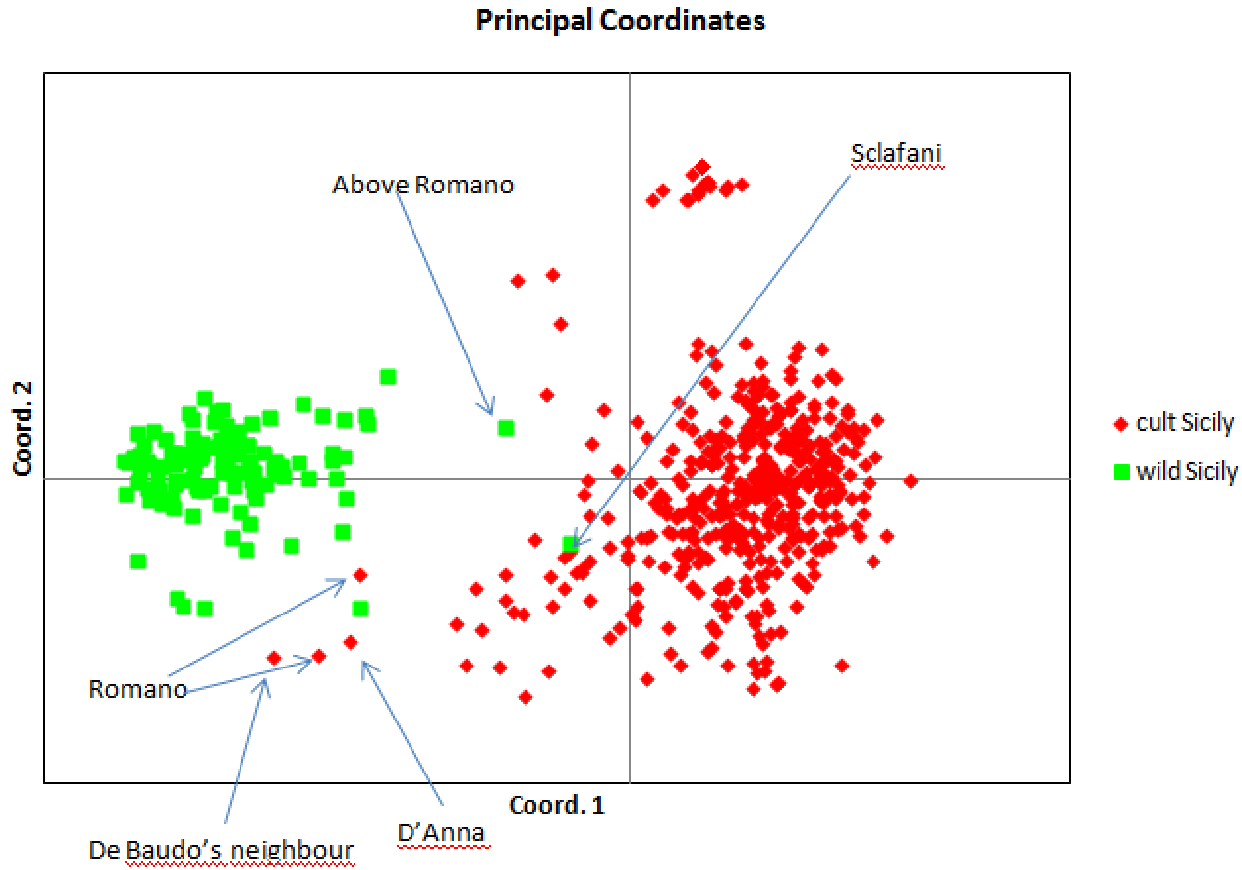
PCA of all leafy kale (cult) and all *Brassica rupestris* (wild) populations



