ECPGR *Brassica* Working Group

European Cooperative Programme for Plant Genetic Resources
ECP/GR

Ferdinando Branca

Information on BRESOV results relevant for the Brassica WG

Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A)
Università degli Studi di Catania

STVDIVM GENERALE SICILIAE 1434

EUBRASWILD project meeting 20-21 February 2024, Catania

ECPGR *Brassica* Working Group

Brassica oleracea L. crops and its wild relatives

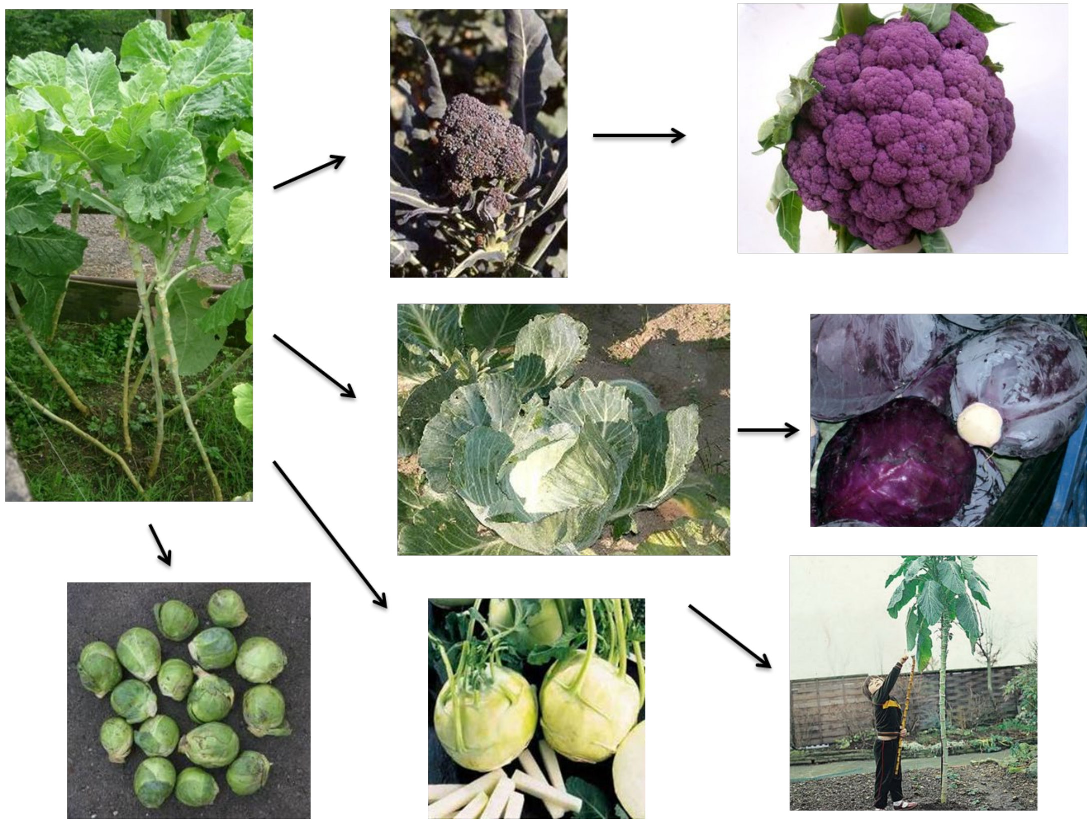
Phenotyping of the BRESOV *Brassica oleracea* core collection

Morphotyping of the BRESOV *Brassica oleracea* core collection

Domestication of the *Brassica oleracea* L. crops

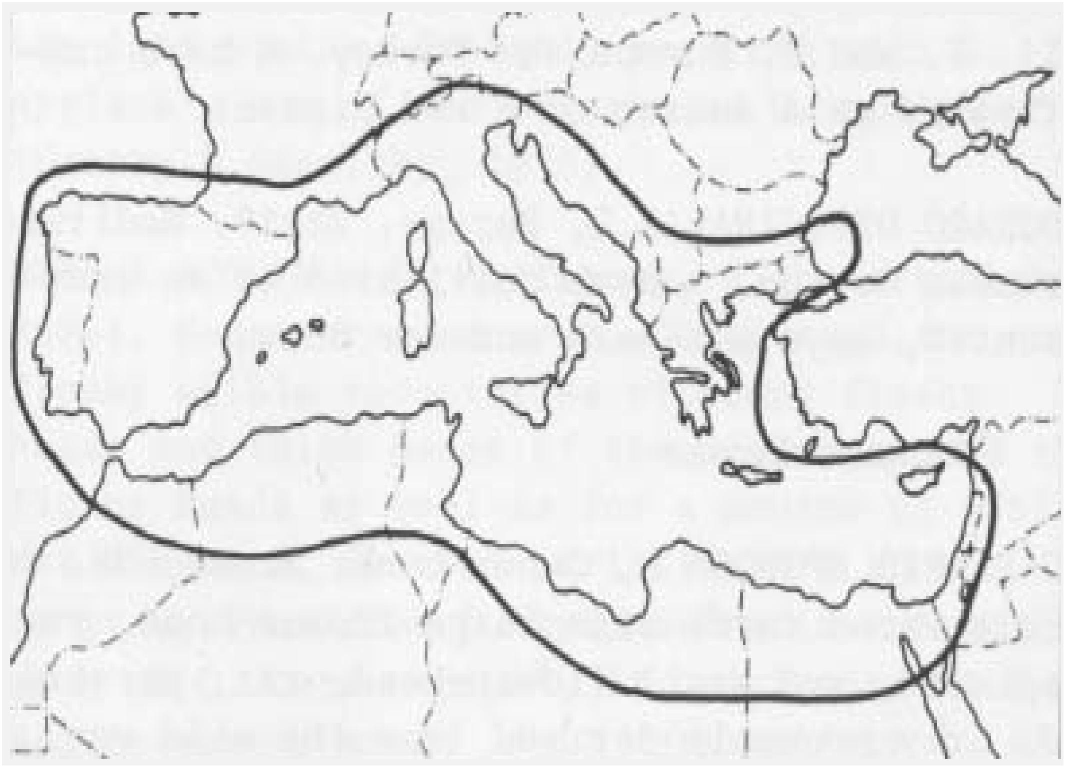
Domestication of the Brassica oleracea L. crops

Brassica oleracea: Cole crops



Domestication of the *Brassica oleracea* L. crops

The Mediterranean origin of the *B. oleracea* L. crops



Domestications of the *Brassica oleracea* L. has been along the coasts of the Mediterranean basin (Vavilov,1928)

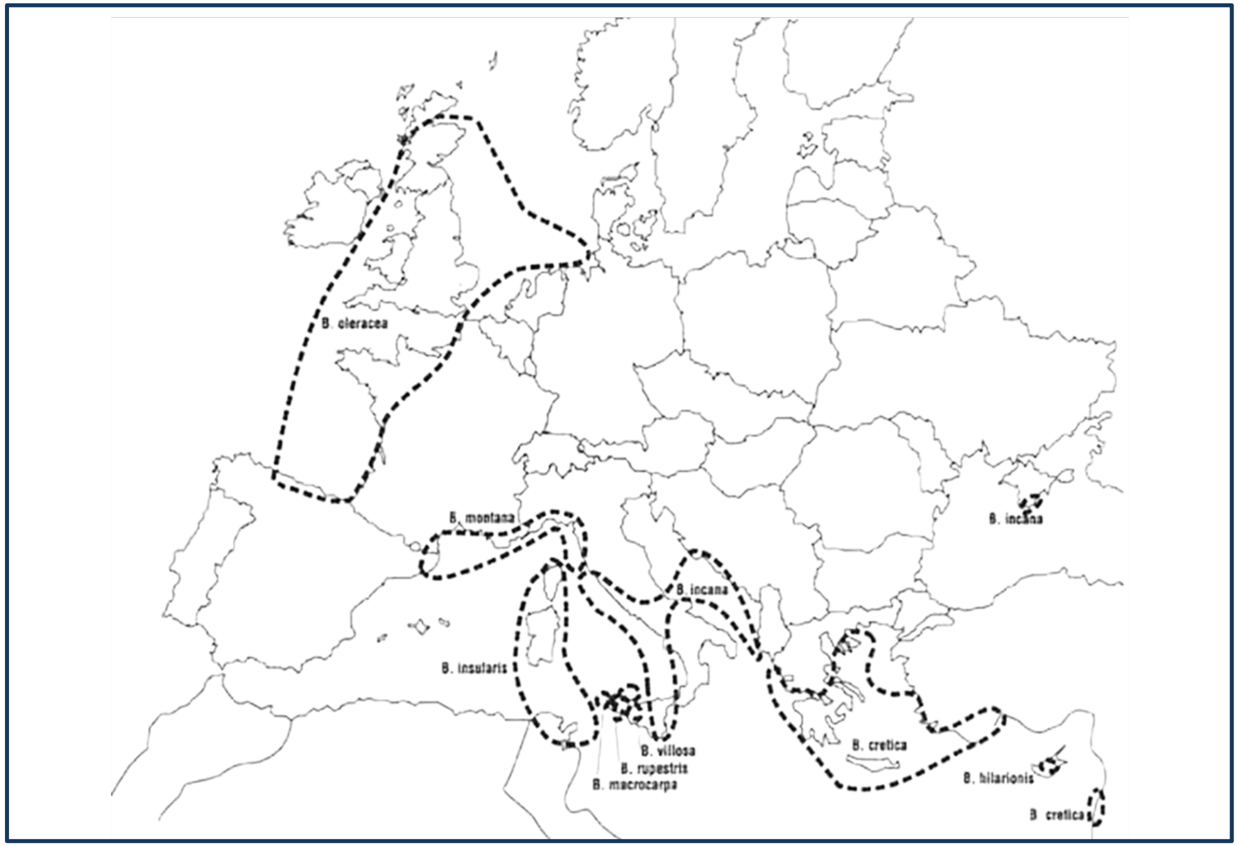
a) Archeological remains;

b) Characteristics of the plants *in situ, on farm;*

c) Other hystorical references (hystorical informations, language comparations).

Domestication of the *Brassica oleracea* L. crops

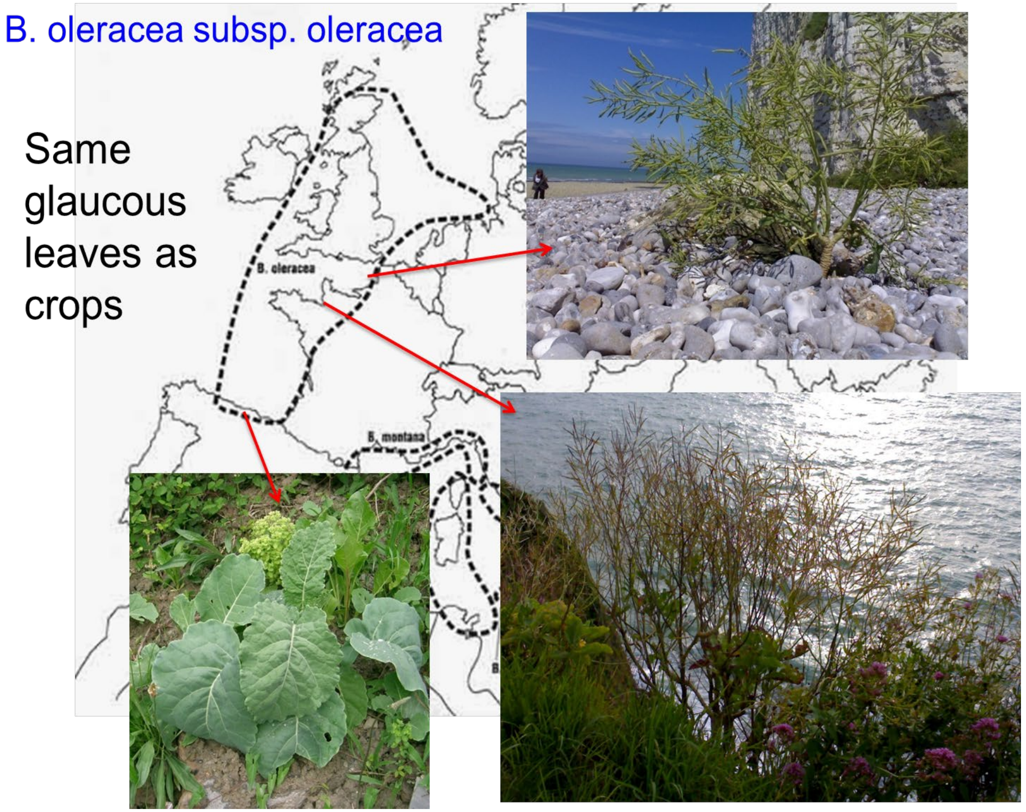
Living plants: *B. oleracea* group C-genome (n=9)



Domestication of the *Brassica oleracea* L. crops

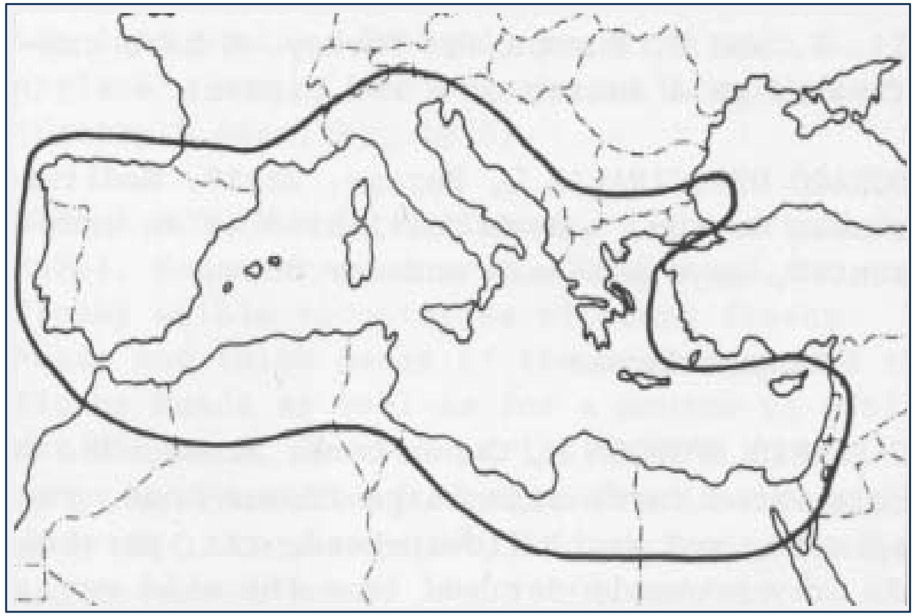
Since '80 of the last year a new Hypothesis supported the domestication of cole crops has been from the wild population of *Brassica oleracea* L. widespread along the english channel cliffs.

Living plants: *B. oleracea* group
C-genome (n=9)



Domestication of the *Brassica oleracea* L. crops

The Mediterranean origin of the *B. oleracea* L. crops



Domestication of the *Brassica oleracea* L. crops

Origin Poliphyletic of the *Brassica oleracea* L. crops

(Snogerup, 1980 – morphology and other considerations)

B. oleracea ssp. oleracea → cabbage (East Europe)

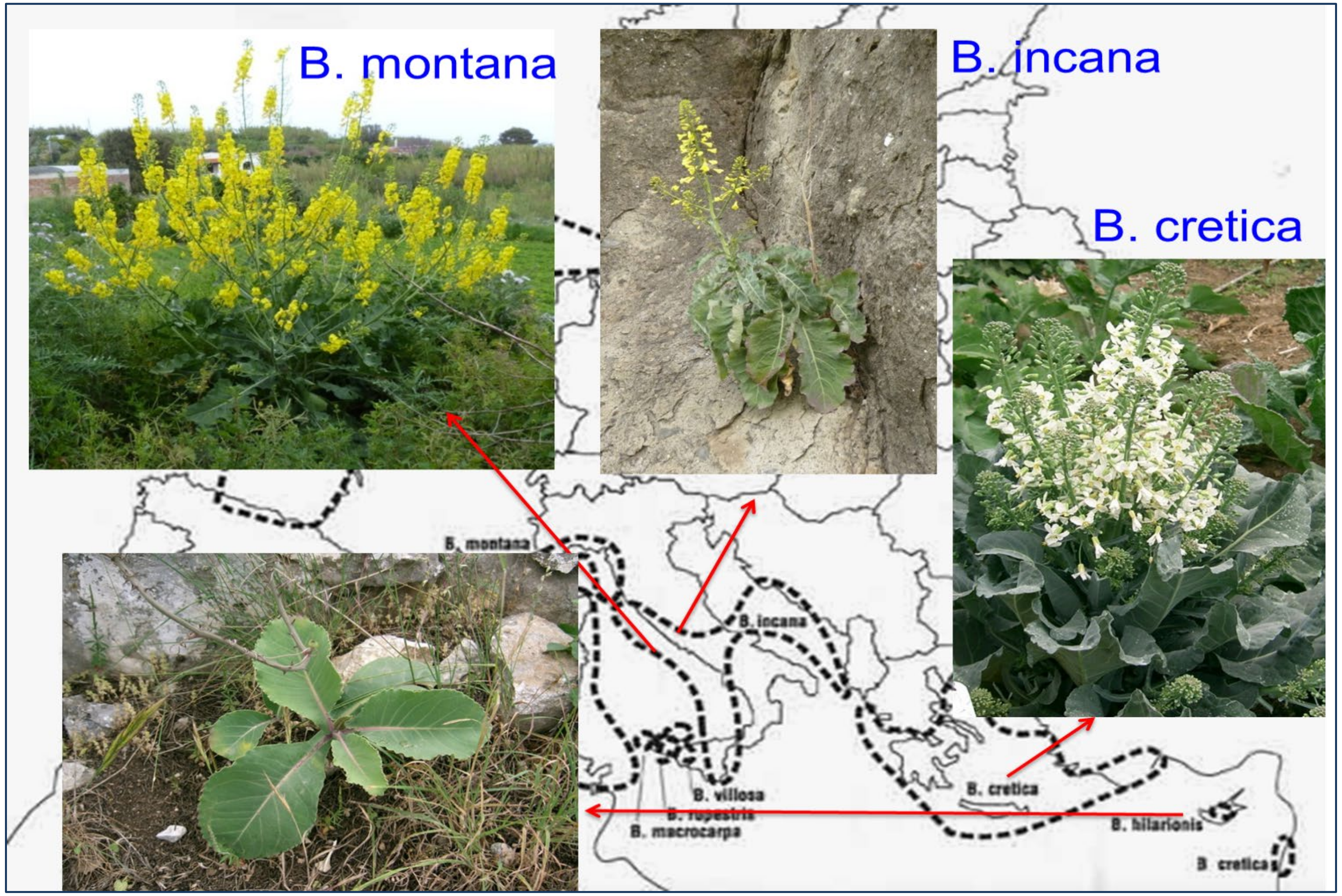
B. cretica → Brached kale (Greece)

