Genetic Resources

ORIGINAL ARTICLE

Genetic Resources (2024), 5 (10), 1–16
DOI: 10.46265/genresj.PCWY8016
https://www.genresj.org
ISSN: 2708-3764

Collecting Mediterranean wild species of the *Brassica oleracea* group (*Brassica* sect. *Brassica*)

Lorenzo Maggioni [*,a], **Smiljana Goreta Ban** [b,c], **Sokrat Jani** [d], **Nenad Jasprica** [e], **Simone Treccarichi** [f], **Nina Išić** [c] **and Ferdinando Branca** [f]

[a] *European Cooperative Programme for Plant Genetic Resources (ECPGR), c/o Bioversity International, Via di San Domenico, 1, Rome, 00153, Italy*
[b] *Centre of Excellence for Biodiversity and Molecular Plant Breeding, Zagreb, Croatia*
[c] *Institute of Agriculture and Tourism, K. Huguesa 8, 52440, Poreč, Croatia*
[d] *Institute of Plant Genetic Resources, Agricultural University of Tirana Rruga 'Siri KODRA', Tirana, Albania*
[e] *Institute for Marine and Coastal Research, University of Dubrovnik, Kneza Damjana Jude 12, 20000, Dubrovnik, Croatia*
[f] *Dipartimento di Agricoltura, Alimentazione e Ambiente (DiA3), Università di Catania, Via Valdisavoia 5, 95123, Catania, Italy*

Abstract: Within the framework of the project EUBRASWILD (Capturing *Brassica* Wild Relatives Diversity in Southeastern Europe), several collecting missions were organized, targeting wild *Brassica* complex species (2n = 18), belonging to the gene pool of *Brassica oleracea* L. These crop wild relatives have repeatedly shown their potential to contain useful alleles for biotic and abiotic stress resistance, and nutritional or health-beneficial traits that can be easily intercrossed with the related crop. The missions described in this paper aimed to collect taxa that are poorly represented in public genebanks or databases for long-term conservation. This report describes missions carried out by national teams in Albania, Croatia and Italy (Ponza and Sicily), including highlights of newly discovered locations.

Keywords: *Brassica cretica, Brassica drepanensis, Brassica incana, Brassica macrocarpa, Brassica montana, Brassica oleracea, Brassica rupestris, Brassica villosa*, Crop wild relatives, Collecting missions

Citation: Maggioni, L., Goreta Ban, S., Jani, S., Jasprica, N., Treccarichi, S., Išić, N., Branca, F. (2024). Collecting Mediterranean wild species of the *Brassica oleracea* group (*Brassica* sect. *Brassica*). *Genetic Resources* 5 (10), 1–16. doi: 10.46265/genresj.PCWY8016.

Introduction

The cole crops (*Brassica oleracea* L.) comprise several types of commercially important vegetables, including cabbage, cauliflower and broccoli (Branca, 2008). These crops share the same C genome with 2n = 18 chromosomes and easily intercross with several wild species native to the Mediterranean area, such as *B. cretica* Lam., *B. drepanensis* (Caruel) Damanti, *B. incana* Ten., *B. insularis* Moris, *B. macrocarpa* Guss., *B. montana* Pourr., *B. rupestris* Raf., *B. villosa* Biv., included in *Brassica* section *Brassica* (Snogerup *et al*, 1990; Bothmer *et al*, 1995). These crop wild relatives are potential providers of agronomically useful traits and can serve as a source of suitable alleles that may have been lost during the domestication process. For example, in *B. incana*, resistance against various fungal diseases has been identified, such as Verticillium wilt (*Verticillium longisporum*) and Sclerotinia sclerotiorum (Happstadius *et al*, 2003; Mei *et al*, 2011; Taylor *et al*, 2018); in *B. insularis* against *Leptosphaeria maculans* (Mithen and Magrath, 1992) and *Pyrenopeziza brassicae* (light leaf spot disease) (Bradburne *et al*, 1999); in *B. villosa* against downy mildew (*Hyaloperonospora brassicae*) (Coelho

*Corresponding author: Lorenzo Maggioni (l.maggioni@cgiar.org)

Received: 11.03.2024 | Accepted: 25.06.2024 | Published online: 29.07.2024

et al, 2018). Recently, resistance to *Xanthomonas campestris* was identified in one accession of *B. montana* collected in 2005 by L. Maggioni on the island of Ponza, Italy, differently from what was reported in Sheng *et al* (2020) (F. Branca, pers. comm.). Various types of insect resistance were studied in *B. villosa*, *B. incana* and *B. montana* (Palaniswamy and Bodnaryk, 1994; Ellis *et al*, 1999; Pelgrom *et al*, 2015; Vosman *et al*, 2015). Furthermore, Zhang *et al* (2016) carried out a comparative transcriptome analysis to evaluate the resistance against *Plasmodiophora brassicae* in broccoli and *B. macrocarpa*. Their analysis revealed the activation of different metabolic pathways in *B. macrocarpa*, which enabled it to overcome the pathogen infection. The opportunity to breed for higher content of potentially beneficial (antioxidant and anti-cancer) glucosinolates has been studied (Picchi *et al*, 2020; Arena *et al*, 2022; Bianchi *et al*, 2024) and used for the development of the 'Beneforte' broccoli, resulting from a cross with *B. villosa* from Sicily (Mithen, 2014). The wide genetic variability expressed by *Brassica* wild relatives has also facilitated the detection of distinct allelic patterns associated with morphometric traits in wild relatives compared to their cultivated counterparts (Treccarichi *et al*, 2023).

The importance of wild relatives of *B. oleracea* and the high priority for their seed conservation were highlighted as far back as 1981 in a plan of action report prepared by international experts for the International Board for Plant Genetic Resources (IBPGR), with the title *Genetic Resources of Cruciferous Crops* (IBPGR, 1980). A plan of action was subsequently accepted and supported by IBPGR as the 'Germplasm Conservation of Wild (n = 9) Mediterranean Brassicas Project' (Gustafsson, 1982; Gustafsson *et al*, 1985). Six collecting missions were funded between 1982 and 1988 targeting Cyprus, France, Greece, Italy, Spain, Tunisia, Turkey and the United Kingdom (Gómez-Campo *et al*, 1994). An agreement was signed in 1981 between IBPGR and the genebank of the Universidad Politécnica, Madrid (UPM), to hold a global collection of wild relatives of cruciferous crops, as part of the register of base collections, with agreed responsibilities for long-term conservation and distribution to bona fide users (Maggioni, 2010). Therefore, all samples were deposited at UPM, as well as safety-duplicated in the country of origin and other genebanks.