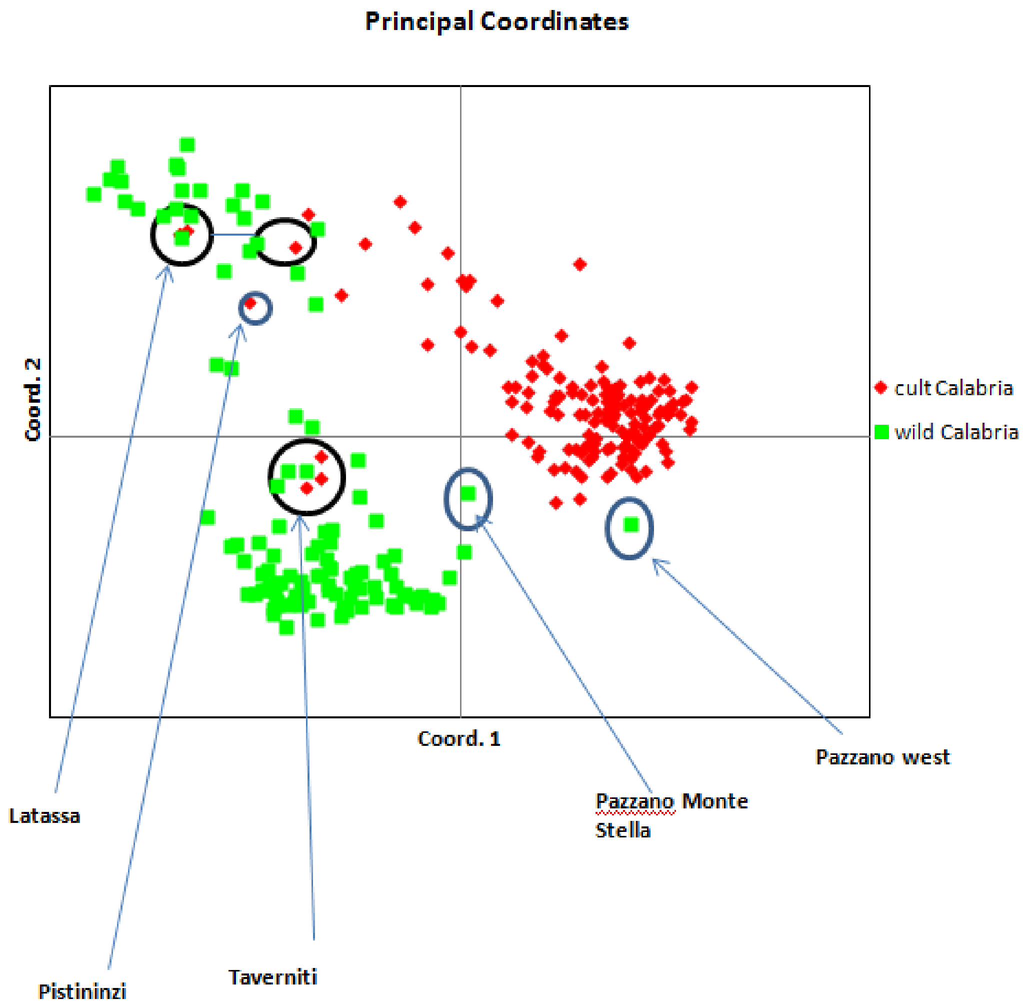


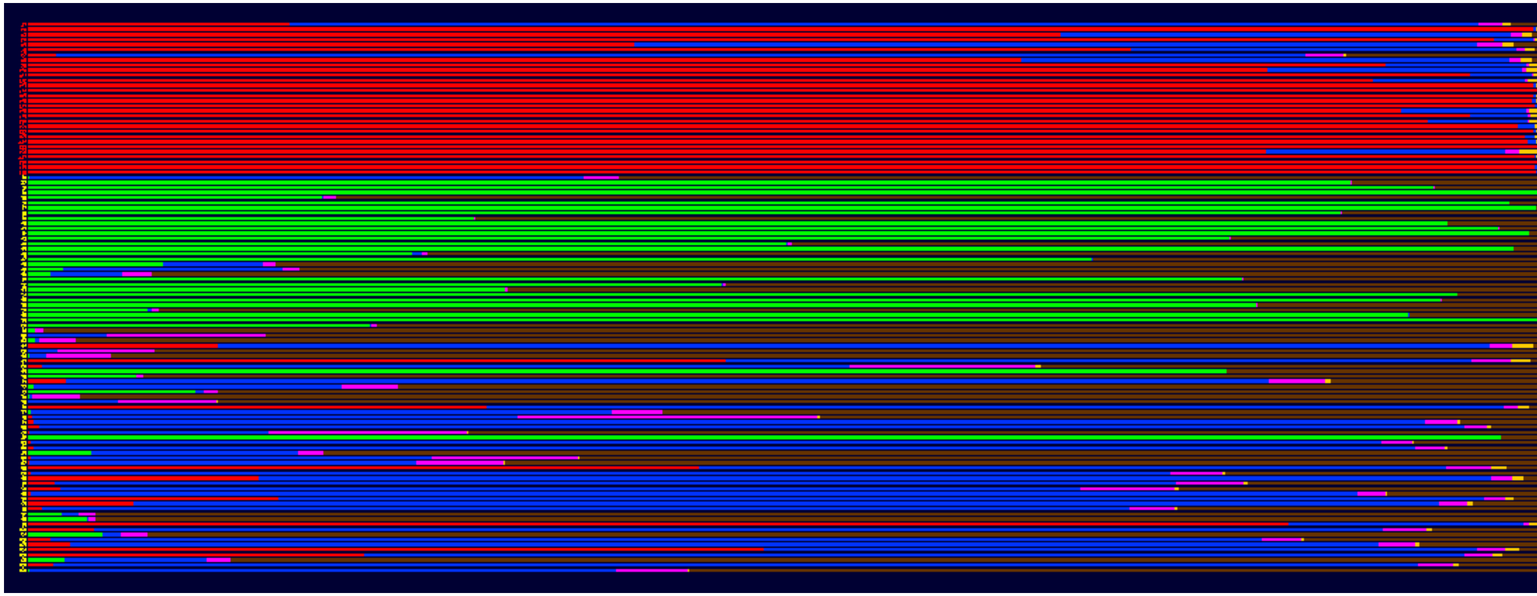



PCA of Sicilian leafy kales vs. B. rupestris populations



PCA of Calabrian leafy kales vs. *B. rupestris* populations and AMOVA





Average category probability after 37000 sweeps.
Rows 1-30, cultivated samples;
Rows 31-60, wild samples;
Rows 61-108, putative hybrids.

Red = Pure cultivated;
Green = Pure wild;
Blue = F1;
Pink = F2;
Light brown = Backcross with cultivated;
Dark brown = Backcross with wild.

Project for Phase VIII (2009-2013) of ECPGR of the Brassica Working Group

Brassica wild species

An European core collection of about 26 accessions of wild *Brassica* species was set up by the contribution of several EU genebanks

These have been characterized and evaluated for several traits, such as bio-morphological, oil content, nutraceutical compounds, and by some SSr primers






ECPGR Brassica Working Group project

INSTCODE	ACCENUMB	GENUS AND SPECIES	SPECIES CODE