→ ? Broccoli and cauliflower

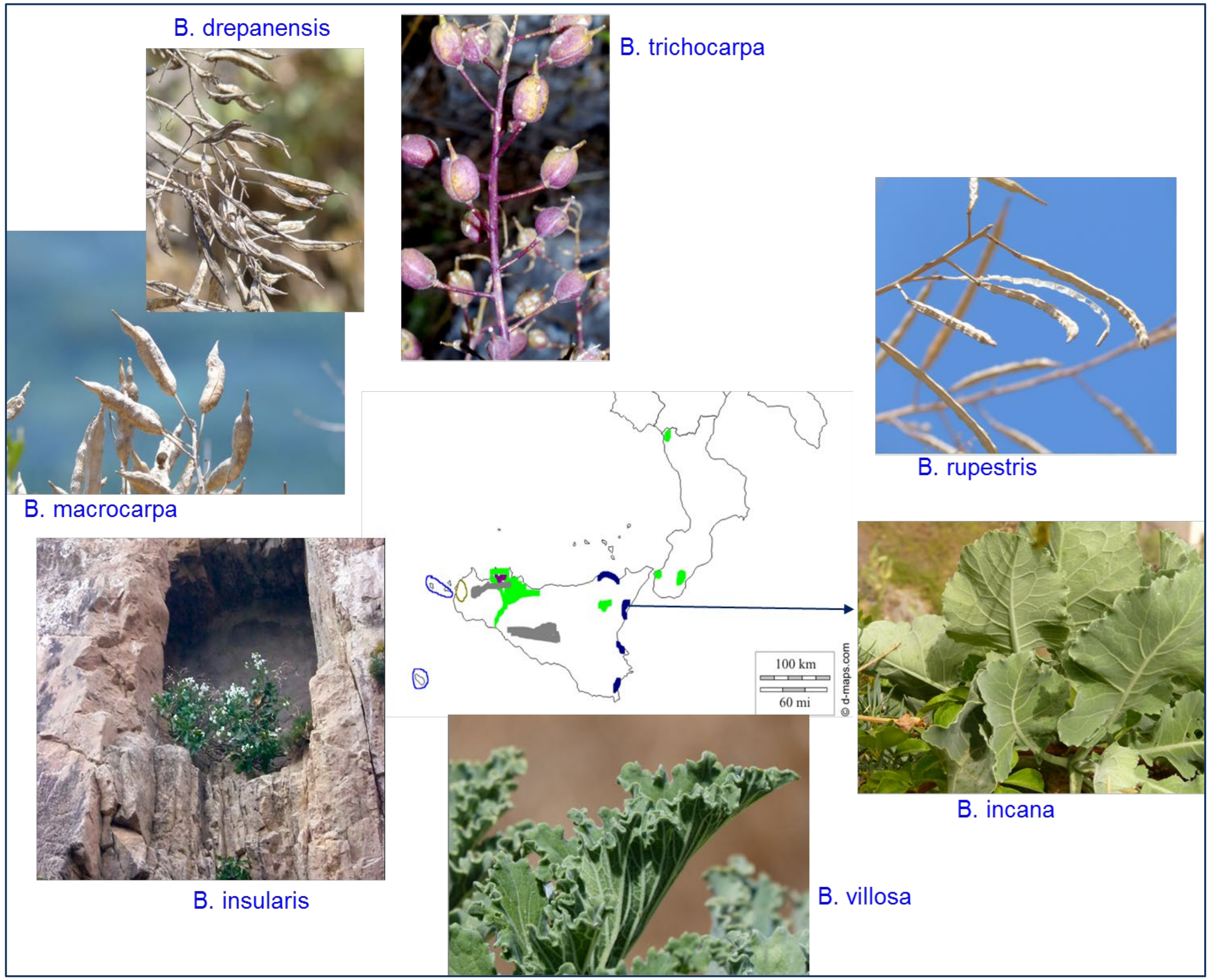
B. incana-rupestris → kale not branched single stem

Sicily-South Italy kale, sprouting broccoli, main head broccoli, and cauliflower (Branca et al., 2018).

Domestication of the *Brassica oleracea* L. crops



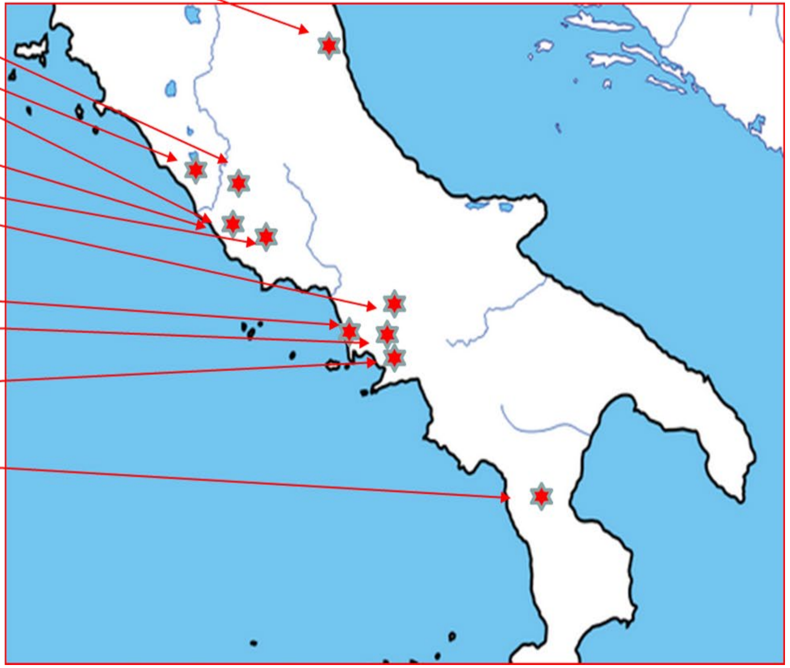
Domestication of the *Brassica oleracea* L. crops



Domestication of the *Brassica oleracea* L. crops

Roman landraces
(Plinio; Columella -I century b.C.)

- Quae Marrucini
- Tiburis
- Sabellico
- Lacuturnenses
- Aricinum
- Quae Signia
- Caudinis faucibus horti
- Cumanum
- Pompeianum
- Stabiae
- Bruttiani



Domestication of the *Brassica oleracea* L. crops




The variability observed in Sicily among the landraces and the Brassica wild relatives (n=9) and the related genetic flux have permitted to diversity some crops belonging to *Brassica oleracea* L., which are represented by several varietal groups characterized by high organoleptic traits appreciated by local consumers.



Phenotyping of the BRESOV *Brassica oleracea* core collection

Biochemical phenotyping

- 139 *Brassica oleracea* complex species (n=9) accessions belonging to UNICT and UNLIV were screened for:
  - antioxidant capacity,
  - ascorbic acid,
  - glucosinolates profile and amount.

Crop name	Common name	Number of accessions	Provider
B. oleracea var. *acephala*	Kale	68	63 from UNILIV
			5 from UNICT
B. oleracea var. *botrytis*	Cauliflowers	18	18 from UNICT
B. oleracea var. *italica*	Broccoli	3	3 from UNICT
	Composite cross populations (CCPs)	8	8 from UNICT
B. oleracea var. *italica* x *B. oleracea* var. *botrytis*	CCPs		3 from UNICT
B. oleracea var. *botrytis* x *B. oleracea* var. *botrytis*	CCPs		3 from UNICT
B. rupestris x *B. oleracea* var. *botrytis*	CCPs		2 from UNICT
	Crop wild relatives (CWRs)	42	42 from UNICT
Brassica balearica Pers.	CWRs		2 from UNICT
Brassica barrelieri (L.) Janka	CWRs		2 from UNICT
Brassica bourgeaui (Webb ex Christ) Kuntze	CWRs		3 from UNICT
Brassica cretica Lam.	CWRs		2 from UNICT
Brassica desnottesii Emb & Maire	CWRs		1 from UNICT
Brassica drepanensis (Caruel) Ponzo	CWRs		2 from UNICT
Brassica hilarionis Post.	CWRs		1 from UNICT
Brassica incana Ten.	CWRs		7 from UNICT
Brassica macrocarpa Guss.	CWRs		3 from UNICT
Brassica montana Pourr.	CWRs		2 from UNICT
Brassica soulie (Batt.) Batt.	CWRs		1 from UNICT
Brassica rupestris Raf.	CWRs		7 from UNICT
Brassica tyrrhena Giotta, Piccitto & Arrigoni	CWRs		1 from UNICT
Brassica villosa Biv.	CWRs		7 from UNICT
Brassica villosa Biv. subsp. *tinei* Raimondo & Mazzola	CWRs		1 from UNICT
	Total number 139		

- ✓ 63 accessions by the Brassica collection of the University of Liverpool (UNILIV);
- ✓ 76 accessions by the Brassica collection of the University of Catania (UNICT).

- Data showed several differences among the species.

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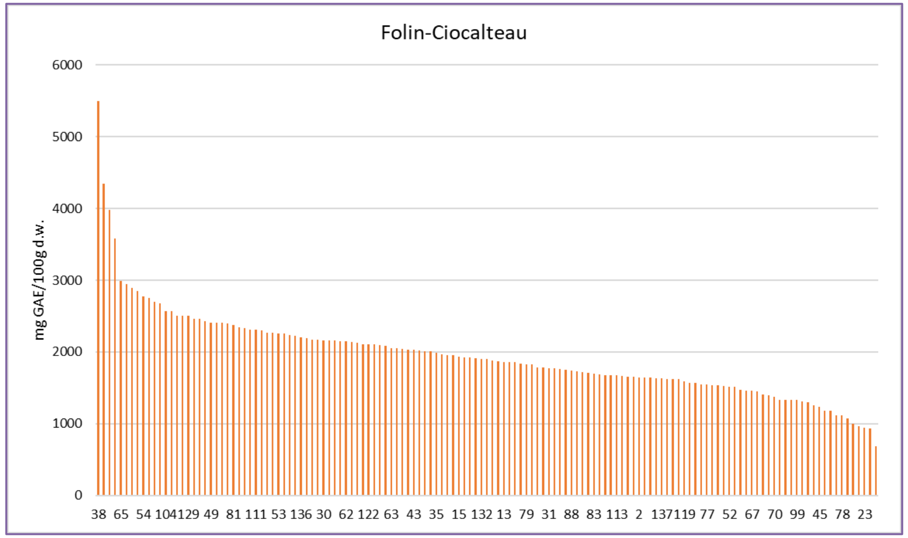
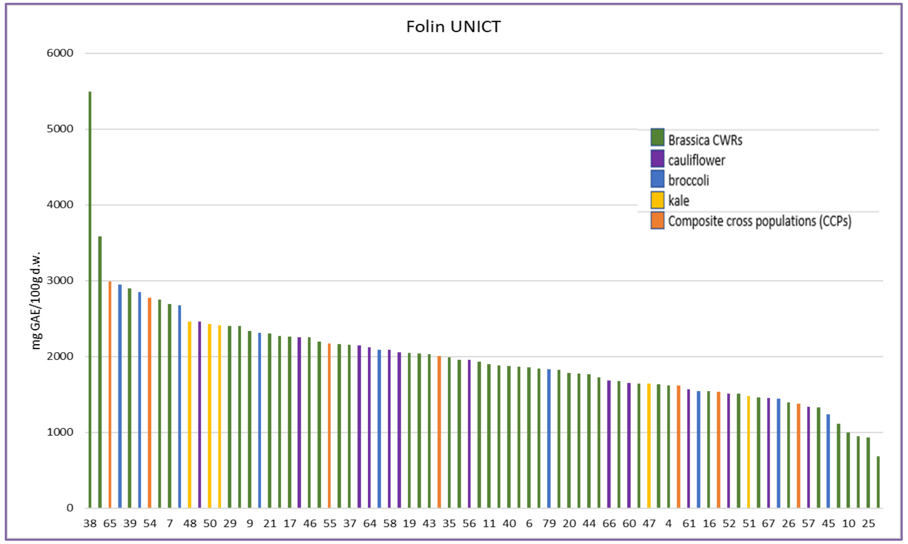
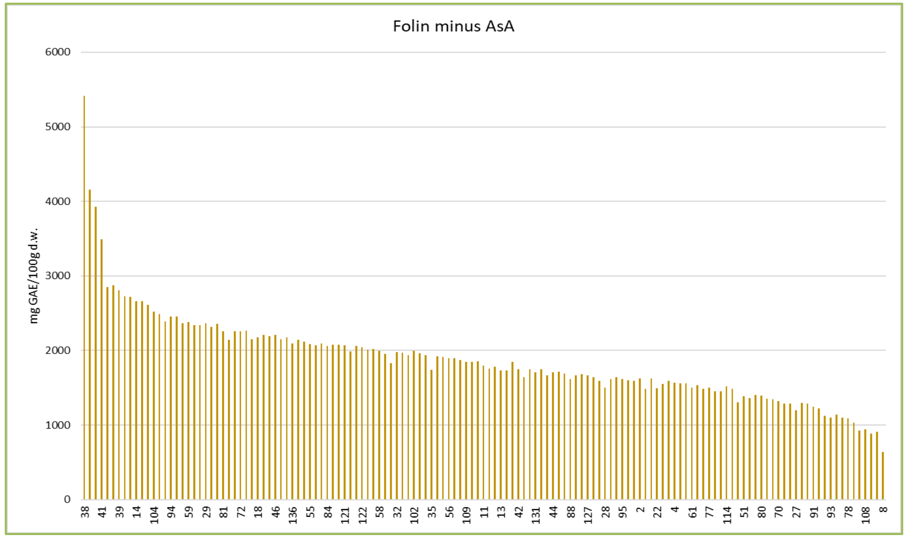
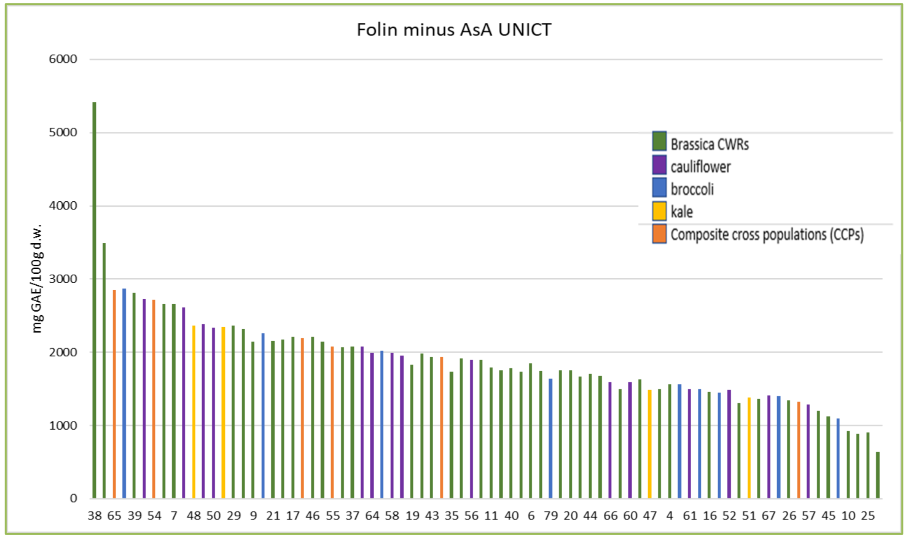