Despite these arrangements, a survey of the status of *Brassica* wild relative accessions (Maggioni *et al*, 2013) revealed that most species and localities were underrepresented in collections. The main geographical gaps were the Adriatic coasts (Albania, Croatia and Italy), the Aegean coasts (Greece and Turkey), northern Cyprus and the coastal cliffs of Tunisia. Moreover, the availability of accessions with a Standard Material Transfer Agreement (SMTA) was very limited, owing to the lack of sufficient seed, since brassicas are laborious and expensive to multiply. In other cases, there was reluctance to share wild brassicas, even though the entire *Brassica* complex is fully included in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009).

In particular, UPM became unable to guarantee sufficient multiplication and distribution of samples (Gonzalez-Benito, 2010). Similar difficulties were faced by other genebanks. Additionally, some geographic areas had not been covered by the international collecting missions, such as the Croatian coast, observations of new sites have constantly expanded the known distribution range of these wild relatives, and new species were proposed, such as *B. tyrrhena* Giotta, Piccitto & Arrigoni (Giotta *et al*, 2002) and *B. trichocarpa* C. Brullo, Brullo, Giusso & Ilardi (Brullo *et al*, 2013). The most comprehensive distribution map and list of observations of all the C genome wild *Brassica* species still remains the one prepared by Snogerup *et al* (1990), although it is today outdated and would require a thorough revision.

A small project titled 'Capturing *Brassica* Wild Relatives Diversity in Southeastern Europe (EUBRASWILD)', funded by the European Cooperative Programme for Plant Genetic Resources (ECPGR), was recently set up under the coordination of Smiljana Goreta Ban, Institute of Agriculture and Tourism, Poreč, Croatia, involving partners from seven countries of the south Balkan area and Italy. Among the objectives of this project, which was dedicated to monitor, collect and characterize various populations of crop wild relatives in the Brassicaceae family, there was also the exploration, collecting and multiplication of (2n = 18) wild *Brassica* populations. This paper describes the collecting missions carried out in Albania, Croatia and Italy, including background information, methodology and results, focusing on *B. cretica*, *B. drepanensis, B. incana, B. macrocarpa, B. montana, B. rupestris* and *B. villosa*. Knowledge about the distribution range and existing collections in Europe and their gaps for the above species is briefly summarized below. Taxonomy names and synonyms are according to the GRIN Taxonomy (Wiersema and Schori, 2022) and Euro+med (2006) for taxa not treated by GRIN.

Brassica cretica Lam.

[Synonym: *Brassica oleracea* L. subsp. *cretica* (Lam.) Gladis & K.Hammer]

The distribution range of this species covers mainly coastal areas of Albania, Greece, west Turkey, central and south Lebanon (possibly introduced) and Israel at Mount Carmel (also possibly introduced) (Snogerup *et al*, 1990; Barina *et al*, 2011; Flora Ionica Working Group, 2016). Collections documented in the European Search Catalogue for Plant Genetic Resources (EURISCO, http://eurisco.ecpgr.org), consist of 120 accessions, conserved in Greece (43), Spain (42), Albania (17), the United Kingdom (10), Germany (5), Israel (2) and Italy (1). These originate from Greece (85), Albania (17), Turkey (10), Israel (3) Lebanon (3) and unknown (2). Accessions from Albania have recently been added to the Albanian Genebank as a result of the EUBRASWILD activity, whereby a thorough exploration

has filled gaps in knowledge and material, as described in this paper. Other areas of Greece and Turkey are not well covered by the documented collections of this species.

Brassica drepanensis (Caruel) Damanti

[Synonym: *Brassica villosa* Biv. subsp. *drepanensis* (Caruel) Raimondo & Mazzola]

This taxon is endemic to northwest Sicily, Italy, and limited to four locations in the province of Trapani, with an 'endangered' status, according to the IUCN Red List of Threatened Species (Maggioni and Domina, 2020). Urbanization and fires are likely to threaten all known subpopulations of this species, especially below the cliffs of Mount Erice (Snogerup *et al*, 1990). Only thirteen accessions clearly referable to this taxon are documented in EURISCO. Four of these are conserved at IPK in Germany, four at UPM Madrid, Spain and one in Warwick, United Kingdom, all collected around the town of Erice. One sample is conserved at the University of Catania, Italy, received as a duplicate from IPK, Germany. Other three accessions, with undisclosed location of collecting, are conserved in Hungary (2) and the United Kingdom (1). Two accessions documented under the name *B. villosa* subsp. *drepanensis*, conserved at UPM, Spain, were collected outside the recognized distribution area of this taxon, which raises doubts about their correct identification. The population in Erice has been the most collected, which is justified by the fact that it is relatively small and probably the most threatened due to human activities. Other small populations are included in protected areas (Zingaro Nature Reserve and Monte Cofano, Capo San Vito e Monte Sparagio Special Protection Area) (Raimondo *et al*, 1991). However, they are not subject to active conservation and are not regularly monitored. Therefore, they would deserve to also be secured in a genebank. Although local collections of these populations may exist in Sicilian universities or botanic gardens, the Italian national inventory displayed in EURISCO is deficient as far as *B. drepanensis* is concerned.

Brassica incana Ten.

[Synonyms: *Brassica botteri* Vis.; *Brassica cazzae* Ginzb. & Teyber; *Brassica mollis* Vis., *Brassica taurica* (Tzvelev) Tzvelev]

This species is distributed in Tyrrhenian coastal areas of central and south Italy, northeast and east Sicily, Adriatic coastal localities in Puglia and Croatian islands, south Albanian coast and Greek Ionian islands (Snogerup *et al*, 1990; Baldini, 1995; Castellano and Bazan, 2009; Anzalone *et al*, 2010; Barina *et al*, 2011; Maggioni, 2015; Flora Ionica Working Group, 2016; Bartolucci *et al*, 2018). One population growing in Crimea, farther apart from the rest of the distribution range, has been considered as an introduced sample (Snogerup *et al*, 1990) and was interpreted as a feral lineage by Mabry *et al* (2021). Based on EURISCO, 53 accessions are conserved in European genebanks in Spain (28), the United Kingdom (11), Germany (7), Albania (5) and Italy (2). Overall, these samples originate from Italy (37, of which 17 from Campania, 16 from Sicily, 2 from Tuscany, 1 from Lazio and 1 from Puglia), Albania (5), Ukraine (3), Croatia (1) and unknown (7). This collection appears largely unrepresentative of the diversity and geographic distribution of this species, with several gaps from the Italian distribution area, as well as from Croatia and Greece, the latter two totally missing, except for one sample from Korčula, Croatia. Particularly poor is the collection maintained in Italy, which declares to conserve only two samples, even though the country hosts the widest number of existing populations within its territory.

Brassica macrocarpa Guss.