DEU 146	BRA 2850	Brassica balearica	BB
DEU 146	BRA 2990/K10127	Brassica barrelieri	BA
DEU 146	BRA 2998	Brassica bourgeaui	BO1
DEU 146	BRA 2848	Brassica bourgeaui	BO2
DEU 146	K 10120	Brassica cretica	BC2
DEU 146	K 6631	Brassica cretica	BC1
DEU 146	BRA 2919	Brassica desnottesii	BE
DEU 146	BRA 2923	Brassica drepanensis	BD
DEU 146	BRA1810	Brassica fruticulosa	BF1
DEU146	BRA 2918	Brassica incana	BY2
DEU146	K 5997	Brassica insularis	BI
DEU146	CR 2929	Brassica rapa	CR
DEU146	BRA 2945/K7690	Brassica rupestris	BU1
DEU146	BRA1896	Brassica villosa	BV1
DEU146	BRA 2944	Brassica macrocarpa	BM2
DEU146	BRA 1644	Brassica montana	BX2
DEU146	BRA 1727	Brassica fruticulosa	BF2
NLD037	CGN06903	Brassica oleracea	BR
NLD037	CGN14116	Brassica villosa	BV2
NLD037	CGN18472	Brassica montana	BX1
HRI:02	HRI:02:006691	Brassica incana	BY1
HRI:08	HRI:08:0124838	Brassica hilarionis	BH
UNICT 2973	BB	Brassica macrocarpa	BM1
UNICT	S542	Brassica rupestris	BU2
UNICT	S401	Brassica rupestris	BU3
UNICT3944	BU7	Brassica incana	BV3