- **Folin-Ciocalteau**
- **Folin minus AsA**





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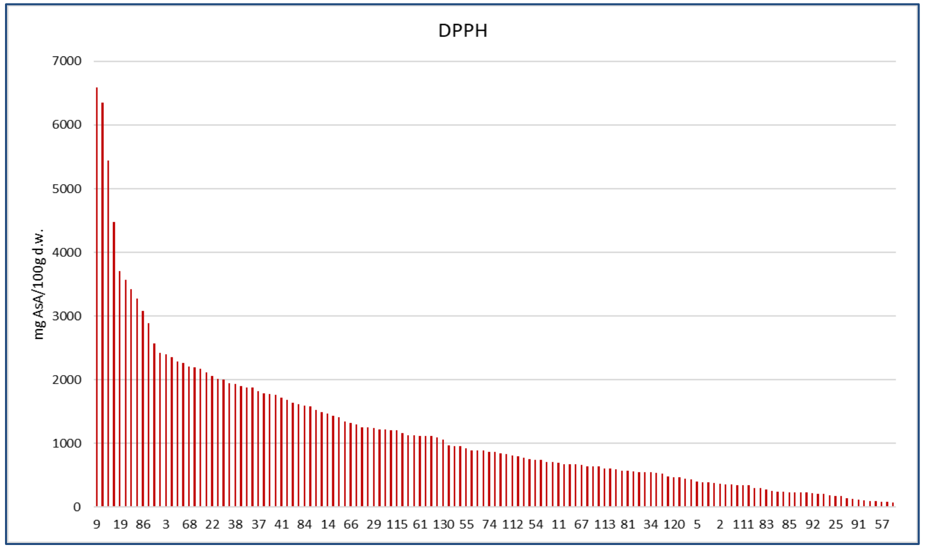
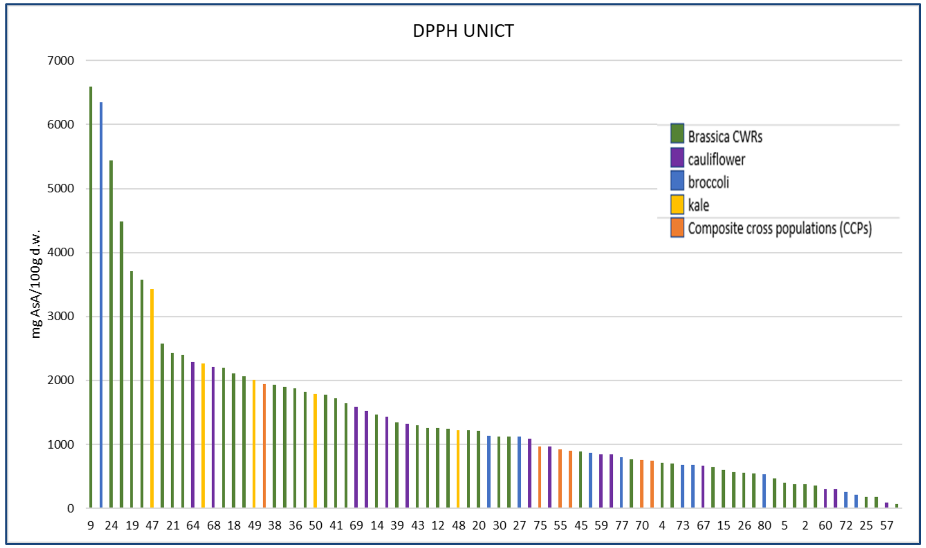
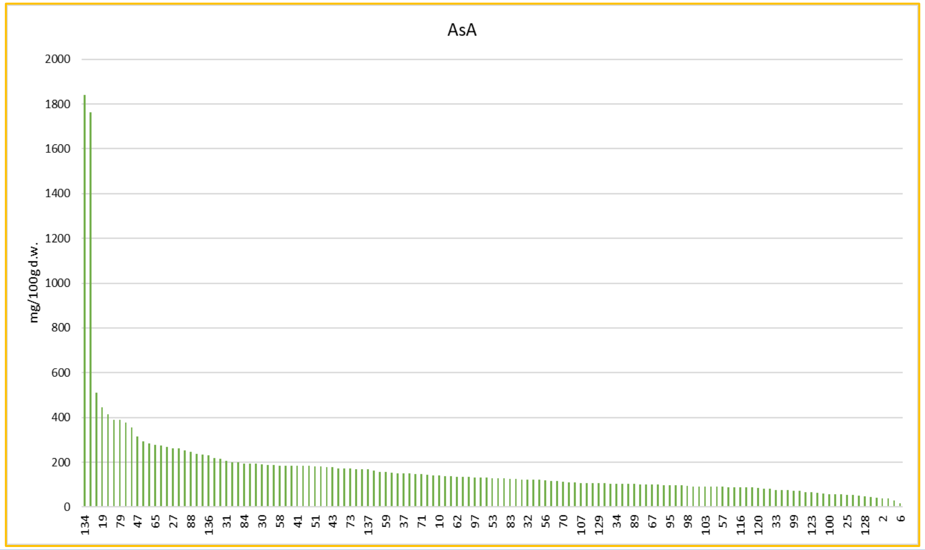
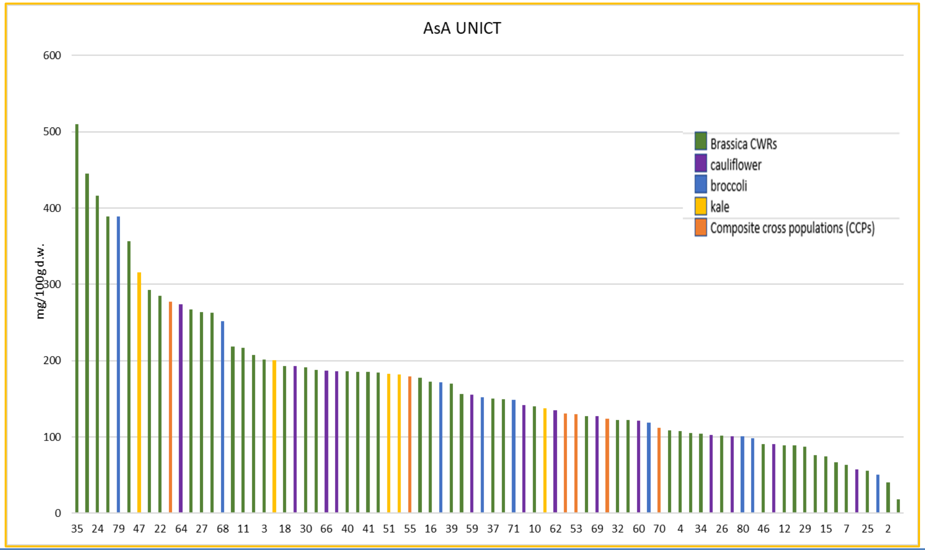




- **DPPH assay**
- **Ascorbic acid**





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- **TOP CWRs**

Brassica balearica Pers.

Brassica cretica Lam.

Brassica tyrrhena Giotta, Piccitto & Arrigoni

***Brassica villosa* Biv. subsp. *tinei* Raimondo & Mazzola**

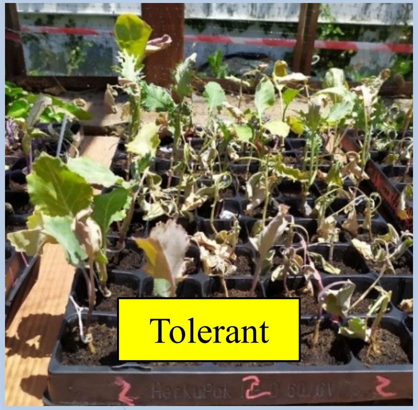
Brassica hilarionis Post.

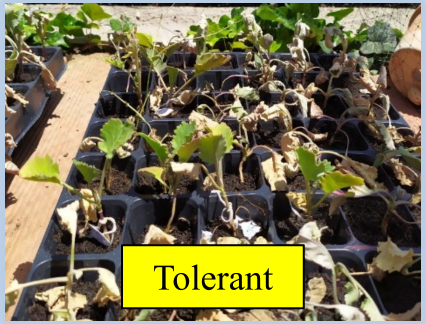
Brassica rupestris Raf.

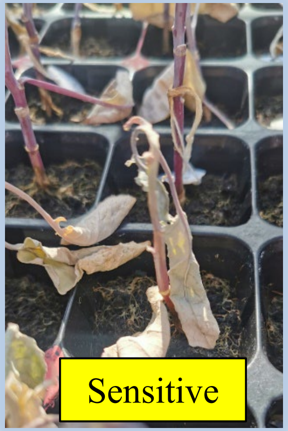
Brassica incana Ten.

Drought stress phenotyping

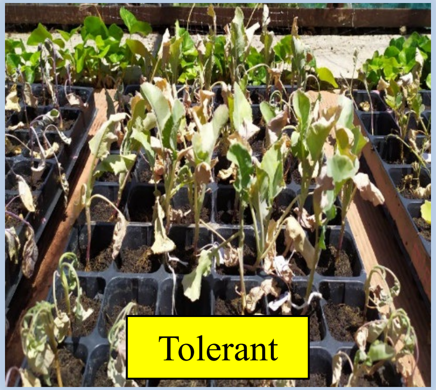
- Selection of 4 accessions 2 *Brassica macrocarpa* tolerant plants one from Marettimo (TP) and one from Favignana islands (**B1800069** and **B1800113**, respectively).
- Selection of 2 sensitive plants.
- Then leaves were collected for RNA extraction and RNA seq.







SPAD ANALYSIS 3d cycle											
	Water stressed plants							Not water stressed plants			
Sample	SPAD	Δ SPAD	Total leaves	Δ leaves	Chlorotic	Dry	Score	SPAD	Total	Chlorotic	Dry
BY (1)	55,4	111,47	9	90	4	0	2	49,7	10	1	0
BY (2)	37,4	92,57	8	88,89	3	1	2	40,4	9	0	1
BY (3)	32,3	59,59	8	80	3	0	2	54,2	10	0	0
BW (1)	39,5	154,30	6	75	4	1	0	25,6	8	0	0
BW (2)	44,4	84,25	7	77,78	3	2	0	52,7	9	0	0
BW (3)	46,7	86,32	8	88,89	4	1	0	54,1	9	1	0
FL (1)	61	103,57	8	88,89	2	0	2	58,9	9	0	0
FL (2)	53,5	144,59	9	100	2	1	2	37	9	0	0
FL (3)	57,3	130,82	7	63,64	2	0	2	43,8	11	0	0
FK (1)	35	80,83	8	114,29	2	1	2	43,3	7	0	0
FK (2)	28,2	55,19	7	77,78	4	0	2	51,1	9	0	1
FK (3)	42,5	86,38	9	112,5	3	0	2	49,2	8	0	0



BRESOV	2° ANNO	GREEN HOUSE	IAS (AZIENDA AGRARIA SPERIMENTALE)				
TASK 2.2	2020	WATER STRESS	TRIAL IN POTS				
SAMPLE	ACCESSIONE	CODICE UNICT	CROP NAME	CODE	COMMON NAME	ORIGIN	FORNITORE
1	BW	UNICT 5088 BR 365	B.oleracea var.italica	BR	Ciurietto maiolino	MODICA (RG)	AZ. PAOLOINO
2	BY	UNICT 5081 BR 360	B.oleracea var.italica	BR	Ciurietto settembrino	MODICA (RG)	AZ. PAOLOINO
3	FK	UNICT 5006 BM 28	Brassica macrocarpa	BM	specie spontanea	FAVIGNANA (TP)	Prof. BRANCA
4	FL	UNICT 5124 BM 30	Brassica macrocarpa	BM	specie spontanea	MARTETTIMO (TP)	VITO VACCARO

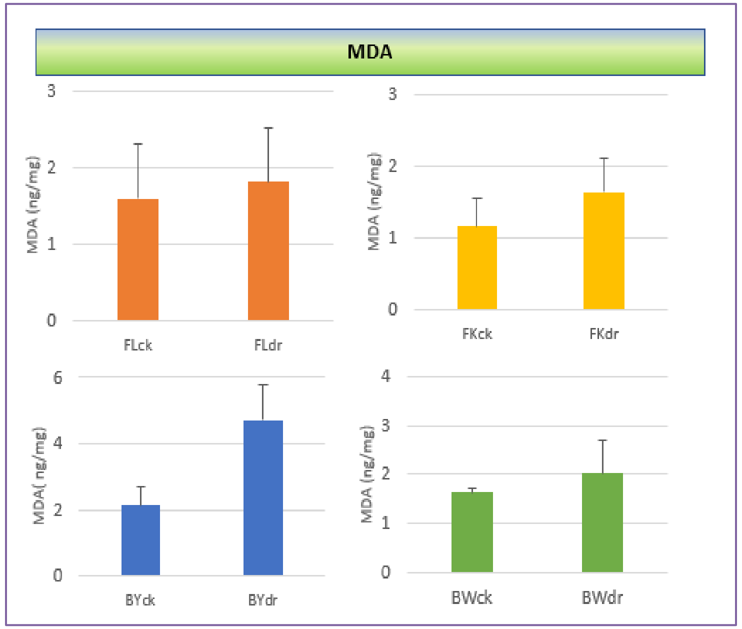
Quantification of 2 metabolites involved in the oxidative stress response

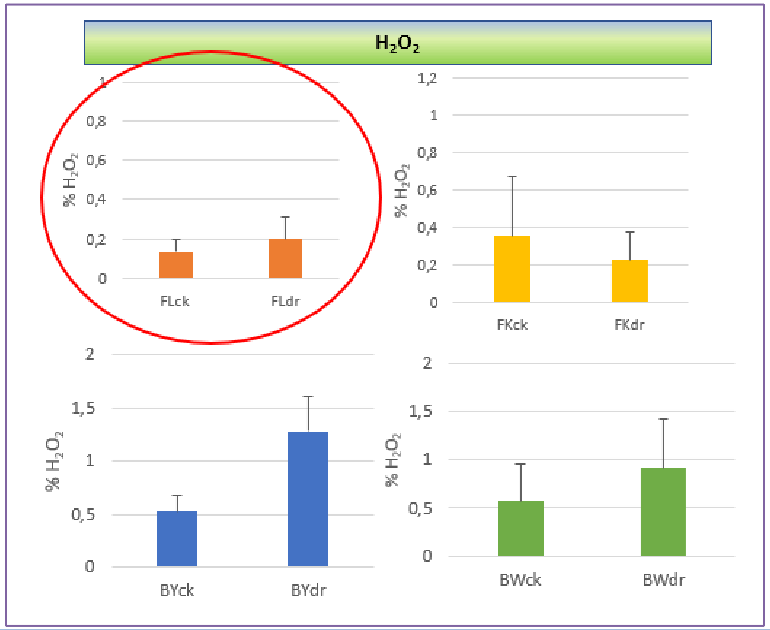
- Malonildialdehyde (MDA) → Lopez-Hidalgo et al. (2021)

Polynsaturated fatty acids degradated by the ROSs, producing MDA.

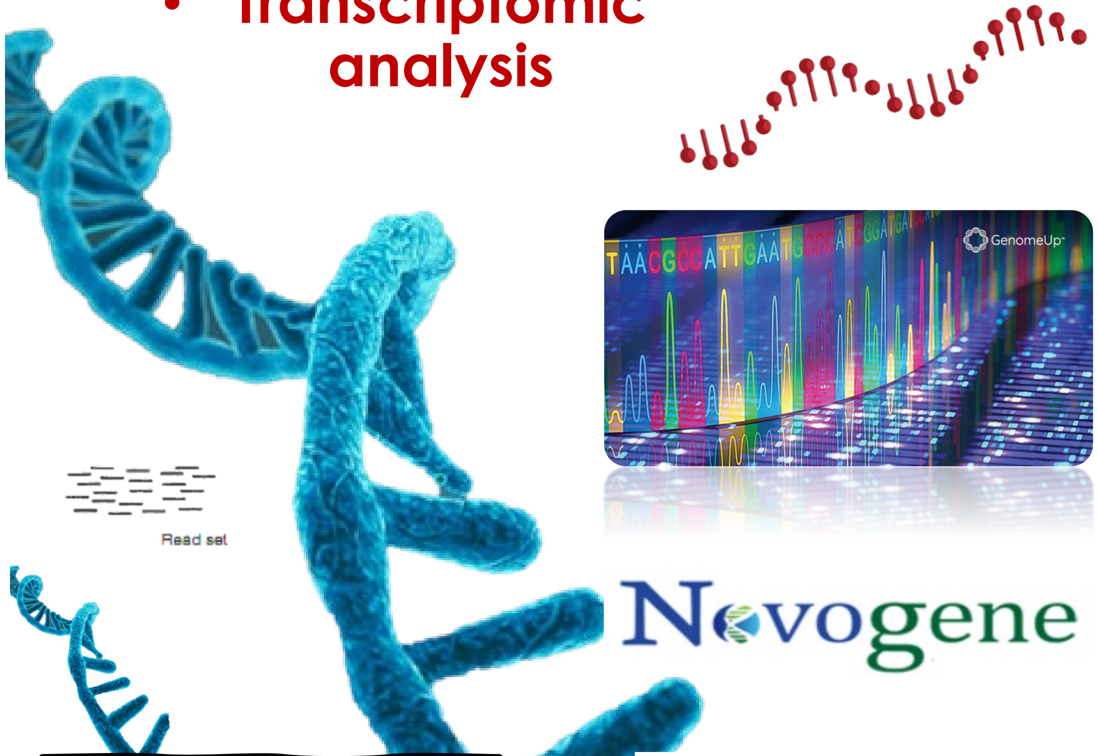
- Hydrogen Peroxide (H_2O_2) → Velikova et al. (2000)