[Synonyms: *Brassica oleracea* L. subsp. *macrocarpa* (Guss.) Gladis & K. Hammer; *Eruca macrocarpa* (Guss.) Caruel]

This is an endemism of Egadi Islands, Sicily, which is present only on the two islands of Favignana and Marettimo, while it has no longer been found on the island of Levanzo (Maggioni *et al*, 1996). This species is classified as critically endangered in the IUCN Red List of Threatened Species, due to its very narrow extent of occurrence, area of occupancy and risk of decline due to the possible human disturbance of its habitat (Branca and Tribulato, 2011). According to EURISCO, 23 accessions are conserved in Germany (2), Spain (4) and the United Kingdom (17). Most of them were collected in Favignana and two of them are from Marettimo.

Brassica montana Pourr.

[Synonyms: *Brassica robertiana* J. Gay; *B. oleracea* L. subsp. *robertiana* (Gay) Rouy et Foucaud]

B. montana is distributed along the northern Mediterranean coasts of Spain (Gerona province), south France and Italy (Liguria and northern Tuscany) (Snogerup *et al*, 1990). It was formerly present in the Apennine mountains of Emilia Romagna, but its occurrence there could not be confirmed recently (Maggioni and Alessandrini, 2019). It reappears further east in the Republic of San Marino near the Italian Adriatic coast and then in Ancona and at Monte Conero (Maggioni and Alessandrini, 2019). On the Tyrrhenian side of Italy, it grows on the Pontine Islands (Anzalone *et al*, 2010). Punctual observations have been reported also further south in Campania, Basilicata and Calabria (Bartolucci *et al*, 2018), however, current presence and actual distribution in southern Italy would require a thorough investigation.

Collections documented in EURISCO with at least approximately known and reliable collecting locations comprise 57 accessions. These originate from Gerona province in Spain (16), south France (26) and Italy (15, of which 9 from Liguria, 4 from Tuscany and 2

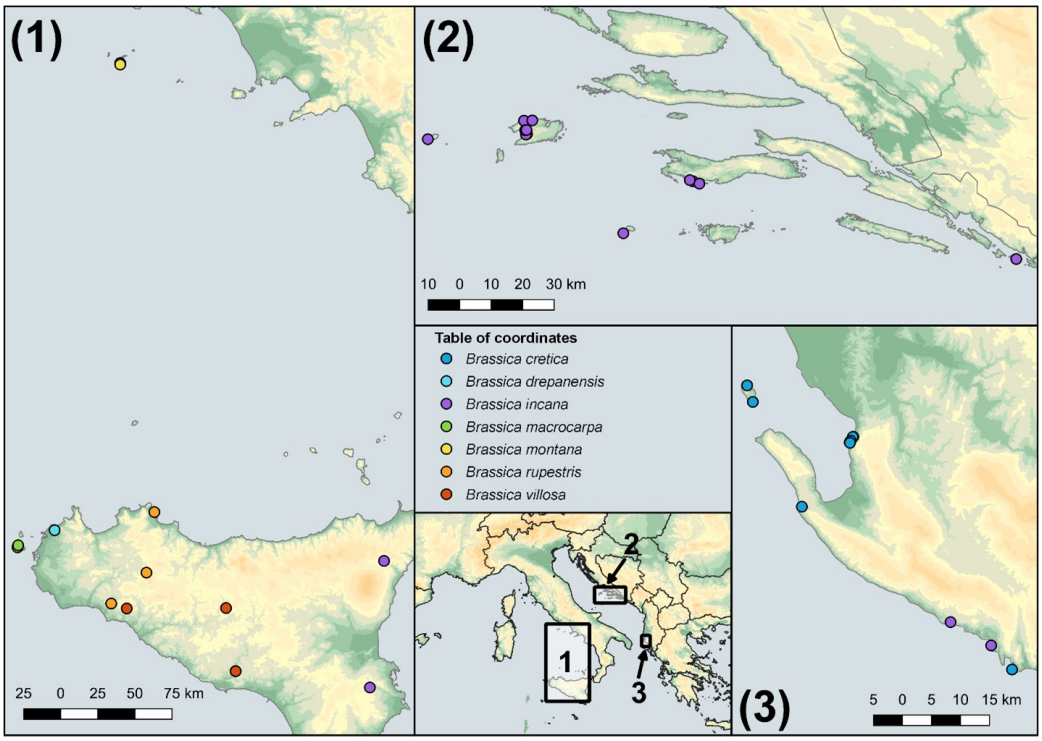

Figure 1. Collecting sites in Italy (1), Croatia (2) and Albania (3). Map created with QGIS v3.28.11-Firenze, (CC BY-SA 3.0)

from Marche regions). Gaps in *ex situ* collections are especially evident for central and south Italy.

The recent account that one sample from Ponza was highly resistant to *Xanthomonas campestris* (Sheng *et al*, 2020) raised increased interest and the need to secure the conservation of populations from the Pontine islands.

Brassica rupestris Raf.

[Synonym: *Brassica oleracea* L. subsp. *rupestris* (Raf.) Gladis & K. Hammer]

It is distributed mainly in north and west Sicily (Snogerup *et al*, 1990) and also found in south-eastern Calabria (Hammer *et al*, 1987). More recently, few sites have been identified also in eastern Sicily (Branca pers. comm.) and southwestern Calabria (Maggioni, pers. comm.). According to EURISCO, European genebanks only hold 22 accessions from western Sicily, conserved in Spain (13), Germany (4), the United Kingdom (3) and Italy (2). The genetic diversity of a few localized populations from the provinces of Palermo and Reggio Calabria has been studied extensively (Maggioni *et al*, 2014). Otherwise, a thorough analysis of the geographic distribution and diversity of this species is lacking and serious gaps exist in the *ex situ* European collections.

Brassica villosa Biv.

[Synonyms: *Brassica villosa* Biv. subsp. *tinei* (Lojac.) Raimondo & Mazzola; *Brassica villosa* Biv. subsp. *bivoniana* (Mazzola & Raimondo) Raimondo & Mazzola; *Brassica villosa* Biv. subsp. *villosa*]

B. villosa is endemic to the northwestern and central part of Sicily, in a few cases coastal, otherwise mostly found at inland cliffs localities (Snogerup *et al*, 1990). Various taxonomic treatments exist for this taxon, which is structured into various subspecies according to Malfa *et al* (2020), also including *B. drepanensis* as a subspecies of *B. villosa*. According to EURISCO, only 26 accessions of *B. villosa* are conserved in European genebanks, excluding those samples referring to *B. drepanensis*. These are conserved in the United Kingdom (11), Spain (7), Germany (5), Italy (1), the Netherlands (1) and Sweden (1). In some cases, the location of the collecting is not indicated, or it is placed in eastern Sicily, which makes the taxonomic attribution doubtful. Based on these data, the overall European *ex situ* collection of *B. villosa* is probably underrepresented. A thorough taxonomic revision including molecular markers and a better definition of the actual distribution range of *B. villosa* would also be beneficial and enable defining gaps in the existing collections.