		IBPGR
1	Vegetative stem length	4.2.54
2	Vegetative stem width (Diameter)	4.2.55
3	length/ diameter	4.2.56
4	Plant height	4.2.3
5	Plant diameter	4,2,4
6	Plant shape	4.2.2 UPOV_3
7	Leaf anthocyanin coloration	UPOV_5
8	Leaf color (of fully developed leaf)	4.2.24
9	Leaf blade shape	4.2.16
10	Leaf blade length	4.2.12
11	Leaf blade width	4.2.13
12	Leaf angle - petiole attitude (at middle of plant)	4.2.15
13	Leaf lamina attitude	4.2.23
14	Leaf blade density of curling (leaves at middle of plants)	4.5.10 UPOV_14
15	Leaf blade blistering	4.2.21
16	Leaf lobes	
17	Petiole length	
18	Petiole width	
19	Petiole enlargement	4.2.27
20	Petiole and/or midwein color	4.2.33
21	Branching plant	
22	heading habit!!!	4.2.34
23	Average leaves per plant main stem	4.2.11
24	Average leaf scars	4.2.10
25	Folding leaf section	

Brassica balearica

Brassica bourgeaui

Brassica cretica

Brassica desnottesii

Brassica drepanensis

Brassica fruticulosa

Brassica hilarionis

Brassica incana

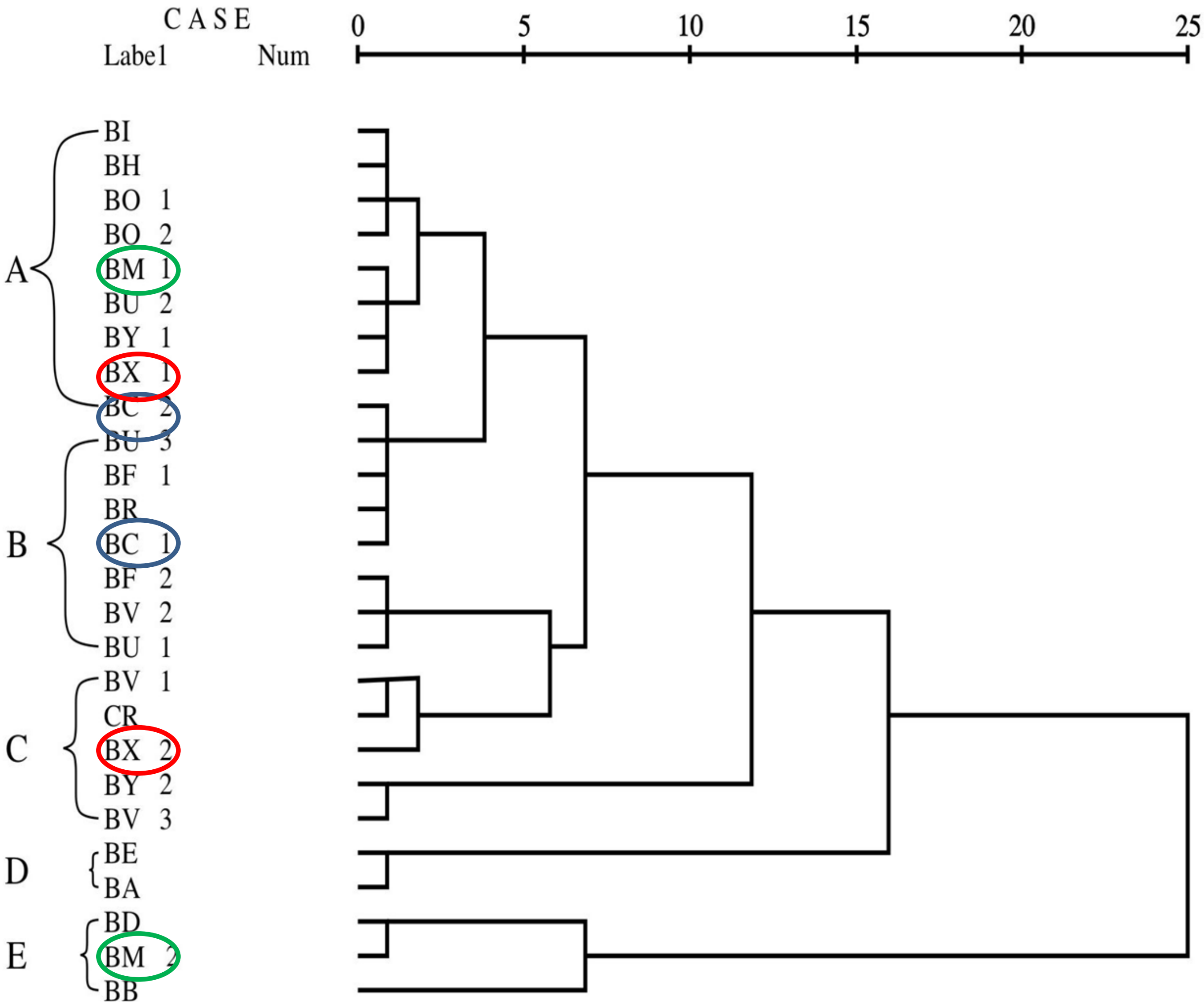
Brassica macrocarpa

Brassica montana