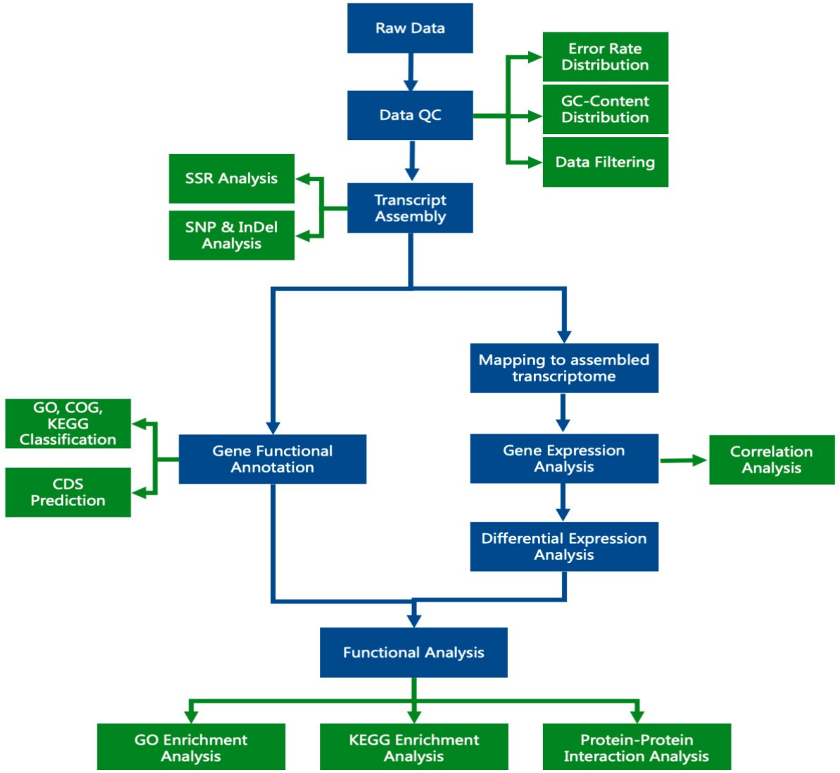
Produced in oxidative stress condition

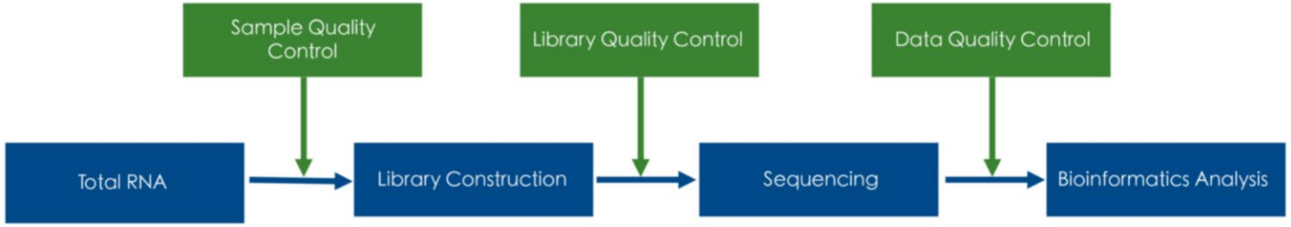




- **Transcriptomic analysis**







SOFTWARE

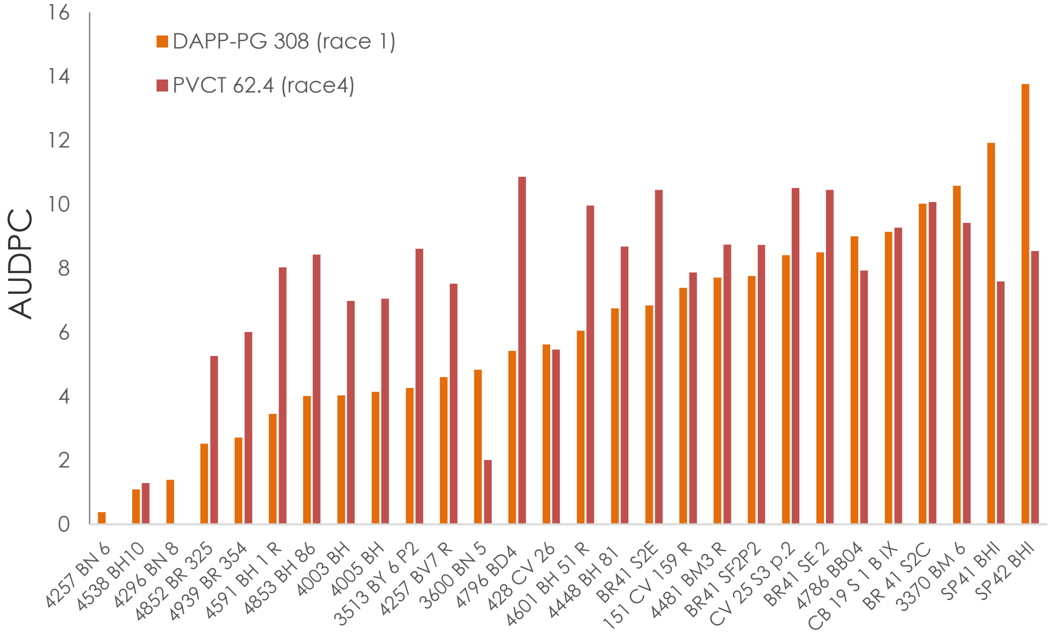
Trinity (Gene assembly)
Corset (Hierarchical clustering)
RSEM (Gene expression)

• *Xanthomonas campestris* pv. *campestris* phenotyping

- Selecting **new source of resistance** to **Black rot disease** caused by the vascular bacterium ***Xanthomonas campestris* pv. *campestris* in *B. oleracea* complex** accesion to be used in breeding programs.

- Evaluation of the response of *Brassica* accessions to the inoculation to strains of the most widespread races in Eu, i.e **race 1 and 4**.

- **Twenty-eight accessions** from ten species maintained at Di3A, University of Catania, *Brassica hylarionis*, *B. oleracea*, *B. villosa*, *B. incana*, *B. drepanensis*, *B. macrocarpa*, *B. bourgeaui*, tested for the first time.



Brassicas accessions	UNICT code
B. bourgeaui	4786 BBO4
B. hylarionis	SP41 BHI1; SP42 BHI2
B. drepanensis	4796 BD 4
B. incana	3512 BY5/1; 3513 BY6/2
B. macrocarpa	4481 BM3R; 3370 BM6
B. villosa	4275 BV7R
B. oleracea var. botrytis	3154 CV5S3/2; 428 CV26; 3151 CV159R/2/1; 421 CV19
B. oleracea var. italica	71 BR41S2C; 709 BR41S2/2; 710 BR41S2E; 4852 BR325; 4939 BR354
B. oleracea var. acephala	4853 BH 86; 4003 BH; 4005 BH; 4591 BH1R; 4538 BH10; 4601 BH51R; 4448 BH 81

A **significantly different response of the accessions** to the inoculation with the two *Xcc* strains was observed. **AUDPC** (area under the disease progress curve) values ranged from 0.38 to 13.75 and from 0 to 11.50 when the accessions were inoculated with the **race 1 or race 4**, respectively.

Morphotyping of the BRESOV *Brassica oleracea* core collection

Materials and methods

- 182 accessions of different morphotypes of *Brassica oleracea* complex species (n=9) provided by the genebank of UNICT, UNILIV and VURV were sowing in October 2018.

- The seeds were sown in cellular trays in a cold greenhouse under natural light (4.6 to 9.2 MJ.m-^{2}d-^{2}) and temperature (15.4 ± 5.8 ± C°) conditions, from October to December 2018 in the Experimental Agricultural Institute "IAS" (Istituto Agrario Sperimentale) situated in the South of Italy (37°31010" N 15°04018" E; 105 m above sea level (m a. s. l.), Catania), using organic growing practices.

- The plants were transplanted on 27 December 2018 in a cold greenhouse (36°51'13.3" N 14°29'32.0" E, Contrada Randello, Ragusa). For each accession three plants were transplanted and analyzed.

The experimental field (Contrada Randello, Ragusa).

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B. oleracea crops and CWRs accessions used for morphological characterization and morphological traits studied.

Crop name	Common name	Number of accessions	Provider
B. oleracea var italica	Broccoli	37	21 from UNILIV
			12 from UNICT
			4 from VURV
B. oleracea var botrytis	Cauliflower	26	15 from UNILIV
			7 from UNICT
			4 from VURV
B. oleracea var capitata	Cabbage	60	29 from VURV
			27 from UNILIV
			4 from UNICT
B. oleracea var acephala	Kale	18	12 from UNICT
			6 from UNILIV
B. oleracea var alboglabra	Chinese kale	18	18 from UNILIV
B. oleracea var gongylodes	Kohlrabi	17	10 from UNILIV
			5 from VURV
			2 from UNICT
B. villosa	CWR	2	UNICT
B. drepanensis	CWR	2	UNICT
B. incana	CWR	2	UNICT
Total number		182	

The majority of the accessions studied were provided by Liverpool University germplasm with 97 accessions, while 43 accessions were provided by UNICT and 42 accessions by VURV.

Index	Descriptors
IA	Inflorescence appearance (d)
PB	Plant branches (n)
PLS	Plant leaf shape (1-7)
PGH	Plant growth habit (1-9)
PLN	Plant leaves number (n)
PSL	Plant stem length (cm)
LHR	Leaf hairiness (0-7)
LA	Leaf area (cm^2)
LL	Leaf length (cm)
LW	Leaf width (cm)
LD	Leaf division (incision)
LPL	Leaf petiole length (cm)
LPW	Leaf petiole width (cm)
RLA	Root left angle (°)
RRA	Root right angle (°)
BRD	Basal root diameter (mm)
MRD	Main root diameter (mm)
MRL	Main root length (cm)
LRD	Lateral root diameter (mm)
RA	Roots area (cm^2)
RFW	Roots Fresh weight (g)
RDM	Roots dry matter (g)

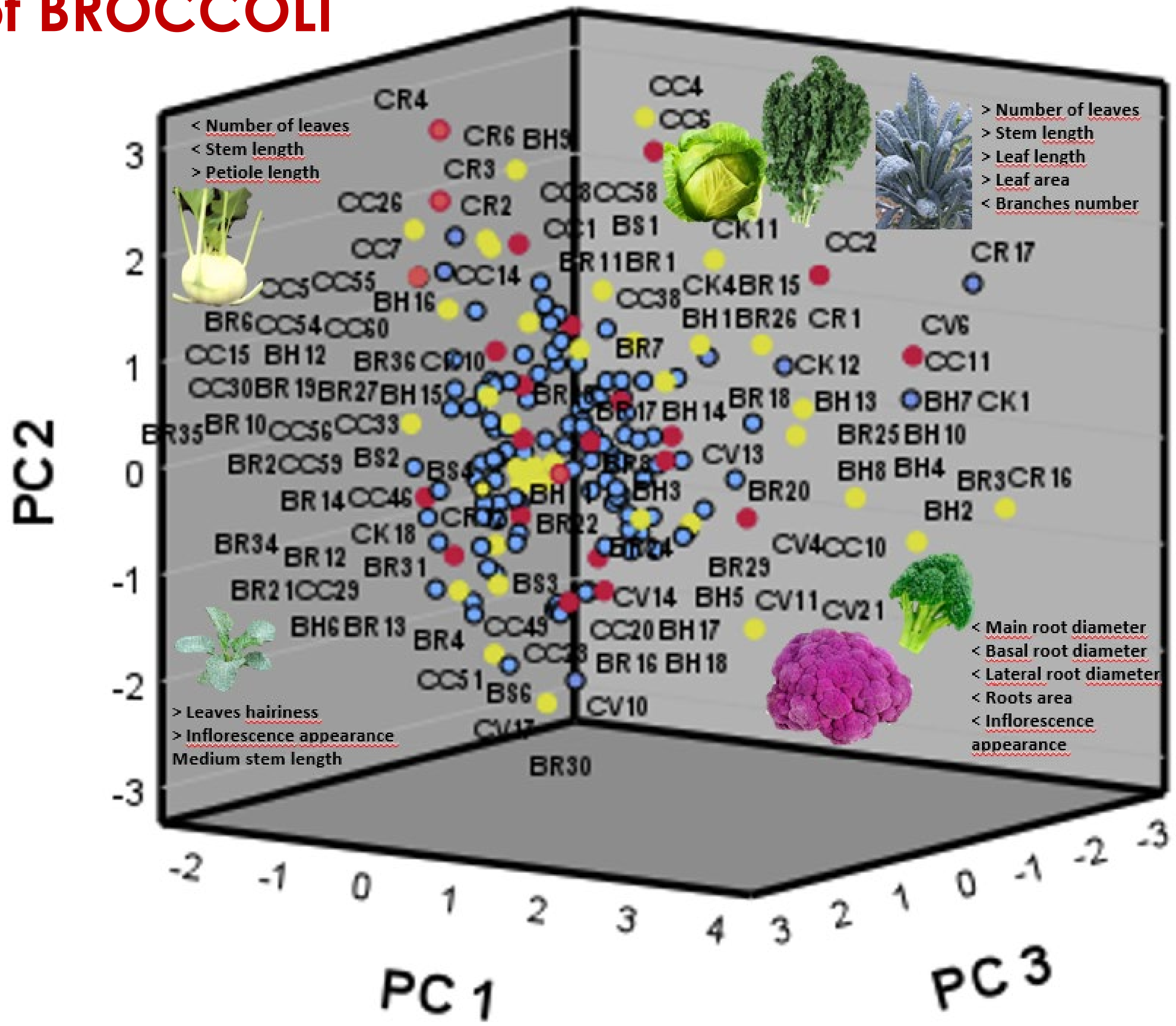
Correlation matrix among the different bio-morphometric descriptors evaluated

	IA	PB	PLS	PGH	PSL	PLN	LL	LW	LA	LPL	LPW	LD	LHR	LWN	RLA	RRA	BRD	MRD	LRD	MRL	RA	RFW
PB	-0.040																					
PSL	-0.080	0.024																				
PGH	0.087	-0.294	0.338**																			
PSL	0.116	0.042	0.383**	0.222**																		
PLN	0.116	0.043	0.383**	0.220**	0.899**																	
LL	0.064	-0.099	-0.133	-0.106	-0.059	-0.056																
LW	0.006	-0.037	0.018	-0.023	-0.088	-0.086	-0.041															
LA	0.062	-0.098	-0.085	-0.120	-0.079	-0.074	0.739**	0.603**														
LPL	0.011	-0.010	0.111	0.123	0.221**	0.222**	0.101	0.179*	0.181*													
LPW	0.133	-0.075	-0.168	-0.148	-0.104	-0.101	0.449**	0.026	0.380**	-0.038												
LD	0.087	-0.103	0.139	0.284**	0.340**	0.337**	0.070	0.061	0.087	0.195**	-0.042											
LHR	0.351**	0.001	-0.048	0.153*	0.038	0.036	-0.041	-0.091	-0.082	-0.069	0.016	0.005										
LWN	0.087	-0.054	0.037	0.153*	-0.042	-0.041	-0.059	-0.104	-0.101	-0.103	-0.046	-0.266	0.226**									
RLA	-0.006	0.095	0.051	0.005	-0.042	-0.036	0.068	0.033	0.084	-0.033	0.048	-0.064	0.014	0.035								
RRA	0.101	-0.078	-0.116	-0.115	-0.167	-.161*	0.182*	0.035	0.171*	0.072	0.192**	0.014	-0.004	-0.118	0.262**							
BRD	-0.026	-0.065	0.182*	0.199**	0.263**	.261**	-0.089	0.097	0.004	.157*	-0.129	0.150*	-0.004	0.039	-0.077	-0.231						
MRD	0.027	-0.104	0.172*	0.156*	0.124	0.122	-0.075	-0.022	-0.077	0.006	-0.052	-0.056	-0.010	0.090	-0.002	-0.210	0.395**					
LRD	0.013	-0.076	0.218**	0.211**	0.211**	0.210**	-0.157	0.091	-0.051	0.008	-0.118	0.100	-0.050	0.011	-0.032	-0.215	0.411**	0.580**				
MRL	0.025	-0.105	0.199**	0.144	0.259**	0.260**	-0.167	0.129	-0.026	0.071	-0.124	0.090	0.010	0.007	0.115	-0.080	0.340**	0.392**	0.571**			
RA	-0.014	-0.104	0.262**	0.225**	0.279**	0.277**	-0.176	0.037	-0.114	0.035	-0.148	0.138	-0.054	-0.059	-0.129	-0.290	0.598**	0.382**	0.568**	0.611**		
RFW	-0.042	-0.154	0.219**	0.275**	0.215**	0.214**	-0.106	0.055	-0.053	0.081	-0.115	0.155*	-0.045	-0.026	-0.071	-0.201	0.590**	0.378**	0.423**	0.495**	0.806**	
RDM	-0.052	-0.123	0.211**	0.255**	0.115**	0.114**	-0.059	0.065	-0.079	0.077	-0.118	0.175*	-0.039	-0.023	-0.073	-0.212	0.582**	0.398**	0.432**	0.485**	0.826**	0.864**

**. The correlation is significant at the 0.01 level (bilateral).

*. The correlation is significant at the 0.05 level (bilateral).

Diversity of BROCCOLI



Spatial distribution of the different accessions characterized in the experiment in relation to the main three principal components (PCs).

- UNICT
- UNILIV
- VURV

Diversity of BROCCOLI





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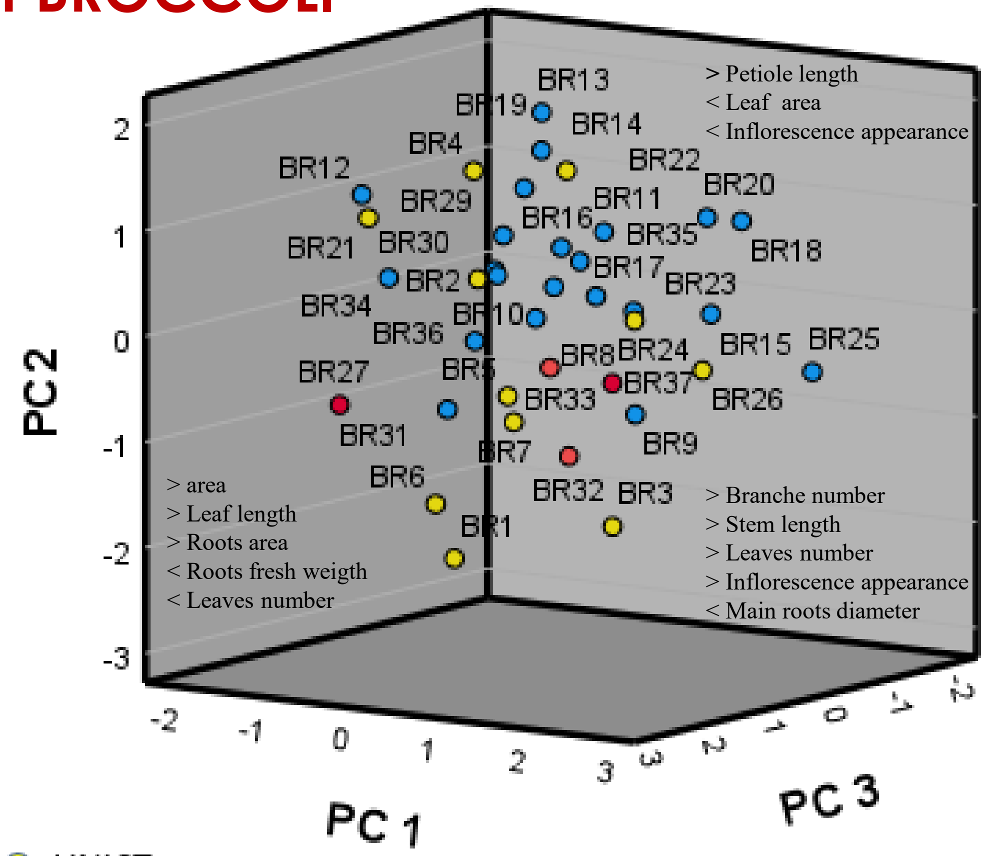
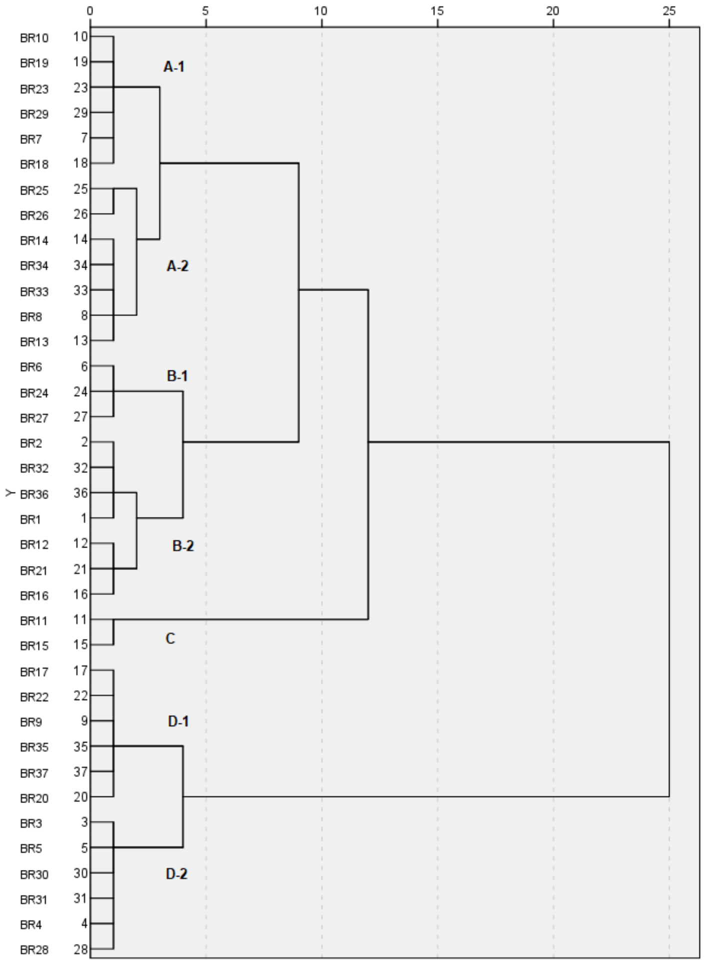
Diversity of BROCCOLI



UNICT
UNILIV
VURV

Spatial distribution of the Broccoli accessions.