Collecting missions – Methods and results

Collecting missions were carried out by project partner teams in Albania, Croatia and Italy (Lazio and Sicily). The composition of the teams, preparatory steps, itineraries and results are summarized below for each mission. All the collection sites are shown on a map of Europe in Figure 1. Information on collected accessions is summarized in Table 1.

Table 1. Summary of surveyed populations and collected accessions. Accessibility: A, The entire population or up to 80% of the plants are accessible; B, Between 20% and 80%; C, Less than 20% accessible and D, The entire population is inaccessible unless special equipment is used.

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
***Brassica cretica* Lam.**	ALB	Vlore, Tuneli, Depo ujit	Jul 2020	SJ;NH;LF019, SJ;NH;LF020, SJ;NH;LF021	40.421485; 19.488230	ALB026	AGB4451; AGB4452; AGB4453;	Seed	Small population; Accessibility: B
	ALB	Vlore, Uji Ftohtë, Moli ujit	Jul 2020	SJ;NH;LF022, SJ;NH;LF023	40.421293; 19.487295	ALB026	AGB4454; AGB4455	Seed	Small population; Accessibility: C
	ALB	Vlore/Bar-Restaurant Kala	Jul 2020	SJ;NH;LF024, SJ;NH;LF025, SJ;NH;LF026, SJ;NH;LF027	40.412923; 19.480489	ALB026	AGB4456; AGB4457; AGB4458; AGB4459	Seed	Few individuals; Accessibility: C
	ALB	Vlore/Resort Marina Bay	Jul 2020	SJ;NH;LF029, SJ;NH;LF030	40.415465; 19.482669	ALB026	AGB4461; AGB4462	Seed	Few individuals; Accessibility: B; endangered by constructions
	ALB	Radhimë	Jul 2020	SJ;NH;LF028	40.412221; 19.481160	ALB026	AGB4460	Seed	Few individuals; Accessibility: B
	ALB	Sazan Island – South side	Jul 2021	SJ;NH;LF039, SJ;NH;LF040, SJ;NH;LF041	40.480726; 19.286550	ALB026	AGB4471; AGB4472; AGB4473;	Seed	Large population; Accessibility: B
	ALB	Sazan Island – North side	Jul 2021	SJ;NH;LF042, SJ;NH;LF043	40.506662; 19.276134	ALB026	AGB4474; AGB4475;	Seed	Large population; Accessibility: B
	ALB	Alican – Karabuni	Jul 2021		40.315463; 19.379214			Not collected	Relatively large population; Accessibility: D
	ALB	Palermo Rock	Jul 2021		40.051141; 19.792803			Not collected	Small population; Accessibility: B
***Brassica drepanensis* (Caruel) Damanti**	ITA	Erice	25 Jul 2021		38.03505556; 12.59147222	ITA331	UNICT 5288	Seed	Abundant; Accessibility: B

Continued on next page

Table 1 continued

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
***Brassica incana* Ten.**	ALB	Himarë /Potam beach	Jul 2020	SJ;NH;LF032, SJ;NH;LF033, SJ;NH;LF034, SJ;NH;LF035, SJ;NH;LF036	40.089685; 19.752504 40.088730; 19.750805	ALB026	AGB4464; AGB4465; AGB4466; AGB4467; AGB4468	Seed	Relatively large population; Accessibility: A/B
	ALB	Gijpe			40.128361; 19.672164			Not collected	Small population; Accessibility: B
	HRV	Sušac	Jul 2022	IPT521	42.752716, 16.490663	HRV050	IPT521	Seed	<20 plants; Accessibility: B
	HRV	Kosor	Jul 2022	IPT518	42.901346, 16.761636	HRV050	IPT518	Seed	20 plants; Accessibility: A
	HRV	Stupa	Jul 2022	IPT522	42.894560; 16.786568	HRV050	IPT522	Seed	10 plants; Accessibility: A
	HRV	Obljak	Jul 2022	IPT520	42.904644, 16.749480	HRV050	IPT520	Seed	20 plants; Accessibility: A
	HRV	Koločep	Oct 2021	IPT517	42.668715, 18.014589	HRV050	IPT517	Seed	30 (–40) plants; Accessibility: B
	HRV	Svetac	May 2023	IPT 618	43.019725, 15.728069	HRV050	IPT 618	Seeds and small plants	20 plants; Accessibility: B
	HRV	Vis (Oključina)	May 2023	IPT 619	43.074649, 16.102745	HRV050	IPT 619	Seeds and small plants	15 plants; Accessibility: B
	HRV	Vis (Gradac)	May 2023	IPT 620	43.075623, 16.134977	HRV050	IPT 620	Seeds and small plants	10 plants; Accessibility: B
	HRV	Vis (St. Duh)	May 2023		43.036415, 16.114780			Not collected	3 plants; Accessibility: B
	HRV	Vis (hiking trail from St. Duh church to St. Nikola church)	May 2023	IPT 621	43.034960, 16.110665	HRV050	IPT 621	Seeds	5 plants; Accessibility: B
	HRV	Vis (Crvene stijene)	May 2023	IPT 622	43.047342, 16.107393	HRV050	IPT 622	Seeds and small plants	20–30 plants; Accessibility: A

Continued on next page

Table 1 continued

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
***Brassica incana* Ten.**	HRV	Vis (St. Mihovil church)	May 2023	IPT 623	43.048160, 16.112421	HRV050	IPT 623	Seeds	5 plants; Accessibility: B
	ITA	Sortino	9 June 2022		37.133350, 15.031676	ITA331	UNICT 5310	Shoots for cutting	Very few individuals; Accessibility: C
	ITA	Francavilla	20 Jul 2021		37.90494444; 15.12491667	ITA331	UNICT 5311	Seed, shoots for cutting and small plants	Abundant; Accessibility: C
***Brassica juncea* (L.) Czern.**	ALB	Himarë	June 2020	SJ;NH;LF037, SJ;NH;LF038	40.101337; 19.741662	ALB026	AGB4469; AGB4470	Seed	
***Brassica macrocarpa* Guss.**	ITA	Favignana	25 Jul 2021		37.92108333; 12.31330556	ITA331	UNICT 5289	Seed	Abundant; Accessibility: C
	ITA	Favignana	25 Jul 2021		37.93400000; 12.31694444	ITA331	UNICT 5308	Seed	Abundant; Accessibility: C
	ITA	Marettimo	25 Jul 2021		37.94880556; 12.08336111	ITA331	UNICT 5309	Not collected	Very few individuals; Accessibility: D
***Brassica montana* Pourr.**	ITA	Ponza	June 2021	LM21-01; LM21-02	40.888889; 12.953056	ITA331 and Orto Botanico Roma	UNICT 2306 UNICT 5316	Seed	Very few individuals; Accessibility: A; endangered by landslides
	ITA	Ponza	June 2021		40.879751; 12.953690			Not collected	Very few individuals; Accessibility: D