Brassica rupestris

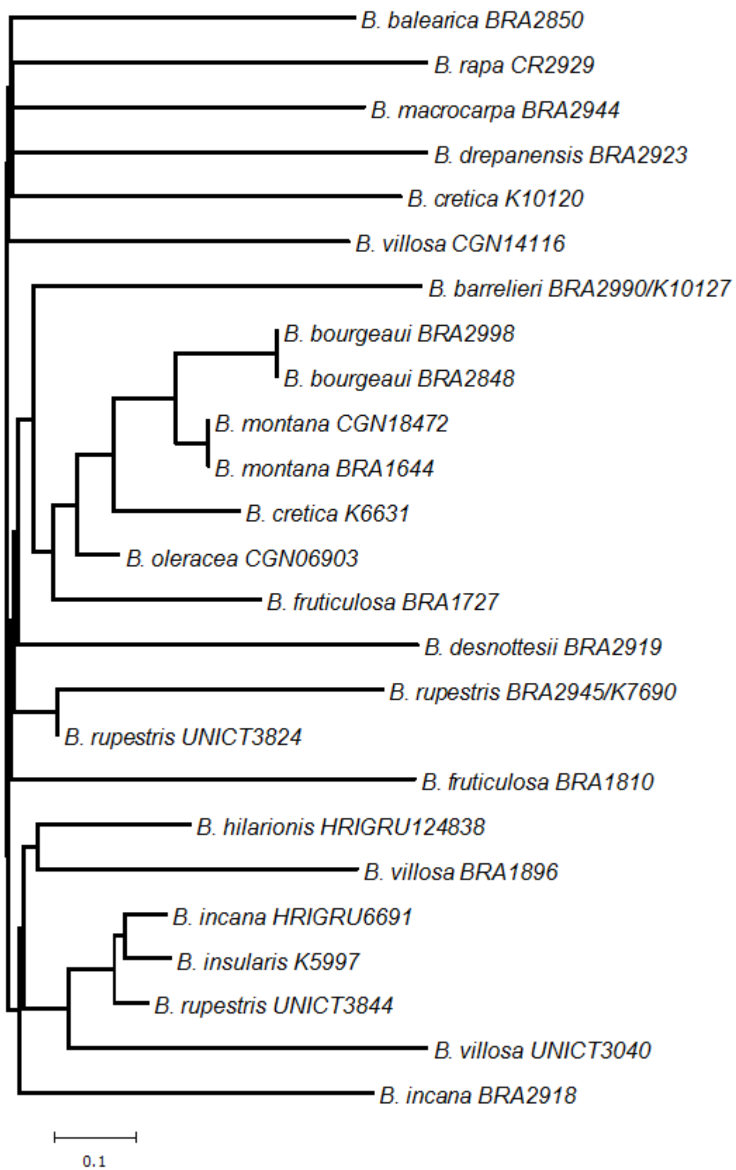
Brassica villosa

Cluster analysis of the accessions characterized



Putative SSr primer for MADS box genes

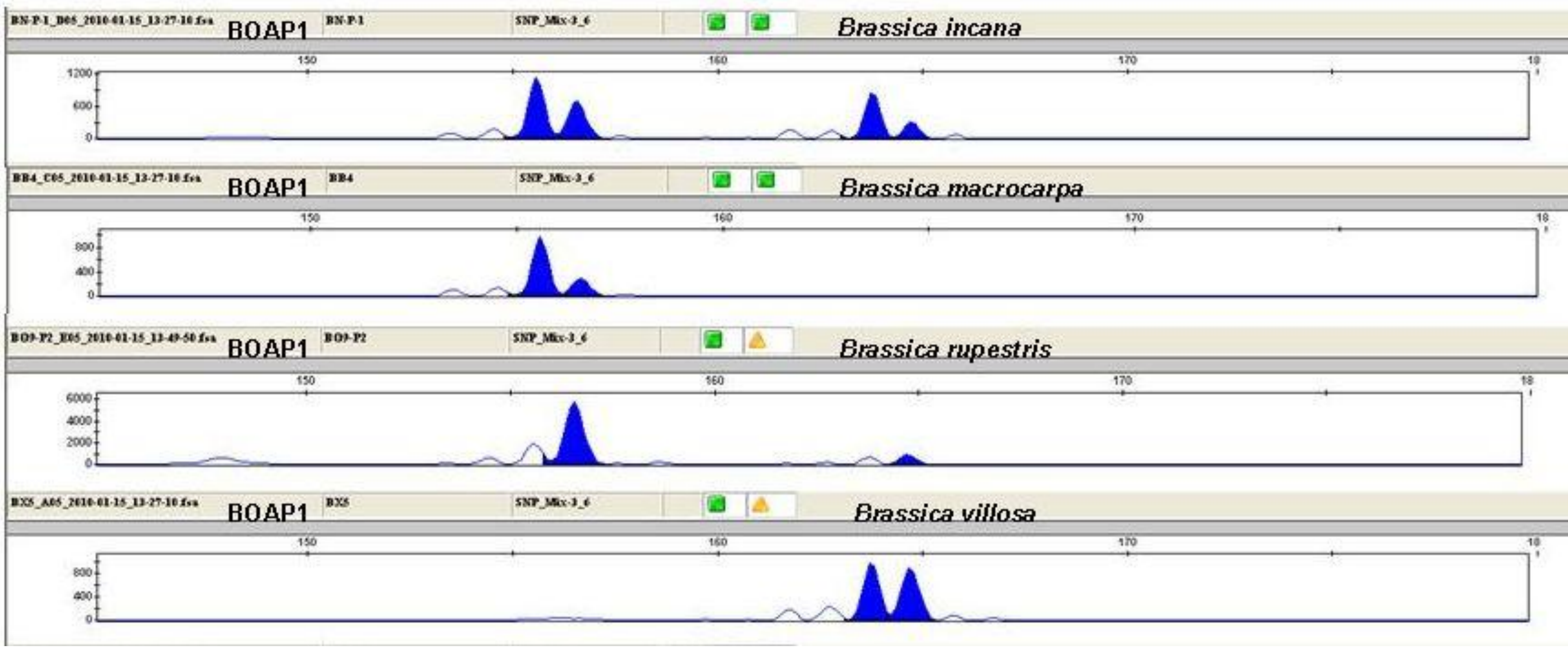
Sign	Markers	Primer forward	Primer revers
P2	BoTHL1	GCCAAGGAGGAAATCGAAG	AAGTGTCAATAAGGCAACAAGG
P3	BoAP1	GGAGGAACGACCTTGATT	GCCAAAATATACTATGCGTCT
P5	PBCGSSRBo39	AACGCATCCATCCTCACTTC	TAAACCAGCTCGTTCGGTTC
P6	BoPLD1	GACCACCGACTCCGATCTC	AGACAAGCAAAATGCAAGGAA
P7	BoABI1	TATCAGGGTTTCCTGGGTTG	GTGAACAAGAAGAAAAGAGAGCC