Dendrogram based on the genetic similarity matrix of Broccoli accessions.

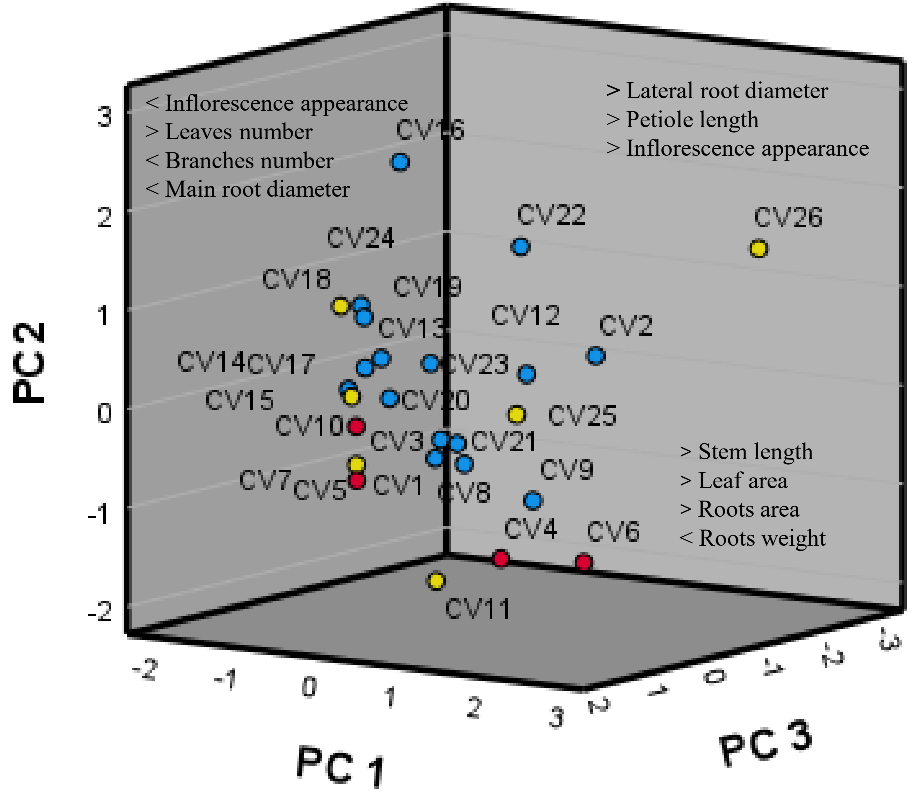
Diversity of CAULIFLOWER





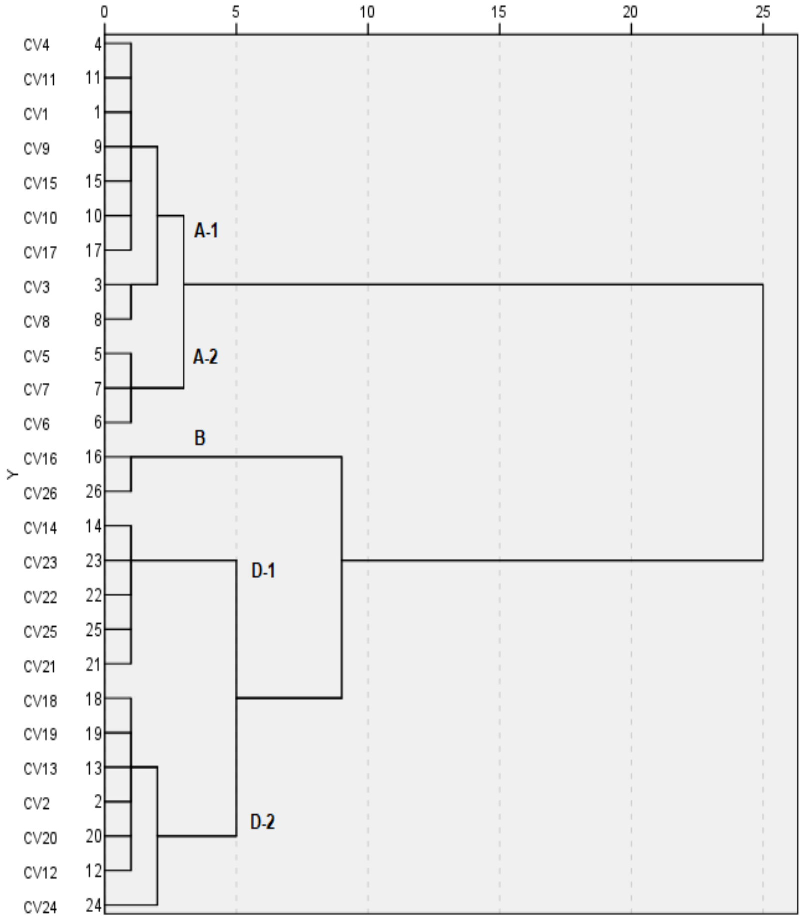



Diversity of CAULIFLOWER



UNICT
UNILIV
VURV

Spatial distribution of the CV accessions.



Dendrogram based on the genetic similarity matrix of CV accessions.

Diversity of CABBAGE





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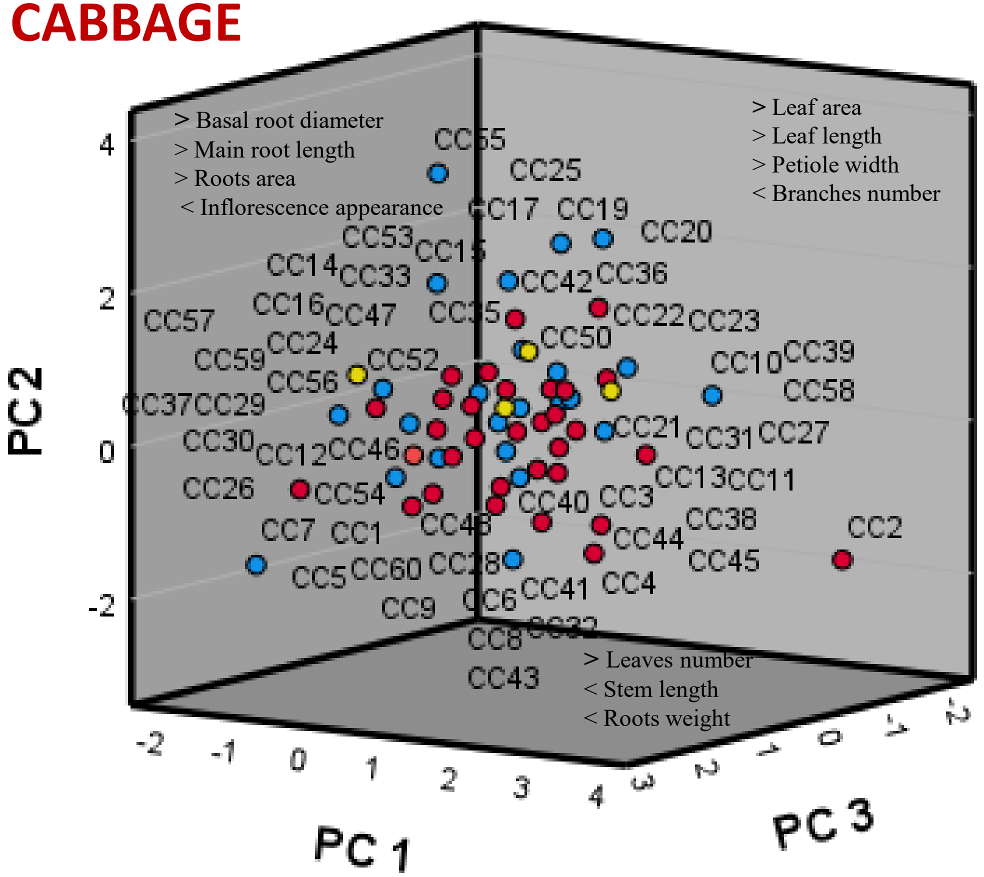
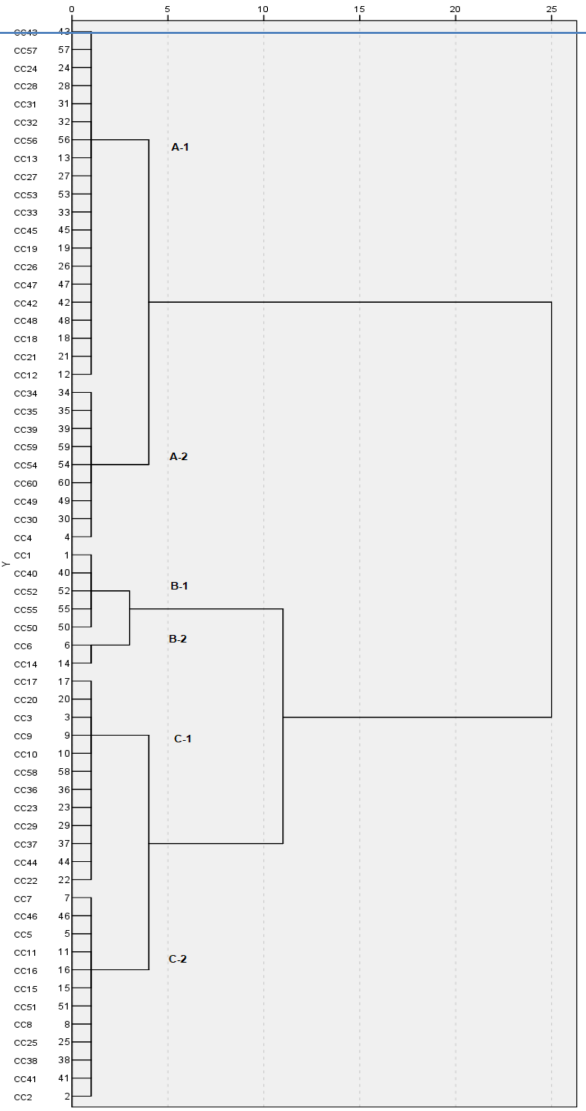
Diversity of CABBAGE



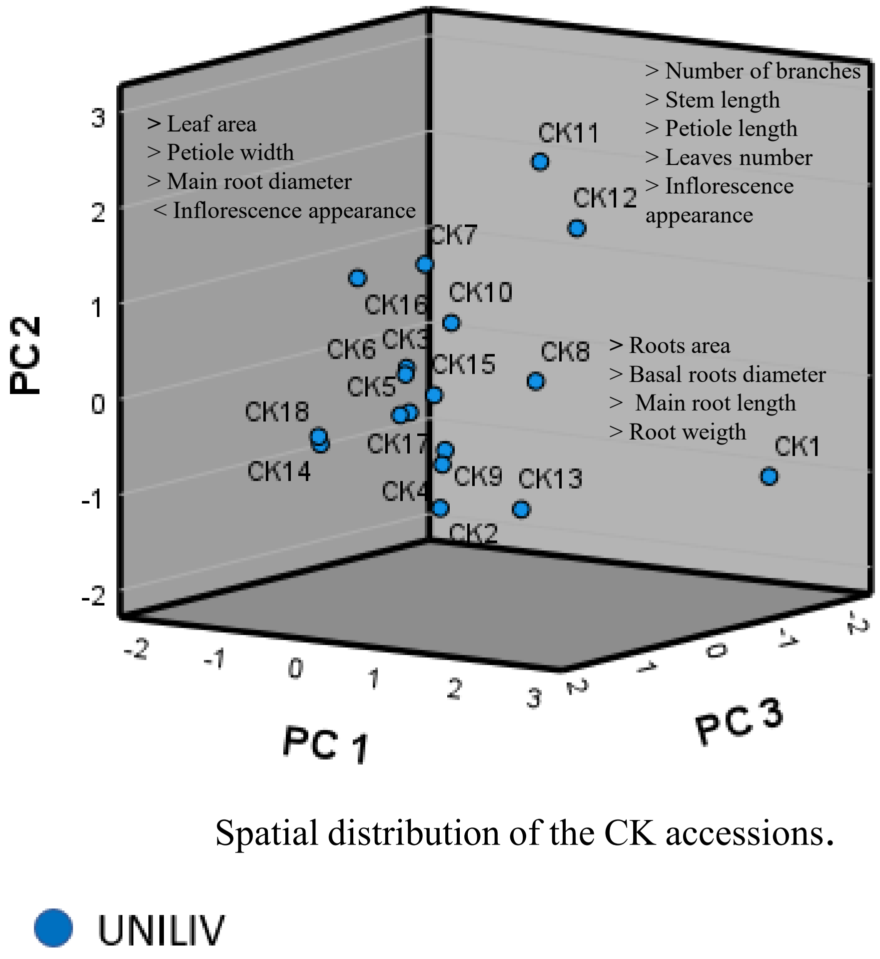
- UNICT
- UNILIV
- VURV

Spatial distribution of the cabbage accessions.

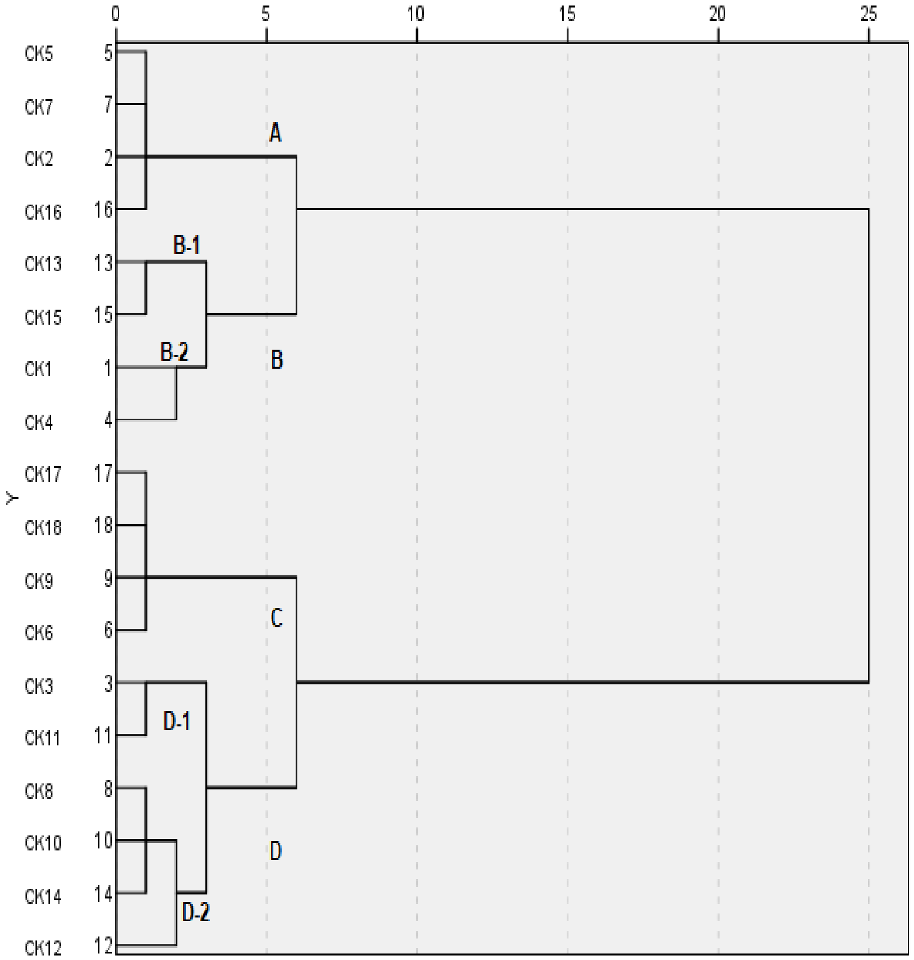


Dendrogram based on the genetic similarity matrix of CC accessions.

Diversity of CHINESE KALE



Spatial distribution of the CK accessions.



Dendrogram based on the genetic similarity matrix of CK accessions.