Continued on next page

Table 1 continued

Taxon	FAO Country Code	Locality	Collecting date	Collecting numbers	GPS coordinates	Receiving Genebank (FAO Code)	Genebank accession numbers	Collected material	Status of the population
***Brassica rupestris* Raf.**	ITA	Corleone	25 May 2021		37.798463, 13.307139	ITA331	UNICT 5383 UNICT 5326 UNICT 5327 UNICT 5328	Seed	Abundant; Accessibility: B
	ITA	Palermo	25 May 2021		38.16830556; 13.35211111	ITA331	UNICT 5290	Seed	Abundant; Accessibility: B
***Brassica rupestris* subsp. *tardarae* (Ilardi & al.) Raimondo**	ITA	Sambuca di Sicilia	25 May 2021		37.615472; 13.053333	ITA331	UNICT 5286	Seed	Less than 100 individuals; Accessibility: C
***Brassica villosa* Biv.**	ITA	Caltabellotta	25 May 2021		37.57669444; 13.16616667	ITA331	UNICT from 5329 to 5335	Seed	Few individuals; Accessibility: C
***Brassica villosa* subsp. *tineoi* (Lojac.) Raimondo & Mazzola**	ITA	Butera	23 Mar 2022		37.21597222; 14.00886111	ITA331	UNICT 5291	Shoots for cuttings	Abundant; Accessibility: C
	ITA	Marianopoli	23 Mar 2022		37.597965; 13.923801	ITA331	UNICT 5292	Shoots for cuttings	Very few individuals; Accessibility: B
	ITA	Butera	20 Oct 2022		37.21577778; 14.01016667	ITA331	UNICT 5312	Seed	Endangered; Accessibility: C

Albania

Under the leadership of Sokrat Jani, the Institute of Plant Genetic Resources, Agricultural University of Tirana, organized a mission targeting the exploration, identification and collection of seed samples of wild relatives of *Brassica* (*B. cretica* Lam. and *B. incana* Ten.) in Ionian coast area and Sazan Island.

The collecting team was composed of Sokrat Jani, Institute of Plant Genetic Resources, Tirana, Nexhip Hysolakoj, Regional Agency of Protected Areas of Vlora and Lavdosh Ferruni, Organic Agriculture Association of Albania.

The travel target areas were the Ionian coast of Vlora and Himarë, and Sazan Island, a small uninhabited island with an area of 5.7km^2 and a coastline of about 21km, at the Gulf of Vlora. During the years 2019–2021, nine field missions were undertaken, three each year respectively around 2–20 April, which coincides with the blooming phase; 10–20 May, which coincides with the fruiting period and 20 June–10 July, which coincides with the seed ripening period. To reach Sazan Island, the team used the boat of the Regional Agency of Protected Areas of Vlora. There are no regular roads for driving on the island, therefore the group explored the area on foot.

Information available about the sites to visit was based on literature, herbarium specimens and personal knowledge. The first documentation about the presence of two maritime *Brassica* species in the Ionian coastal region of Albania was published in Flora of Albania for two localities, Spile (Himarë) for *B. incana*, and Vlora for *B. oleracea* subsp. *oleracea* (Paparisto and Qosja, 1976). A brief description of *B. incana* at Himarë is given in Flore de l'Albanie (Qosja *et al*, 1996). Based on the material collected during their field trips to Albania from 2008 to 2010, Barina *et al* (2011) presented some confirmations for chasmophytic *Brassica* species in three localities, of which two are the same as those of Paparisto and Qosja (1976). In Spile Bay (Potam beach) at Himarë, they confirmed the occurrence of *B. incana*, while *B. cretica* was observed near Vlora, about 4km north of the village of Radhimë, and in the Porto Palermo Peninsula. Subsequently, during a field trip on 4 April 2019 a group consisting of Lorenzo Maggioni (ECPGR Secretariat), Eva Thörn (ECPGR Executive Committee), Imke Thormann (Federal Office of Agriculture and Food, Germany), Fetah Elezi and Sokrat Jani from Agricultural University of Tirana, surveyed the presence of a small population of 12 reproductive plants and some vegetative individuals of *B. cretica* in the front part of the cliff, on both sides of the coast road tunnel at Uje i Ftohtë, as well as a small population on the southwest side of the coast, near Bar Kalaja. Further south, by the Potam beach (Himarë), at the base of the cliff, on its northwestern side, a medium-sized population of about 30 reproductive plants and some vegetative individuals of *B. incana* was surveyed by the same group. Both species were then in the flowering phase.

These expeditions aimed at gathering information, exploring more localities for possible other population sites, finding and identifying species, assessing the situation, evaluating vegetation status and habitats, and collecting seed material for conservation and use.

Samples were identified in the field, based on morphological characters. During the ripening period, seed samples were collected from most populations. This was done according to a collection protocol trying to capture the maximum variability of the population (from as many plants as possible), while avoiding harm to the populations themselves (harvesting only a small part of the seeds produced by any plant). Mature siliquas were collected from individual plants and kept separately in cotton bags. The size of the populations was estimated, distinguishing vegetative and reproductive individuals, as well as human and livestock accessibility. GPS coordinates were taken with a GPSMAP 60CSx. After return from the field, the collected seed samples, after being processed, were included in the Albanian Genebank, Tirana, for long-term storage. As the terrain was difficult, the working group was supported with the necessary tools and equipment, such as cars, boats, drones, professional cameras, etc., by the Regional Agency of Protected Areas, Vlora.

Results

Out of 23 sites visited with a suitable habitat, wild brassicas were observed in 13 of them. The results obtained from the exploration are described below.

Ionian coast area of Vlora

The presence of *B. cretica* was observed in five sites in the Vlora Bay area (two sites near the tunnel area in Uje i Ftohtë, and respectively one each in Marina Bay Resort, Bar-Restaurant-Kala, and Radhimë, part of Kala road) and one on the Karaburun peninsula (in the western part, from Alicani to Bristani Bay):

Tunnel area of Uje i Ftohtë, in front of the cliff, northwest side: Two populations of *B. cretica* were identified there, respectively to the left and right of the tunnel entrance, called Depo ujit and Moli ujit, with 12 and eight reproductive plants, respectively, and very few vegetative individuals (new plants). These sites were respectively rated average and slightly accessible by humans and livestock. Seed was collected from three and two plants, respectively, separately in cotton bags.