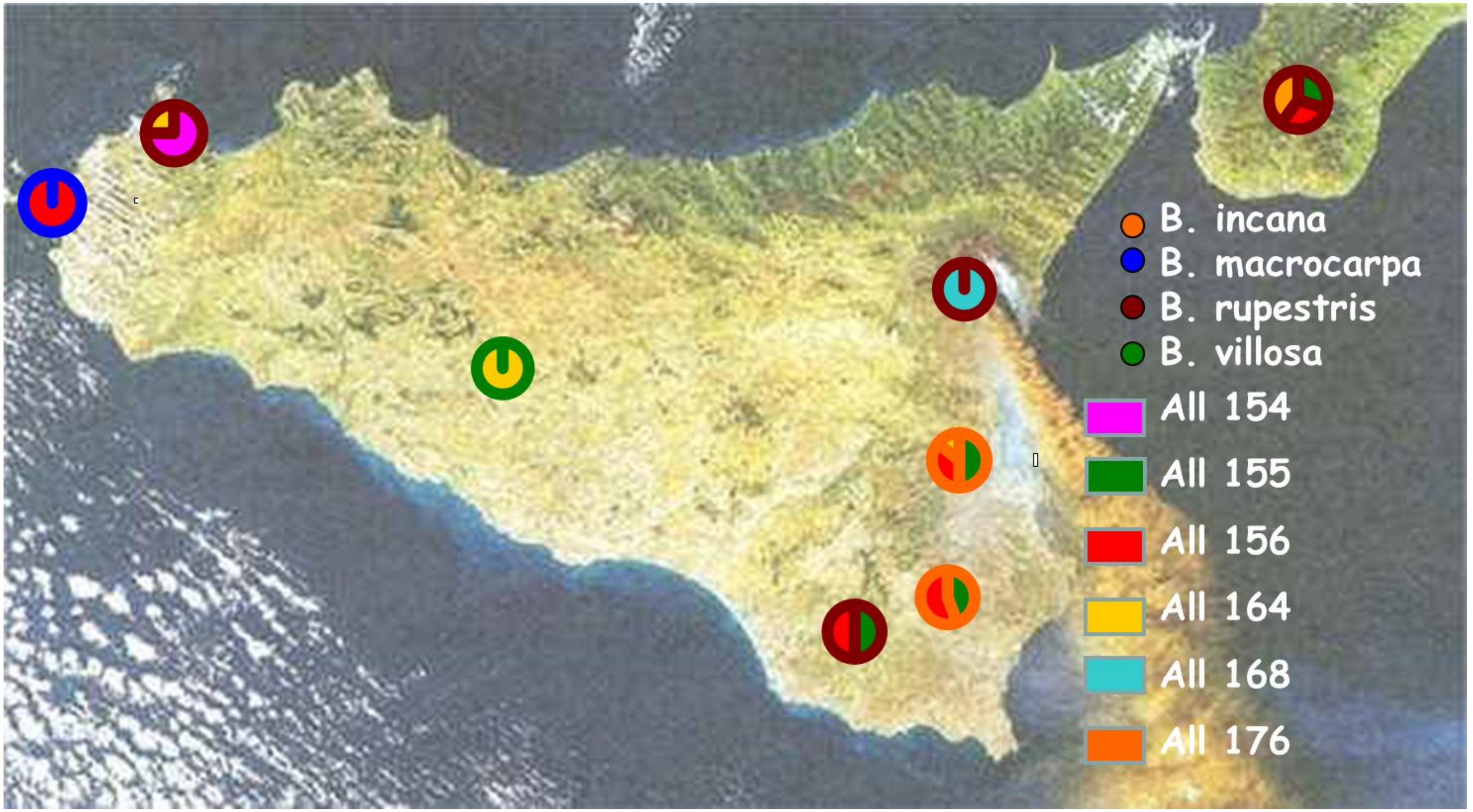
19th CRUCIFER GENETICS WORKSHOP AND BRASSICA 2014

Wuhan 3.30-4.2 2014

Tetrasomy ascertained for BoAP1 SSR primer for some genotypes of crop wild relatives of Brassica widespread in Sicily



Allele frequency of different alleles observed for Bo AP1 SSr primer









Prof. César Gómez Campo

**Banco de Germoplasma Vegetal-
Universidad Politécnica de Madrid**

- **Wild plant species**
- **Endemic species of Iberian Iberian Peninsula and the Macaronesian region**
- **Brassicaceae**

Mediterranean *Brassica* diversity *will provide you an interesting backdrop for discussions and debate towards individuating, delineating and consolidating new research directions for future agriculture innovation.*






Thank you for your attention