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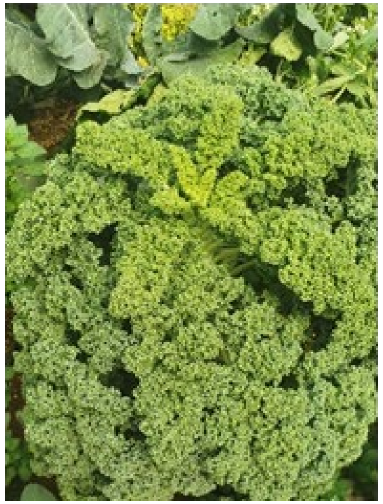
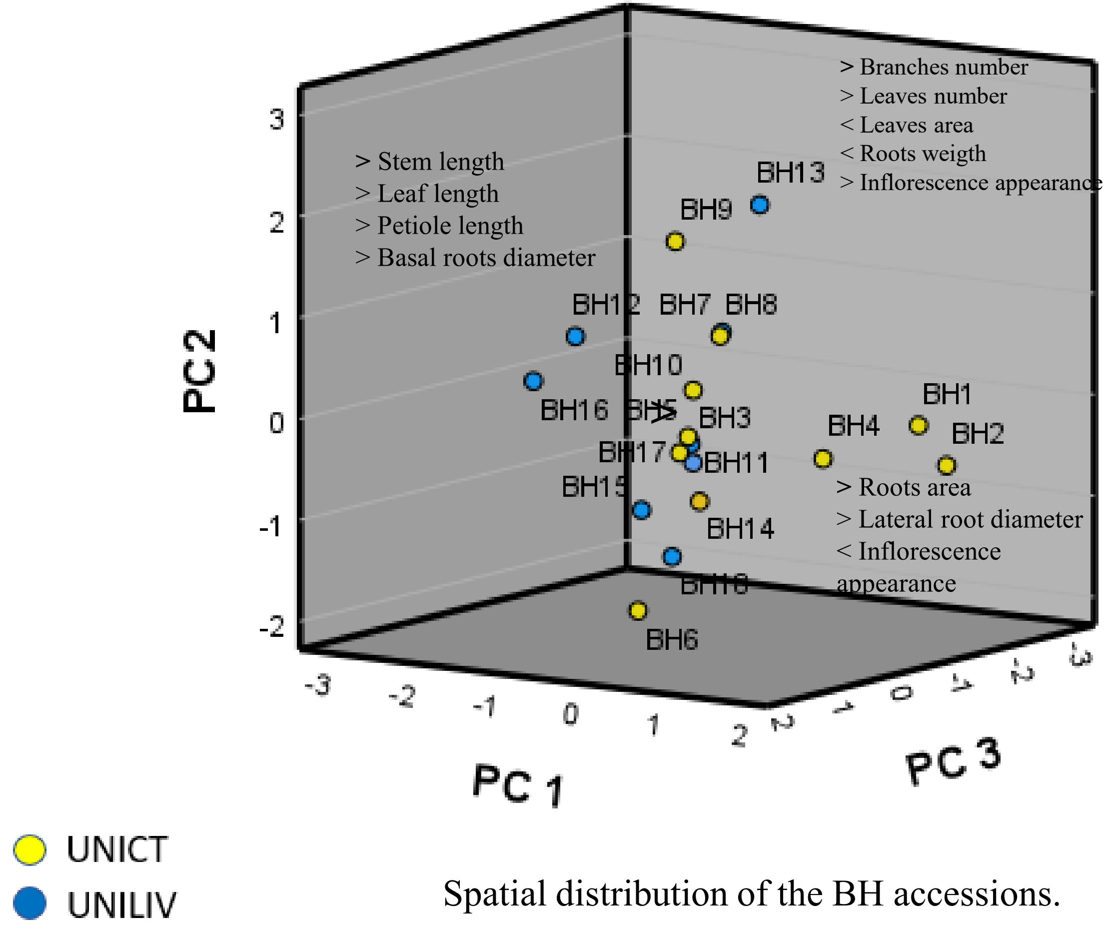
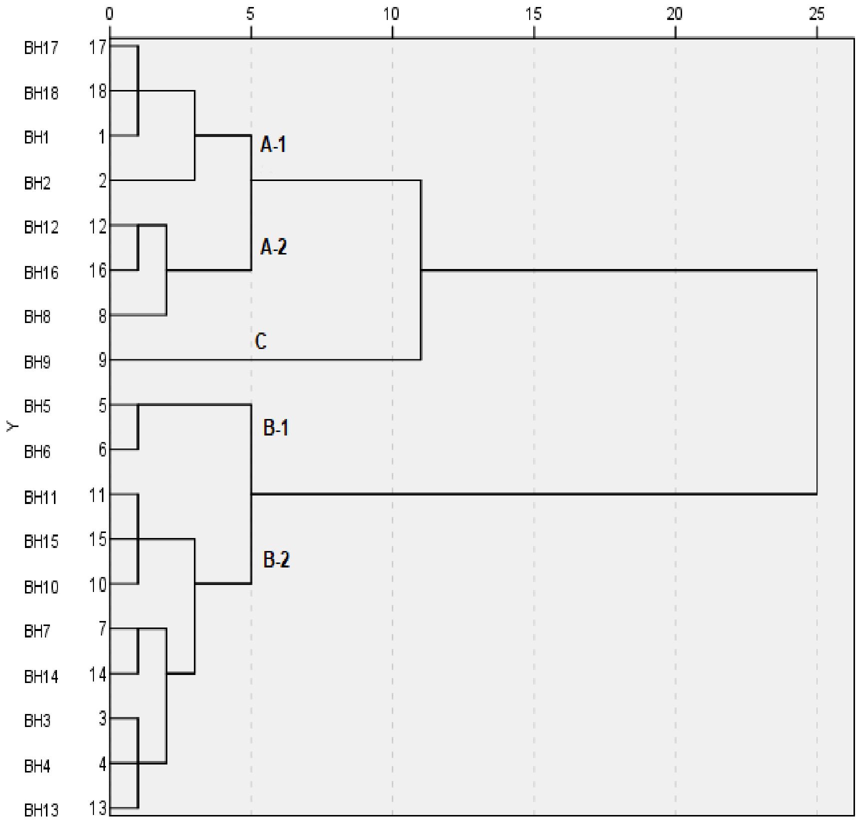



Diversity of KALE



Spatial distribution of the BH accessions.



Dendrogram based on the genetic similarity matrix of BH accessions.

Diversity of KOHLRABI



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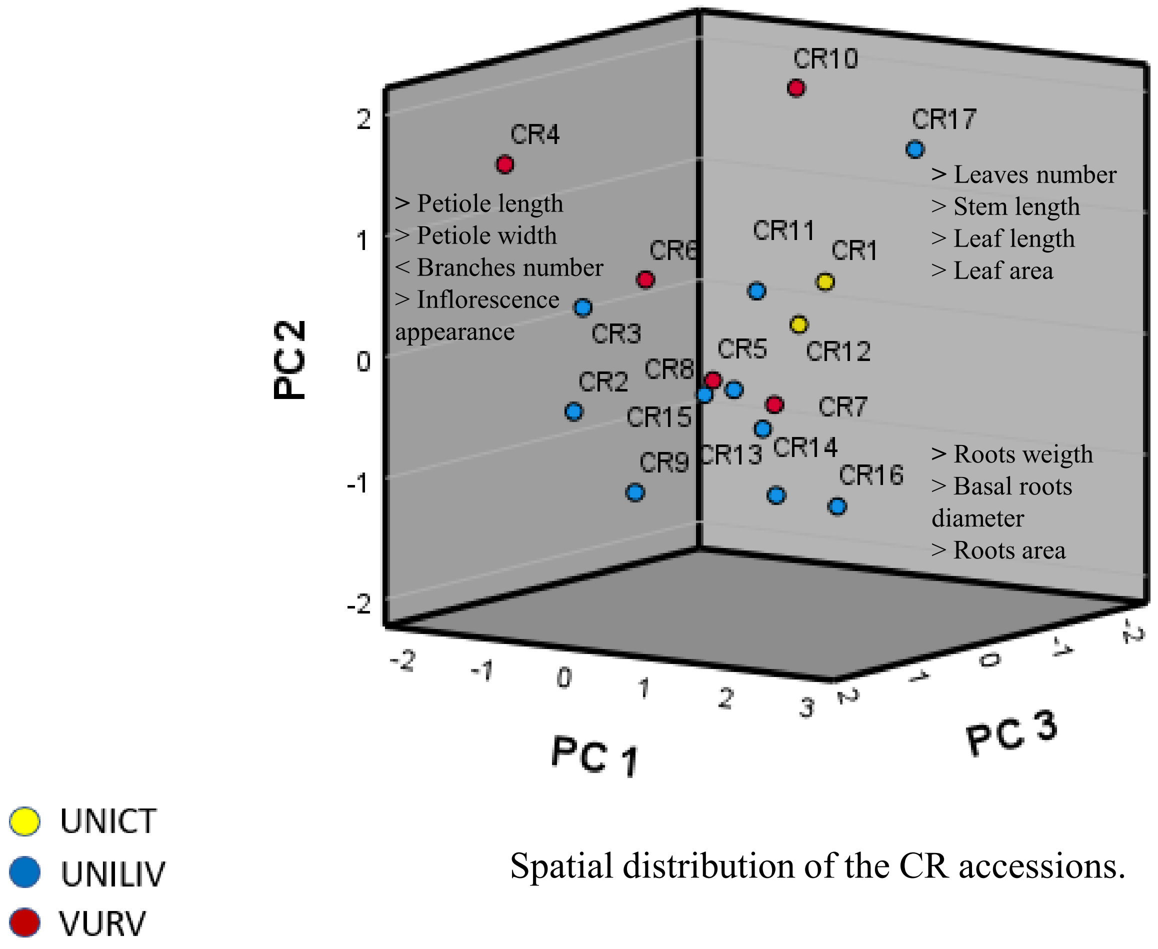
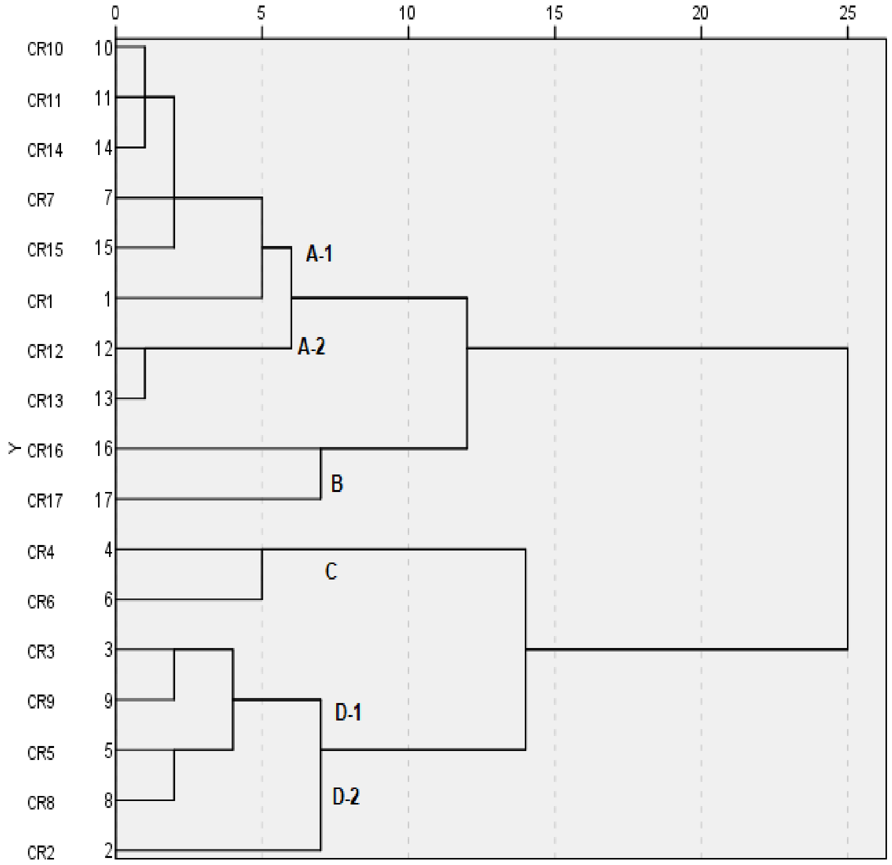


Spatial distribution of the CR accessions.



Dendrogram based on the genetic similarity matrix of CR accessions.

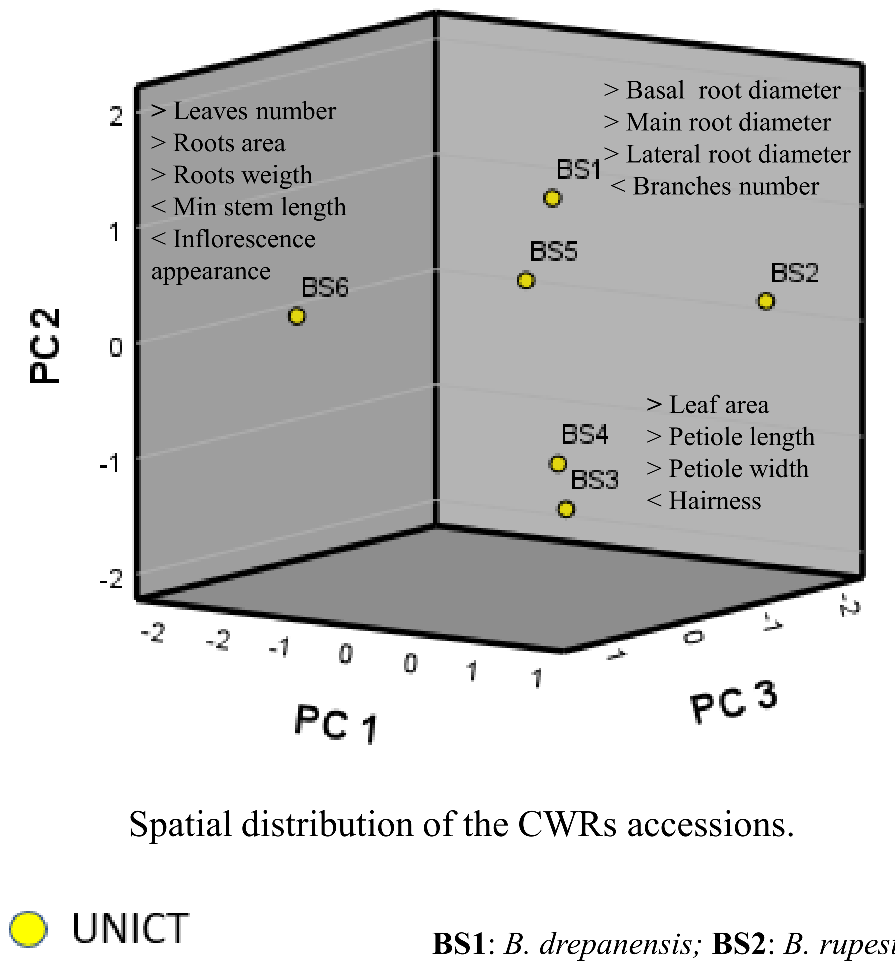
Diversity of CWRs

B. drepanensis

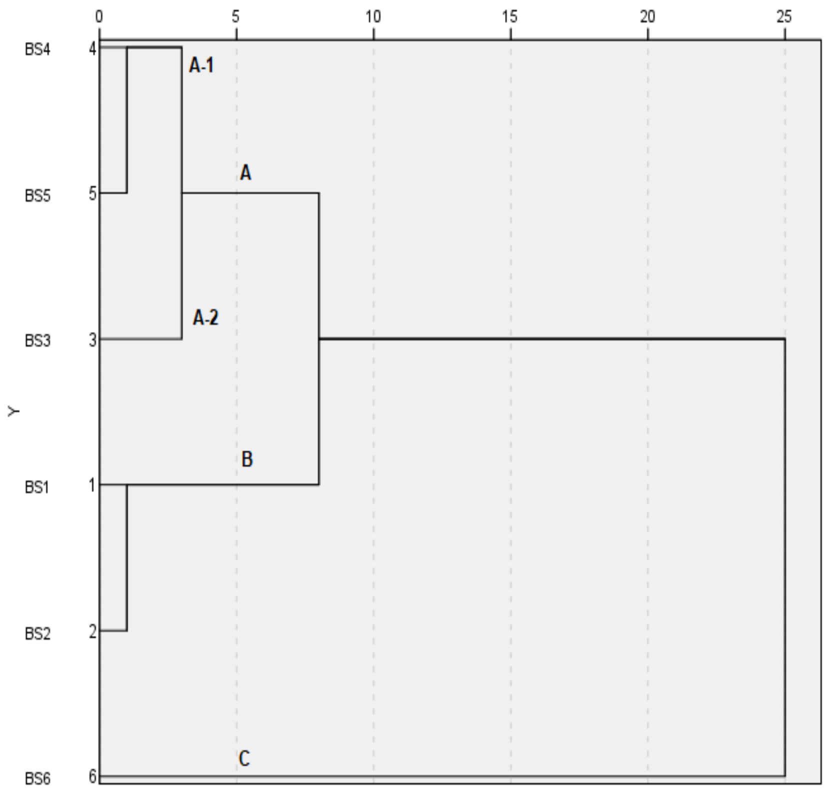
B. villosa

B. incana

B. rupestris



Spatial distribution of the CWRs accessions.



Dendrogram based on the genetic similarity matrix of CWRs accessions.

BS1: *B. drepanensis;* **BS2**: *B. rupestris;* **BS3**: *B. incana;* **BS4**: *B. incana;* **BS5**: *B. villosa;* **BS6**: *B. villosa*.

Molecular Markers for Detecting Inflorescence Size of *Brassica oleracea* L. Crops and *B. oleracea* Complex Species (n = 9) Useful for Breeding of Broccoli (*B. oleracea* var. *italica*) and Cauliflower (*B. oleracea* var. *botrytis*)

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Table 6. List of *B. oleracea* complex species (*n* = 9) utilized in the experiment, with cauliflowers and broccoli F1 and landraces, respectively, and crop wild relatives.