Bar-Restaurant Kala area, south side: the site was first surveyed in 2019, and it was surprising in 2020 to note the disappearance of a small population of *B. cretica* from every accessible site along the base of the cliff, due to mowing of the area by resort gardeners. Not a single plant remained on the cliff, except at the bottom of it, in the inaccessible part, where only ten reproductive plants and very few young plants could be counted. Seeds were collected in 2020 from four plants, separately in cotton bags, with the help of two young climbers.

Radhimë, part of Kala road, west side: It seemed to be a newly established population, with 18 small reproductive plants and very few mature siliquas and

many new plants, possibly formed by migrations from a now extinct *B. cretica* population of Jonufra (4km north of Radhimë), which was an old population documented by Paparisto and Qosja (1976) and later confirmed by Barina *et al* (2011). Seed was collected from only one plant.

Resort Marina Bay area, north side: At this site, two populations of *B. cretica* were located very close to each other in the same area and only 200–300m away from each other (Marina Bay and Resort Gjyla), with overall 12 reproductive plants and very few young plants; these were moderately accessible to humans and endangered of disappearance due to constructions in the tourist resort. Seed was collected from the two matured plants.

Karaburuni peninsula, west side/Alican – Brisani Bay: A site was explored with an area of 4,678m^2 and a relatively large population of *B. cretica*, with a density of 1–2 reproductive plants/100m^2. The first individual plants were identified in the Alican site, on vertical rocks, an area isolated and inaccessible to humans or livestock. Its extent, with few individual plants, was linear to the coast of the entire Gulf of Brisan. No seeds were collected, as the entire population was located on steep cliff tops and therefore inaccessible unless special equipment is used.

Skele neighbourhood of Vlora: In addition to the target species (*B. cretica* and *B. incana*), a small population of eight to ten plants initially identified as *B. rupestris* Raf. was found in a home garden in the coastal neighbourhood Skele of the city of Vlora. From the conversation with the owner of the home garden, it was learned that that population may have originated from a maritime limestone rock area about 7–8km away, called Kuzumbaba, where he had taken soil five years before to use for the home garden; but with the soil, it seems, the seeds also came. However, following a survey of the described area by our team, no plants of *B. rupestris* could be found. Seed was collected from home garden plants and sent to the Albanian Genebank, Tirana. After closer observation, these plants more probably belong to a domesticated type of leafy kale.

Ionian coast area of Himarë

Three sites were found with a good presence of *B. incana*, respectively in Potam, Gjipe bay and possibly *B. cretica* in the Porto Palermo peninsula.

Potam beach: In the southern part of the bay of Spile (Himarë), in a rocky strip with a length of about 2–3km, on maritime limestone rocks, the presence of a relatively large population of *B. incana* was reconfirmed, linear to the coast, with 28 reproductive plants and many new vegetative individuals, which was previously observed and documented by Paparisto and Qosja (1976) and later confirmed by Barina *et al* (2011); every part of it was accessible along the base of the cliff. Seed was collected from five plants.

Himarë Seaport, south side: Besides the two target species (*B. cretica* and *B. incana*), in a home garden, near the Himarë Seaport, a small population of four plants of *B. juncea* (L.) Czern, apparently spontaneously grown was explored. Seed was collected from two plants. Unfortunately, our monitoring in the spring of 2022 revealed that this population had disappeared.

Other localities: other parts of the coast of Himarë were surveyed, north and south of the bay of Spile (Himarë), but new *Brassica* sites were found only in the Gjipe bay (near Gjipe canyon), about 15km north of Himarë and on the peninsula of Porto Palermo, about 9km south of Himarë, at a site known as The Rock of Porto Palermo. It was surprising to notice the disappearance of the *B. cretica* population in the Cave site of Porto Palermo, which was described in 2010 by Barina *et al* (2011). However, a new and smaller population was found, about 1km northwest, in a segment with straight and steep rocks, in the place known as The Rock of Porto Palermo. It seems that livestock may have influenced the migration of *Brassica* vegetation from one site to the other. We estimated that in Gjipe bay and The Rock of Porto Palermo, near the Falling Rocks Beach, 18 and 30 reproductive plants of *B. incana* and *B. cretica* (to be verified) were growing, and some vegetative individuals, but it was not possible to collect seeds from either site.

Sazan Island area, east and northeast side

The large presence of *B. cretica* on the island was surprising, mainly concentrated in two sites, the South Cape and the North Cape. The largest area with more reproductive plants and young plants was located in the south, in the Gully of Jehnem site, where the density was 30–40 reproductive plants/100m^2. Seeds were collected from the two sites, respectively from three and two plants.

Croatia

Under the leadership of Nenad Jasprica, Institute for Marine and Coastal Research, University of Dubrovnik, and Smiljana Goreta Ban, Institute of Agriculture and Tourism (IPTPO), Poreč, two expedition missions were organized, targeting several wild *Brassica* species in the southern Croatian islands and islets. The goals of the expeditions were to check the status of the *Brassica* species populations, to collect seeds or young plants from previously known sites and to estimate the possible losses that happened during previous periods.

Apart from N. Jasprica and S. G. Ban, the collecting team also consisted of Nina Išić and Dean Ban from the Institute of Agriculture and Tourism, Poreč, Branka Salopek Sondi from the Ruer Bošković Institute, Zagreb, and Mirta Tkalec and Nataša Bauer from the Faculty of Science, University of Zagreb.

Preliminary information on the distribution, habitat, ecology and phenology of insular brassicas in Croatia was obtained from the Flora Croatica Database (Nikolić (2005) and onwards). This database contained data from the literature, field observations and references to herbarium specimens, and allowed further detailed study with the aim of completing knowledge on the subject.

The first expedition started on 5 July 2022, travelling to Split and taking the ferry to Vela Luka located on the Korčula Island. The exploration of the island's localities took place over two days, 6-7 July 2022. On 6 July, the team visited the islands Sušac (Lastovo Islands Nature Park), and islets Obljak, Kosor and Stupa (Korčula Archipelago) by speedboat, and on 7 July the collecting team visited two previously targeted localities (the pebble beach of Vaja and the Bay of Samograd) near the Račišće village on the northern coastline of Korčula Island. In addition, the island of Koločep near the city of Dubrovnik was also previously visited on 7 July 2020, 1 October 2021 as well as on 15 July 2022. During the first expedition, mature siliques were collected from four localities: the island of Sušac, and the islets of Obljak, Kosor and Stupa. GPS coordinates were determined with an Android device. Seeds from one plant were collected on Sušac Island, while from each of the other three islands, seeds were collected from approximately ten plants. Siliquas were kept in paper bags. The seeds were counted with Contador optical seed counter and weighted.