Accession Code	Laboratory Code	Origin	Species
UNICT 583	BR 46	Vittoria	BR1
UNICT 658	BR 45 S1	Acireale	BR2
UNICT 658	BR 129	Roccella Valdemone	BR3
UNICT 657	BR 128	Roccella Valdemone	BR4
UNICT 655	BR 126	Adrano	BR5
UNICT 637	BR 106	Cefalù	BR6
UNICT 3675	BR 94 S1	Francavilla	BR7
UNICT 3668	BR 115 S1	Troina	BR8
UNICT 574	BR 36	Biancavilla	BR9
UNICT 3578	BR 165 Marathon	Esasem	BRF1.1
UNICT 651	BR 122 Packman	Petoseed	BRF1.2
UNICT 4145	BR 13 S3 AC	Modica	CI1
UNICT 579	BR 41	Modica	CI2
UNICT 3190	BR 15 S 1 A	Modica	CV1
UNICT 3669	BR 17 S2	Ragusa	CV2
UNICT 3674	CV 19 S2 A	Piazza Armerina	CV3
UNICT 4137	CV 99 S2 B	Adrano	CV4
UNICT 4138	CV 76 S2	Acireale	CV5
UNICT 3652	CV 159	Catania	CV6
UNICT 3900	BR 13 A X CV98/21	Di3A	CV7
UNICT 3895	CV 98/2 X CV 136 EG	Di3A	CV8
UNICT 3089	CV 75 S3AC	Acireale	CV9
UNICT 3906	CV 24 S4	Biancavilla	CV10
UNICT 3671	CV 72 S2	Catania	CV11
UNICT 3876	CV 171 Menhir F1	ISI sementi	CVF1.1
UNICT 3878	CV 173 Freedom	3878 Royal Sluis	CVF1.2
UNICT 3902	CV 33 S1	Royal Sluis	CVF1.3
UNICT 3880	CV 175 White Flash	Sakata	CVF1.4
UNICT 3879	CV 174 Graffiti	ISI sementi	CVF1.5
UNICT 3892	CV 98/2 X BR 13 S3	DISPA 3	CVF1.6
UNICT 3893	CV 136 EG X CV98/2	DISPA 1	CVF1.7
UNICT 342	*Brassica macrocarpa 1*	Favignana	BM
UNICT 733	*Brassica rupestris 1*	San Vito Lo Capo	BU1
UNICT 3270	*Brassica rupestris 2*	Bivongi	BU2
UNICT 732	*Brassica rupestris 3*	Roccella Valdemone	BU3
UNICT 736	*Brassica rupestris 4*	Ragusa Ibla	BU4
UNICT 3040	*Brassica villosa 1*	Marianopoli	BV
UNICT 3512	*Brassica incana 1*	Agnone Bagni	BY1
UNICT 4158	*Brassica incana 2*	Sortino	BY2

Legend: CV—Cauliflower; CI—Ciurietti landrace; BR—Broccoli; BY—*B. incana*; BM—*B. macrocarpa*; BU—*B. rupestris*; BV—*B. villosa*.

Materials & Methods

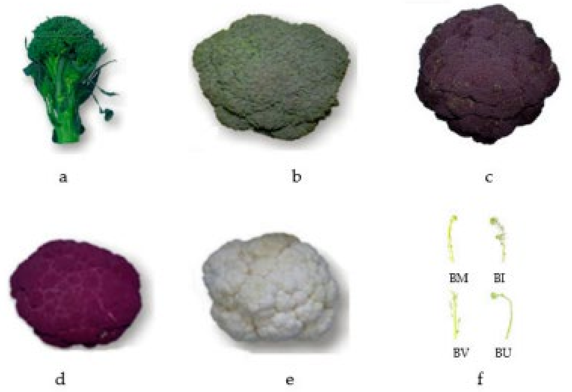

Figure 3. Inflorescence induction in relation of the different morphotypes tested which are, in the following order, (**a**): BR5, (**b**): BRF1.1, (**c**): BR41, (**d**): CV10, (**e**): CVF1.1, (**f**): BM1, BI1, BV and BU1, respectively.

Table 7. List of primers utilized with their sequences and chromosome (C) position.

Name	SSR Motif	Primer Sequence (Forward, Reverse)	C	Position (from–to); (bp)	Code
BoAP1	$(AT)_{9-1}$	GGAGGAACGACCTTGATT GCCAAAATATACTATGCGTCT	C6	33,883,667–33,887,357	P1
BoTHL1	$(CTT)_7$	GCCAAGGAGGAAATCGAAG AAGTGTCAATAAGGCAACAAGG	C9	17,254,558–17,255,176	P2
BoABI1	$(TC)_{16}$	TATCAGGGTTTCCTGGGTTG GTGAACAAGAAGAAAAGAGAGCC	C1	1,229,915,511–12,992,170	P3
BoPLD1	$(CT)_7(AT)_{7-1}$	GACCACCGACTCCGATCTC AGACAAGCAAAATGCAAGGAA	C5	46037340–46,037,606	P4
PBCGSSRBo39	$[GGTCG]_4$	AACGCATCCATCCTCACTTC TAAACCAGCTCGTTCGGTTC	C7	50595248–50595537	P5

Results

Table 1. Inflorescence morphometric traits analyzed.

Accession	IW	IH	ID2	ID1	IS	IA
CVF1.1	1095.8 (21.1) *	11.1 (8.4)	42.3 (8.5)	18.0 (8.7)	0.6 (9.6)	110.0 (21.9)
CV1	965.7 (37.4)	15.4 (14.6)	39.8 (16.4)	20.7 (17.4)	0.7 (16.6)	105.0 (19.4)
CV4	666.6 (42.5)	15.2 (13.2)	34.1 (19.6)	21.1 (15.0)	0.7 (12.1)	112.0 (20.4)
CI1	628.8 (33.7)	16.8 (16.6)	38.1 (18.5)	19.7 (14.6)	0.9 (14.6)	101.0 (22.5)
CVF1.2	605.0 (33.8)	8.9 (16.7)	31.0(10.3)	16.9 (11.8)	0.5 (12.1)	113.0 (13.3)
CV5	567.3 (38.2)	14.5 (15.6)	37.0 (19.8)	19.5 (13.1)	0.7 (17.29)	113.0 (13.5)
CV6	564.9 (37.0)	14.5 (20.7)	34.6 (12.6)	20 (15.1)	0.7 (18.7)	104.0 (16.7)
CV7	554.5 (56.7)	18.8 (20.4)	30.8 (26.9)	19.5 (19.3)	0.9 (29.8)	107.0 (17.7)
CVF1.3	541.5 (54.7)	13.7 (24.4)	32.3 (21.9)	18.9 (29.6)	0.7 (18.3)	112.0 (22.3)
CV8	503.9 (35.4)	16.8 (28.4)	32.4 (18.1)	16.5 (17.9)	1.0 (34.4)	100.0 (27.4)
CVF1.4	467.1 (41.1)	7.5 (20.9)	30.0 (13.3)	14.6 (15.7)	0.5 (11.1)	101.0 (15.6)
CVF1.5	461.8 (47.1)	10.8 (16.1)	33.0 (13.9)	17.5 (16.4)	0.6 (17.4)	110.0 (19.3)
CV9	453.5 (49.7)	11 (17.2)	35.6 (17.9)	18.1 (27.3)	0.6 (22.5)	117.0 (15.8)
CV10	443 (55.9)	12.7 (23.0)	36.4 (24.2)	16.7 (23.8)	0.8 (32.4)	91.0 (26.5)
CVF1.6	438.8 (84.4)	17.6 (24.4)	28.8 (28.1)	16.8 (29.4)	1.1 (34.2)	93.0 (17.7)
CI2	378.3 (46.2)	10.2 (21.0)	36.8 (16.9)	17.2 (19.5)	0.6 (17.8)	113.0 (17.5)
BRF1.1	319.8 (40.9)	14.1 (26.8)	3.5 (19.5)	12.3 (26.7)	1.2 (44.4)	76.0 (29.8)
CVF1.7	317.4 (42.0)	17.2 (22.2)	29.2 (28.1)	14.8 (16.0)	1.2 (33.1)	98.0 (21.6)
CV11	305.7 (68.2)	8.7 (20.7)	31.7 (18.1)	15.4 (22.8)	0.6 (19.7)	92.0 (25.2)
BR1	279 (39.0)	16.6 (18.1)	3.8 (17.3)	11.1 (23.7)	1.5 (28.2)	57.0 (21.5)
BR2	266.9 (33.4)	22.2 (30.9)	3.2 (13.2)	8.5 (32.7)	2.7 (37.4)	58.0 (19.4)
CV2	263.6 (56.1)	11.2 (28.3)	34.2 (18.8)	14.4 (22.0)	0.8 (21.2)	91.0 (23.4)
BR3	226.4 (39.6)	18.2 (12.9)	3.1 (26.8)	7.9 (29.4)	2.3 (30.5)	49.0 (27.8)
BR4	217.7 (58.3)	18.2 (18.2)	2.9 (29.8)	9.5 (31.6)	1.9 (29.4)	54.0 (26.3)
BRF1.2	212.8 (36.3)	12.8 (12.2)	3.1 (15.0)	7. 8 (23.1)	1.9 (16.5)	46.0 (24.1)
BR5	188.3 (51.8)	16.6 (23.4)	2.9 (24.3)	7.7 (28.3)	2.2 (24.2)	46.0 (24.1)
CV3	186.6 (41.3)	8.4 (17.5)	28.6 (16.7)	13.6 (15.1)	0.6 (18.2)	85.0 (24.8)
BR6	164.0 (49.0)	16.5 (17.9)	3.3 (32.4)	8.3 (29.5)	2.0 (52.3)	46.0 (32.8)
BR7	143.9 (42.2)	16.0(29.0)	2.7 (22.7)	7.8 (29.0)	2.1 (22.6)	48.0 (26.7)
BR8	109.5 (30.8)	15.5 (9.5)	2.6 (20.2)	7.9 (25.8)	2.0 (23.4)	41.0 (34.2)
BR9	63.1 (41.7)	16.9 (23.5)	2.7 (18.9)	4.7 (22.3)	3.6 (15.5)	27.0 (15.2)
BU1	33.3 (28.3)	27.6 (15.5)	16.2 (20.2)	3.1 (17.9)	0.2 (21.2)	14.0 (11.7)
BU2	28.7 (1.6)	19.5(1.5)	19.3 (3.3)	4.1 (0.2)	0.21 (0.1)	15.0 (0.9)
BY1	27.7 (3.7)	20.4 (1.0)	22.5 (4.5)	3.3(0.7)	0.1 (0.1)	13.5 (2.1)
BM	26.6 (5.9)	16.7 (4.6)	9.6 (2.5)	2.7 (0.4)	0.3 (0.1)	11.3 (2.6)
BU3	22.4 (0.4)	23.5 (4.0)	19.6 (1.5)	2.0 (0.3)	0.1 (0.1)	9.5 (0.7)
BU4	21.1 (0.8)	19.2 (2.2)	18.9(3.6)	2.2(0.1)	0.1 (0.1)	13.5 (2.1)
BY2	20.6 (1.3)	20.8 (0.6)	18.5 (1.6)	2.8 (0.4)	0.2 (0.1)	11.5 (0.7)
BV	19.7 (0.6)	14.8 (0.4)	19.0 (1.2)	2.4 (0.2)	0.1 (0.1)	10.5 (0.7)

numbers in brackets () * indicate the standard deviation.

Table 2. Pearson's correlation among traits.

Genotype	IW	IH	ID2	ID1	IS	IA
IW	1					
IH	0.024	1				
ID2	0.680 **	−0.035	1			
ID1	0.880 **	−0.066	0.724 **	1		
IS	−0.117	−0.068	−0.638 **	−0.107	1	
IA	0.847 **	−0.033	0.706 **	0.980 **	−0.086	1

** indicates that the correlation is significant at $p < 0.01$.

Table 3. Correlation among all the allelic variants detected by the molecular markers used and the analyzed traits to individuate the most associated alleles of the examined traits.

Allelic Variant	IW	IH	ID2	ID1	IS	IA
P1_155	0.622 **	−0.471 **	0.521 **	0.622 **	0.032	0.677 **
P1_156	−0.101	0.156	0.219	−0.097	0.202	−0.135
P1_164	−0.375	0.072	−0.082	−0.334	−0.283	−0.306
P2_153	−0.288	0.189	0.219	0.308	0.00	−0.264
P2_157	−0.338 *	0.405 **	−0.088	−0.372 *	−0.376 *	−0.372 *
P2_162	−0.152	−0.029	−0.418 **	−0.266	0.196	−0.175
P2_165	−0.461 *	−0.220	0.583 **	0.594 **	−0.014	0.538 **
P2_168	0.160	0.021	0.226	0.205	0.050	0.204
P3_180	0.010	0.033	0.069	0.046	−0.003	0.095
P3_184	−0.455 **	0.440 **	0.123	−0.455 **	−0.477 **	−0.433 *
P3_186	−0.233	0.296	−0.214	−0.257	0.062	−0.187
P3_190	0.257	−0.440 *	0.268	0.303	0.192	0.226
P3_192	0.418 *	−0.324	0.222	0.424 **	0.156	0.436 **
P3_194	0.140	−0.015	−0.068	0.068	0.146	0.174
P4_282	−0.139	0.199	−0.097	−0.184	−0.168	−0.232

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Article

Using Simple Sequence Repeats in 9 *Brassica* Complex Species to Assess Hypertrophic Curd Induction

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Abstract: Five Simple Sequence Repeats (SSRs) were used to assess the relationship between inflorescence characteristics and their allelic variation in 53 *Brassica oleracea* and *Brassica* wild relatives (*n* = 9). Curd morphometric traits, such as weight (CW), height (CH), diameter (CD1), shape (CS) inflorescence curvature angle (CA), and its curd stem diameter (CD2), were measured. The aim of the work was to analyze the relationships among the allelic patterns of the SSRs primers utilized, and their status of homo or heterozygosity registered at each locus, as well as the inflorescence morphometric traits in order to individuate genomic regions stimulating the hypertrophy of this reproductive organ. The relationships found explain the diversity among *B. oleracea* complex species (*n* = 9) for the inflorescence size and structure, allowing important time reduction during the breeding process by crossing wild species, transferring useful resistance, and organoleptic and nutraceutical traits. The five SSRs loci were BoABI1, BoAP1, BoPLD1, BoTHL1, and PBCGSSRBo39. According to the allelic variation ascertained, we evaluated the heterozygosity index (H) for each SSR above cited. The results showed a significant interaction between the H index of the BoPLD1 gene and the inflorescence characteristics, summarized by the First Principal Component (PC1) (*p*-value = 0.0244); we ascertained a negative correlation between the H index and inflorescence characteristics, namely CW, CH, CD1, CD2, CA. The homozygosity BoPLD1 alleles, indicated by the H index, affect the inflorescence characteristics and broccoli and cauliflower yields.

Keywords: *Brassica* complex species; MADH–box genes; SSRs assay; heterozygosity index; allelic variance; curd morphometric traits

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Citation: Treccarichi, S.; Di Gaetano, C.; Di Stefano, F.; Gasparini, M.; Branca, F. Using Simple Sequence Repeats in 9 *Brassica* Complex Species to Assess Hypertrophic Curd Induction. *Agriculture* **2021**, *11*, 622. https://doi.org/10.3390/agriculture11070622

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Article

Molecular Markers for Detecting Inflorescence Size of *Brassica oleracea* L. Crops and *B. oleracea* Complex Species (n = 9) Useful for Breeding of Broccoli (*B. oleracea* var. *italica*) and Cauliflower (*B. oleracea* var. *botrytis*)

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Abstract: The gene flow from *Brassica oleracea* L. wild relatives to *B. oleracea* vegetable crops have occurred and continue to occur ordinarily in several Mediterranean countries, such as Sicily, representing an important hot spot of diversity for some of them, such as broccoli, cauliflower and kale. For detecting and for exploiting the forgotten alleles lost during the domestication processes of the *B. oleracea* crops, attention has been pointed to the individuation of specific markers for individuating genotypes characterized by hypertrophic inflorescence traits by the marker assisted selection (MAS) during the first plant growing phases after the crosses between broccoli (*B. oleracea* var. *italica*)/cauliflower (*B. oleracea* var. *botrytis*) with *B. oleracea* wild relatives (n = 9), reducing the cultivation and evaluation costs. The desired traits often found in several *B. oleracea* wild relatives are mainly addressed to improve the plant resistance to biotic and abiotic stresses and to increase the organoleptic, nutritive and nutraceutical traits of the products. One of the targeted traits for broccoli and cauliflower breeding is represented by the inflorescences size as is documented by the

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- **WP3. Plant Breeding: development of populations, advanced breeding lines and improved genetic material for European Organic Agriculture**

- In order to enhance the biodiversity of cultivated and spontaneous cauliflower of *Brassica oleracea* for making broccoli and cauliflower cultivars adapted to organic cultivation, during the year 2022-2023, the phenotypes selected were subjected to controlled self-fertilization /crossbreeding.

- Starting from November 2022 until January 2023, the genetic materials were subjected to phenotypic, agronomic and qualitative evaluation.

ECPGR *Brassica* Working Group

- BRESOV experimental field at CREA-OF,

Monsampolo del Tronto (AP), Italy, 42°53′ N; 13°48′ E, 184 m a.s.l,

Eutric, Calcaric, Vertic e Fluvic

Cambisol; Haplic Calcisol, soil type nr. 36

Open field selection

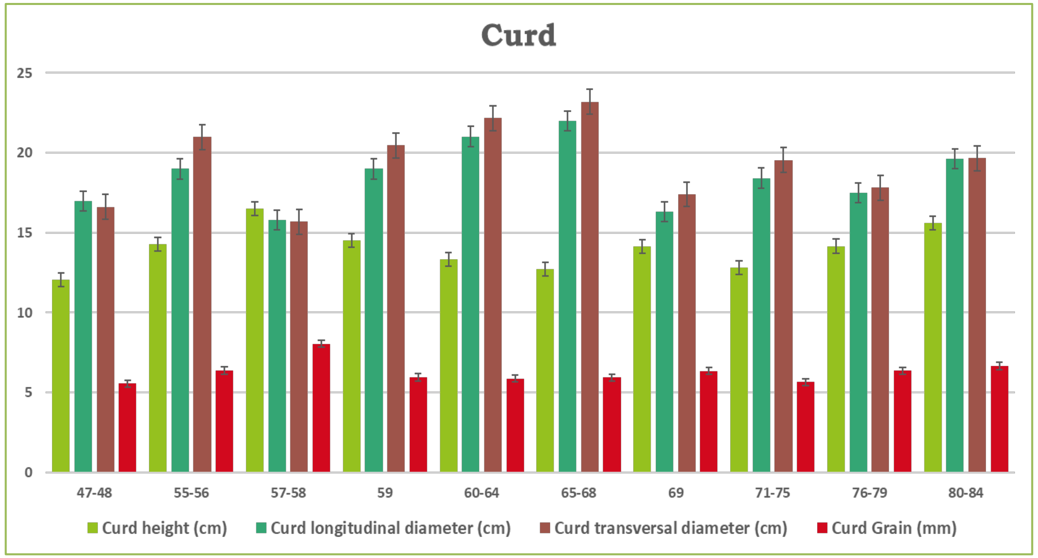
Greenhouse breeding by crosses, back-crosses and self-pollination

European Cooperative Programme for Plant Genetic Resources ECP/GR

Accession	Plot 2022	Curd Area (cm²)	Curd height (cm)	Curd longitudinal diameter (cm)	Curd transversal diameter (cm)	Curd curvature angle (°)	Curd Grain (cm)
(CV52 x CV19) F3	47-48	226.2±22.6	12.0±0.8	17.0±0.1	16.6±0.1	90.0±0.0	0.56±0.01
(CV136 x CV98) F4	55-56	355.8±8.6	14.3±0.2	19.0±0.2	21.0±0.3	103.9±1.5	0.64±0.01
(CV25 x BR115) F4	57-58	189.48±2.1	16.5±0.3	15.8±0.1	15.7±0.4	90.0±5.0	0.81±0.01
(CV159 x CV136) x (BR40 x CV165) F4	59	332.7±10.5	14.5±0.4	19.0±2.9	20.5±2.0	115.0±11.0	0.60±0.01
(CV 141) x (CV 52 x CV 19) F4	60-64	364.9±14.1	13.4±0.4	21.0±0.8	22.2±1.0	110.0±2.2	0.59±0.02
(CV159) x (BR40 x CV165) F4	65-68	391.1±13.1	12.7±0.3	22.0±1.9	23.2±1.9	112.7±3.9	0.59±0.02
(CV19) x (BR115) F4	69	321.0±11.3	14.1±0.1	16.3±0.4	17.4±0.4	100.5±0.5	0.64±0.01
[(CV19)] x [(CV19 x BR115) x (BR115)] F1BC1	71-75	366.6±7.5	12.8±0.1	18.4±0.6	19.5±0.6	102.7±1.8	0.57±0.01
[BR15] x [(CV19 x BR115) x (BR115)] F1BC1	76-79	304.7±3.0	14.2±0.5	17.5±0.5	17.8±0.5	103.2±2.4	0.64±0.03
(CV19 x BR115) x (BR115) F2BC1	80-84	318.7±13.8	15.6±0.6	19.6±1.5	19.7±1.6	103.3±6.7	0.67±0.05

Phenotypic characterization

Morphometric characterization



BRESOV events

✓December 2021 - OrgHort2020 ISHS Symposium in Catania (Italy)

www.orghort2020.it

Acta Horticulturae n. 1354

ISHS Acta Horticulturae 1354

III International Organic Fruit Symposium and I International Organic Vegetable Symposium

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Volumes	1

BRESOV events

UNICT: BRESOV at the International Horticultural Congress in Angers.

Euroseeds: BRESOV at the Euroseeds 2022 Congress in Berlin.

BRESOV events

UNICT: BRESOV Winter School 2022 in Agrigento.

SERIDA: BRESOV at the I National Congress of Legumes in Asturias.

European Cooperative Programme for Plant Genetic Resources
ECP/GR

Crop Wild Relatives Utilization and Conservation for Sustainable Agriculture

cousin

ECPGR *Brassica* Working Group

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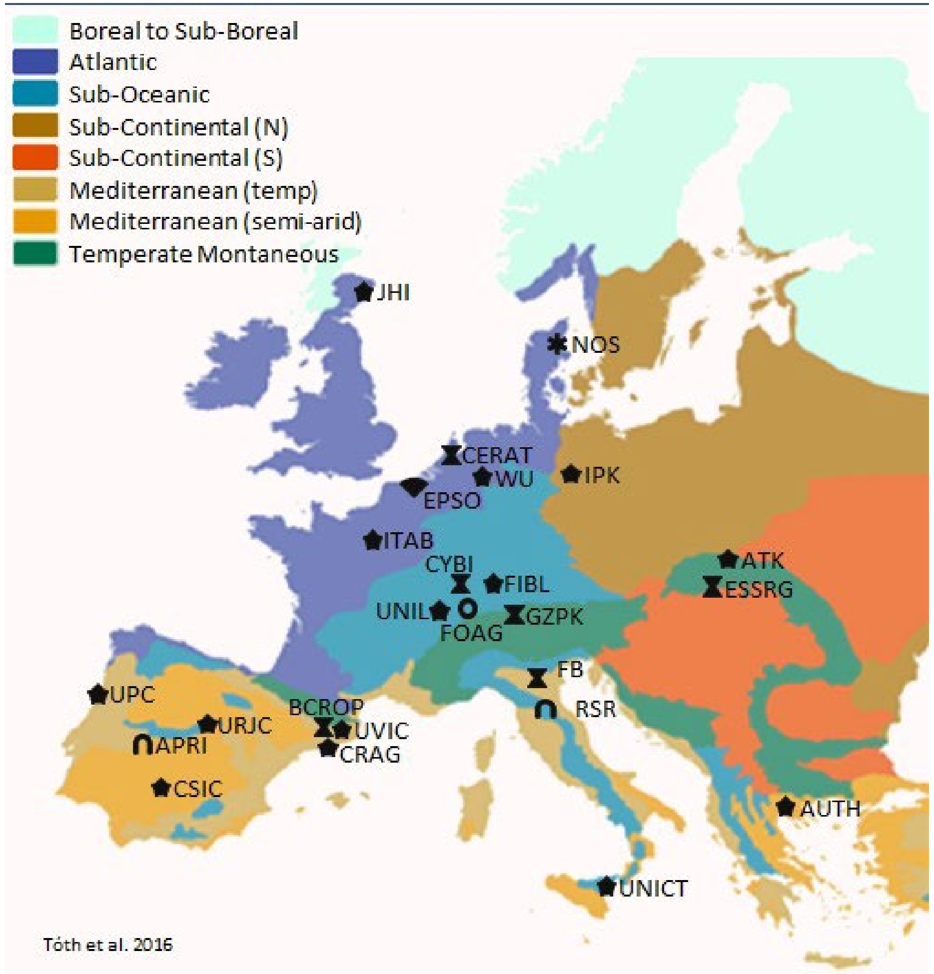

No.	Participant organisation name	Short	Country	Type
1	Universidad Rey Juan Carlos (Coordinator)	URJC	ES	RTD
2	Universidade Católica Portuguesa	UCP	PT	RTD
3	Fundacio Universitaria Balmes	UVIC	ES	RTD
4	Eidg. Departement für Wirtschaft, Bildung und Forschung	FOAG	CH	GO
5	Centre de Recerca en Agrigenomica	CRAG	ES	RTD
6	Rete Semi Rurali	RSR	IT	NGO
7	Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung	IPK	DE	RTD
8	Wageningen University	WU	NL	RTD
9	Agencia Estatal Consejo Superior de Investigaciones Científicas	CSIC	ES	RTD
10	ESSRG Nonprofit KFT	ESSRG	HU	SME
11	Europese Orgnisatie voor Wetenschappelijk Plantenonderzoek	EPSO	BE	other
12	Forschungsinstitut für Biologischen Landbau	FIBL	CH	RTD
13	Getreidezüchtung Peter Kunz	GZPK	CH	NGO
14	Formicablu SRL	FB	IT	SME
15	Asociación Aprisco de Las Corchuelas	APRI	ES	NGO
16	Aristotelio Panepistimio Thessalonikis	AUTH	GR	RTD
17	Cybiome GmbH	CYBI	CH	SME
18	Nordic Seed AS	NOS	DK	LE
19	Institut Technique de l'Agriculture Biologique	ITAB	FR	RTD
20	The James Hutton Institute	JHI	UK	RTD
21	**Universita degli Studi di Catania**	**UNICT**	**IT**	**RTD**
22	Agrártudományi Kutatóközpont	ATK	HU	RTD
23	Université de Lausanne	UNIL	CH	RTD
24	Ceratium BV	CERAT	NL	SME
25	BioCrop Innovations SL	BCROP	ES	SME

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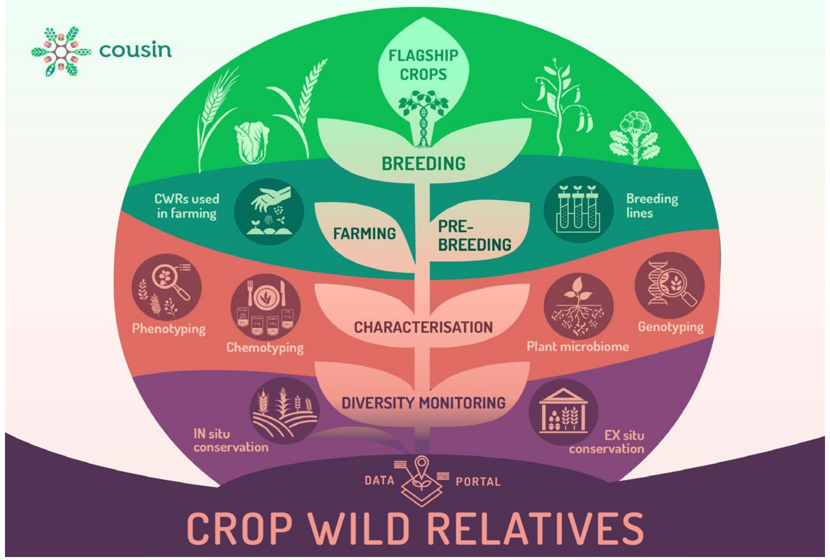
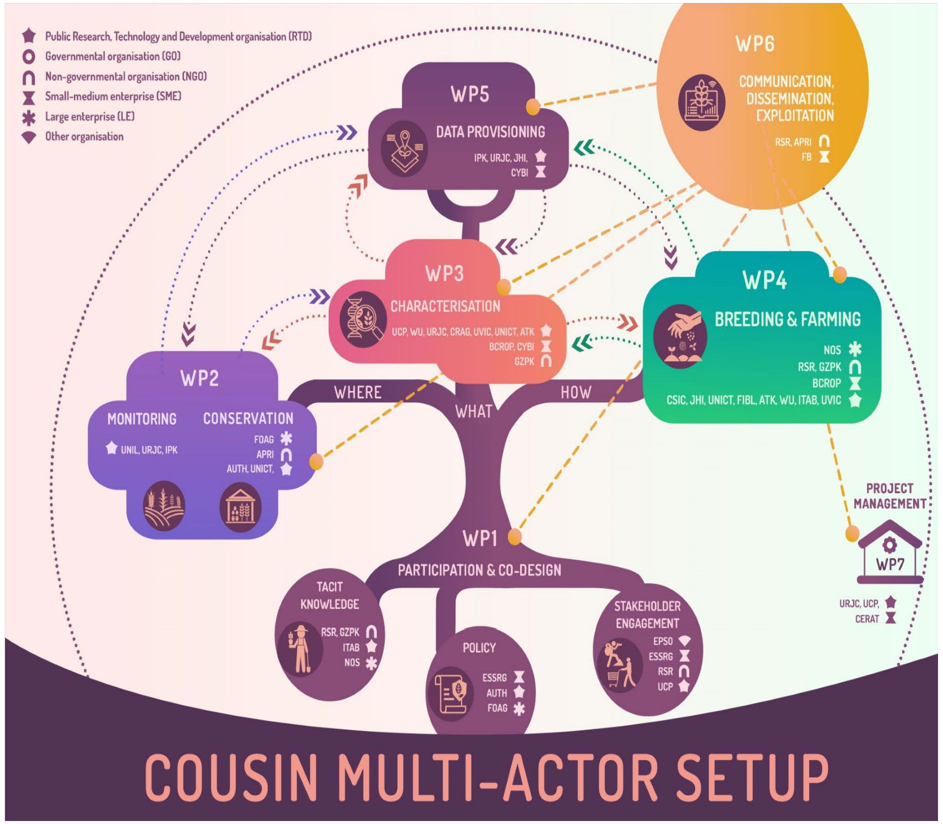


The COUSIN multi-actor setup displaying the key institutions in each work package (WP) and the most relevant



WPs involving UNICT

WP1 : Co-creating the contexts for Crop Wild Relatives

WP3 : Trait characterization of CWRs and pre-breeding germplasm

WP6 : Communication, Dissemination, Exploitation and Training

WP7 : Project Coordination and Management

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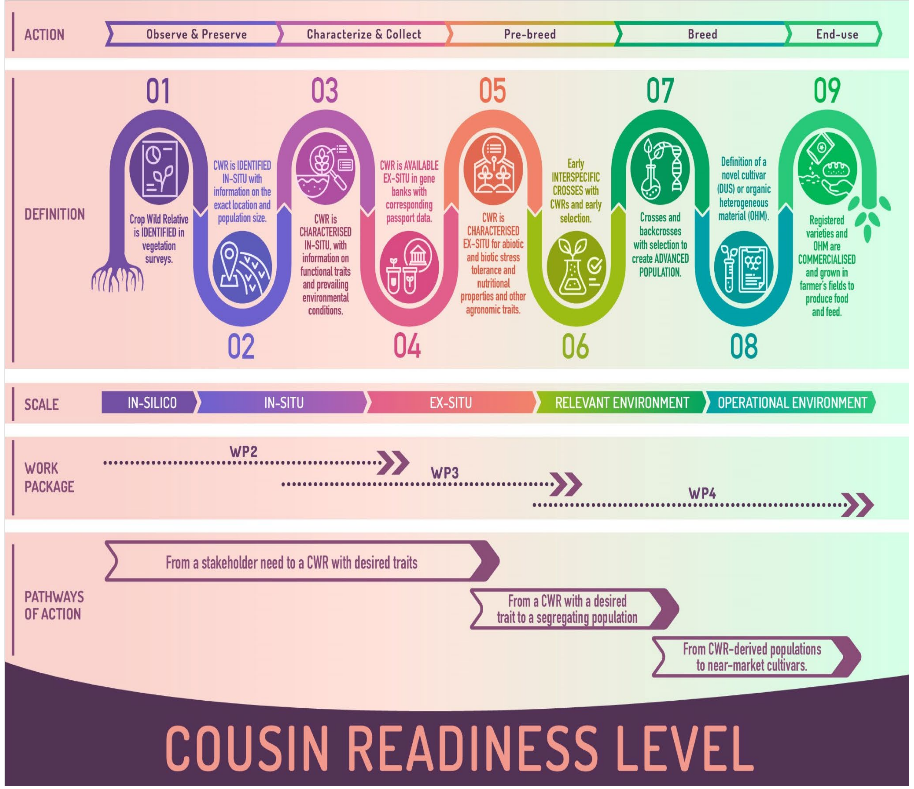

The COUSIN Readiness Levels (CRLs), their type and scale of action, their coverage by WPs 2, 3 and 4 and the three parallel pathways of action implemented in COUSIN.

UNICT Tasks :

- Task 1.1: Transdisciplinary strategy – engaging stakeholders through a cascading engagement strategy
- Task 1.2: Identification of good practices, strategic guidelines for practitioners and establishing pathways to action
- Task 3.1: Assessing CWR biotic and abiotic stress resilience
- Task 3.2: Characterizing CWR benefits in crop management
- Task 3.3: Bioprospecting the nutritional and health value of CWRs
- Task 3.4: Unravel the genetic basis of valuable CWR functional traits, including the CWR ecotype
- Task 3.5: Exploring the microbiome as a tool to enhance crop performance predictions
- Task 4.1 : Generate selection toolboxes for each flagship crop for use of CWRs in breeding activities
- Task 4.2: Implement CWR genetic resources and tools for their use in (pre-)breeding programmes of the five flagship crops across Europe
- Task 4.3: Establish pilots across Europe for CWR-based participatory breeding and CWR use for diversified farming systems
- Task 6.1 : Plan for the Exploitation, Dissemination and Communication of Results
- Task 6.2: Plan for the Exploitation, Dissemination and Communication of Results
- Task 7.2 : Scientific and technical management
- Task 7.3: Exploitation Board and Innovation Management
- Task 7.5: Regulatory and Ethical Issues

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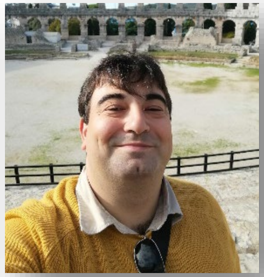
Targets to achieve in COUSIN

- Brassica crops for Biofumigation
- Increase the Nutraceutical value
- Resistance to pest and disease
- Exploitation of Organic Heterogenic Materials (OHMs)
- Cultivars for Organic agriculture

Thank you for your attention!


Prof. Ferdinando Branca


Dr. Simone Treccarichi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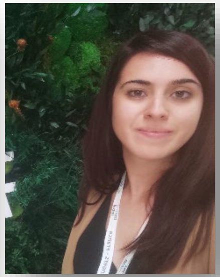
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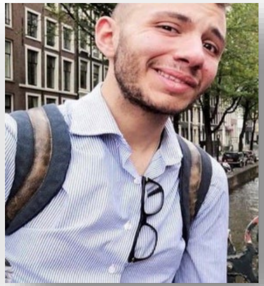
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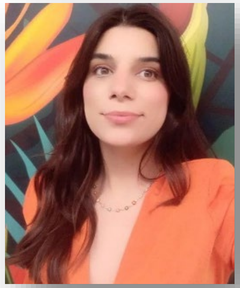
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