The second expedition took place between 22 and 26 May 2023. The expedition included localities previously mentioned in the literature on the island of Vis and the island of Svetac (locally named St. Andrija) (Nikolić (2005) and onwards). The exploration of the two islands took place over two days, 23-24 May 2023. On 23 May, the collecting team visited the Svetac Island by speedboat, followed by several localities on the Vis Island, mainly north-facing cliffs and small coves. On 24 May, the third day of the expedition, the team visited previously mentioned inland localities of Vis, the first one being beneath St. Duh church. Next, the team followed a hiking trail from St. Duh church to St. Nikola church towards Komiža city where the presence of a *B. incana* population was previously recorded. The team then travelled to a location known as Crvene stijene (red rocks), a sport climbing area, where an abundant *B. incana* population was found. The next location was near St. Mihovil church, while the presence of a military zone prohibited the members from visiting the location above Podhumlje. The expedition members spent the fourth day travelling home. GPS coordinates were determined with an Android device. During the second expedition, unripe green siliquas were collected from six localities: the island of Svetac, two coastal localities on the island of Vis (Oključina and Gradac) and three inland localities on the island of Vis (hiking trail near St. Duh church, Crvene stijene and St. Mihovil). Young plants for vegetative propagation were collected from the island of Svetac, two coastal localities on the island of Vis (Oključina and Gradac) and one inland locality on the island of Vis (Crvene stijene). Siliquas were kept in paper bags.

Results

Koločep Island

The population of *B. incana* (locally named *B. mollis*) was quite numerous on the island (more than 50 individuals). It mainly inhabited the vertical sea cliffs oriented towards SW-SE, reaching up to 60m a.s.l. On the vertical profile of the cliffs, it colonized the halotolerant vegetation of the saline areas up to the bushy vegetation and Aleppo pine near the tops of the cliffs.

Sušac Island, southwest side

The population of *B. incana* (locally named *B. cazzae*) was reduced to a single reproductive plant seen by the expedition team near the Sušac lighthouse. Since the plant was on an inaccessible cliff, no seed was collected from this plant. Vegetative individuals were found on the island in the halophilous vegetation of the salt-sprayed rocky cliffs at 90–110m a.s.l., but also in the maquis on the cliff top.

Sušac Island, northwest side

One reproductive plant and several young ones were found near the trail leading to the Sušac lighthouse. Mature siliquas were collected in a paper bag and one entire plant was taken for vegetative propagation.

Obljak, Kosor and Stupa islets

On each of these three islets, the team found more than 20 individuals of *B. incana*. The mature siliquas of about ten plants from each islet were collected in paper bags. On Obljak and Kosor, the plants were found on the entire surface of the islets. Most of the *Brassica* population on Stupa was on the northern slope of the islet. The species occured mainly in the herbaceous vegetation of the salt-sprayed low rocky coasts, but also in the maquis in the upper belt, which is only weakly influenced by the sea aerosol.

Two small bays near the village of Račišće, Korčula Island

The team found no individuals of *B. incana* at these two previously known sites, a result of habitat loss due to anthropogenic impacts. These sites consist of gravel shorelines exposed to wave action, resulting in a thicker or shallower layer of pebbles at the surface. Nitrogen-rich humus often forms beneath the surface.

Svetac Island

The *B. incana* population on the west coast of Svetac Island consisted of about 20 plants, a good portion of them in reproductive stage. The team collected unripe, green siliquas from three mature plants and two young plants. The siliquas were collected in paper bags.

Vis Island

In the locality Oključina the team found about 15 plants, several of them in reproductive stage. The team collected unripe, green siliquas from three plants and took two young plants.

In Gradac cove the team found around ten *B. incana* plants and one plant morphologically similar but with smooth, hairless leaves and stems. Several *B. incana* plants were in the reproductive stage and siliquas were taken from two plants along with two entire young plants. Seeds were taken also from the morphologically similar species, and one young plant was collected just beneath the reproductive one.

In the inland location of St. Duh the team only found three young *B. incana* plants in vegetative stage. Due to the lack of siliquas and a very small number of individuals, no siliquas or plants were taken.

On the hiking trail from St. Duh church to St. Nikola church in Komiža, the team found five *B. incana* plants. Several plants were in reproductive stage, so the team collected a small portion of unripe green siliquas from two plants. Because it was only a small population, no young plants were taken.

The locality Crvene stijene hosted the most abundant population in the Vis Island, counting more than 20 *B. incana* plants, several of them in reproductive stage. The team collected a small portion of green siliquas from five plants and two young plants.

Around five plants were found near St. Mihovil church with only two plants in reproductive stage. The team collected a small portion of green siliquas from the two plants and no young plants were collected.

Italy

Island of Ponza, Lazio, Italy

Lorenzo Maggioni, ECPGR Secretariat, Rome, carried a mission to the island of Ponza on behalf of the University of Catania (UNICT), targeting *B. montana*. Travelling from Rome to Formia and then taking the ferry to Ponza on 13 June 2021, the exploration of the island took place over two days, 14 and 15 June 2021 by foot and using the public bus. Selected dates were suitable to find mature siliquas, based on previous experience. Information available about the sites to visit was based on literature, herbarium specimens and personal knowledge. In their map of vegetation series of Ponza, Stanisci *et al* (2005) indicated the presence of *B. montana* as a companion species at localities Monte Guardia and Faro. Anzalone *et al* (2010) indicate the presence of *B. montana* in the Pontine Islands. Herbarium specimens in the Anzalone Herbarium in Rome [RO-HA] were collected from localities Monte Guardia in 1966 and Forna in 1974. During a previous survey in March 2004, from the trail midway up to the top of Monte Guardia, northwest side, Maggioni could count more than 200 flowering plants in a gully facing the famous cliff and beach of Chiaia di Luna. This gully was subsequently subject to a rock collapse which probably destroyed a large part of this population and the trail became almost inaccessible. Other individual plants could also be observed on formerly cultivated abandoned terraces, closer to the top of the mountain. In July 2005, at the southwest side of Monte Guardia, Maggioni surveyed the presence of a relatively large population at the base of the cliff, all along the trail reaching the lighthouse at Punta della Guardia.

The objective of the present survey was to collect seeds from sites already known and explore the rest of the island for possible other population sites. Mature siliquas were collected from individual plants and kept in separate paper bags. The size of the population was estimated, distinguishing vegetative and reproductive individuals. GPS coordinates were taken with a cellular phone. After return from the field, siliquas were manually threshed in paper trays and the number of seeds was approximately counted (data not shown).

Results

Monte Guardia, northwest side: it was confirmed that the relatively large population observed in 2004 had almost disappeared and only two reproductive plants and very few vegetative individuals could be found on or along the trail. Seed was collected from the two plants [collecting numbers LM21-01 and LM21-02]. Paper envelopes containing respectively ca. 200 and 100 seeds were sent to UNICT. One envelope with ca. 50 seeds of LM21-01 was also sent to the Botanic Garden of the University La Sapienza in Rome, Italy (care of Giuseppe Fabrini).

Monte Guardia southeast side: it was surprising to note the disappearance of *B. montana* from any accessible site along the base of the cliff. No plants could be identified on the cliff either, except at the very end of the trail, on the small promontory Punta della Guardia, hosting the lighthouse. Here, less than ten vegetative plants could be observed in inaccessible positions. In recent years, this area has been subject to landslides that have disturbed the base of the cliff. Also, the abundant presence of *Dittrichia viscosa* (L.) Greuter, widely colonizing the area, might have become a competitor to *B. montana*.

Other localities: other parts of the island were surveyed (descent to Punta del Fieno; Piana d'Incenso up to Punta Incenso; Fortino del Papa and surroundings; and descent to Scogli della Cantina) but no new sites were found. The locality Forna was not thoroughly surveyed and might deserve a more careful check, considering that one herbarium sample was collected at this locality in 1974 by Anzalone, but with no detailed site indications.

Sicily

Under the leadership of Ferdinando Branca, UNICT, Italy, organized three missions targeting several wild *Brassica* species growing in Sicily. The UNICT team, represented also by the PhD students Maria Concetta Di Bella, Simone Treccarichi, Donata Arena and Giulio Flavio Rizzo, collected several samples in natural areas during three expeditions, also evaluating the status of the populations. In total, six expeditions were carried out, travelling by car from Catania, between May 2021 and October 2022, targeting the provinces of Palermo and Agrigento (I), Erice and Egadi Islands (II), Butera and Marianopoli, province of Caltanissetta (III), Sortino, province of Siracusa (IV), Francavilla di Sicilia, province of Messina (V) and Butera and mount Muculufa, province of Caltanissetta (VI).

The aim of the expeditions was to increase the availability of seeds for the biomorphological, biochemical and genetic characterization of these populations which were not available in genebanks. Based on previous personal knowledge and local information, the team

explored the above-mentioned sites to observe the status of the populations (distribution and estimated number of plants) and seed samples were collected based on the biological status of the plants. Information regarding the collecting sites was obtained through Google Earth and by interviews with local residents.

The collecting methodology encompassed either harvesting of siliquas from individual plants, which were kept separate in the case of *B. rupestris* of Corleone and *B. villosa* of Caltabellotta, or bulked from several plants for all other populations for which seeds were collected. Additionally, vegetative cuttings were also collected. Siliqua samples were stored in paper bags to avoid mould formation. Subsequently, siliquas were cleaned at the University of Catania, specifically in the Lab of Biotechnology of Vegetable Crops belonging to Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A). As concerns the cutting method, it consisted of oblique cutting carried out with professional pruning shears. Fresh material was stored in plastic bags to maintain high moisture conditions.

Results

The first expedition (I) was carried out at the end of May 2021 and five different accessions were collected: two accessions of *B. rupestris* from Corleone and Monte Pellegrino (Palermo province), one *B. rupestris* subsp. *tardarae* (Ilardi & al.) Raimondo at Gole della Tardara, near Sambuca di Sicilia (Agrigento province), one accession of *B. villosa* from Caltabellotta (Agrigento province). The first site is located south of Corleone on the Rocca cliffs, where an abundant population of *B. rupestris* is widespread. With regard to Mt. Pellegrino, an important population of *B. rupestris* was found around the cliffs of the Monastery of Santa Rosalia in Palermo, which is the type site for *B. rupestris*. The population of the subsp. *tardarae* was not abundant or much diversified in terms of plant age, whereas the population of *B. villosa* identified around Pizzo Telegrafo and the surrounding areas near Caltabellotta was abundant.

During the second expedition (II) at the end of July 2021 in the province of Trapani, three different accessions were collected. Two accessions of *B. macrocarpa* were collected on the islands of Favignana, in two different stations. Of these two populations, the more abundant was located on Monte Santa Caterina, spreading from the top of the mountain down to the sea coast. On the other hand, on the island of Marettimo, a few individuals were noted in cliffs that were not well accessible around Punta Bassana. Another population in Marettimo was located on the top of Monte Falcone, but this was not visited. Finally, one accession of *B. drepanensis* was collected on Mount Erice.

The third expedition (III) was carried out in March 2022 at Mount Muculufa, near Butera and Marianopoli, Caltanissetta province, monitoring two populations of *B. villosa* subsp. *tineoi* (Lojac.) Raimondo & Mazzola and collecting shoots for establishing rooted cuttings. Of the two identified populations, the former was abundant whereas the latter was represented by few individuals.

The fourth expedition (IV) was organized at Sortino, Siracusa province, in June 2022, monitoring the populations of *B. incana* and collecting shoots for establishing rooted cuttings. The fifth expedition (V) was organized at Francavilla di Sicilia, Messina province, in July 2022, collecting *B. incana* seeds and cuttings for rooting. Finally, the sixth and last expedition (VI) was organized near Ravanusa exploring Monte Muculufa, Caltanissetta province, in October 2022, where a population of *B. villosa* was found in a chalk quarry, endangered by human activities (caves for salt extraction) and fires.

Conclusion

Although the distribution of the wild populations belonging to the gene pool 2n = 18 of *B. oleracea* has been intensely investigated, starting in the 1970s, a full overview of all the existing populations is far from complete. Even for well-known populations, major gaps remain in the available documentation of the European genebanks and access to documented populations is often uncertain or not guaranteed, owing to limited seed availability, difficult and expensive multiplication or other limitations. The ECPGR-funded EUBRASWILD project enabled collecting activities focused on some of the less documented locations within the distribution range of these wild brassicas. Each mission was carried out by local teams within their country, with the intention to eventually make both information and genetic material publicly available. As indicated in Table 1, all accessions have been deposited in the respective genebanks with an assigned accession number. Requests from potential users can be immediately honoured in case of sufficient seed availability. The missions carried out in Albania were for the first time specifically dedicated to these taxa and allowed to describe with a good degree of accuracy several populations of *B. incana* and *B. cretica*, including some locations that had never been described before (*B. cretica* in the Sazan Island and Karaburun peninsula and *B. incana* in the Gjipe bay). In other cases, habitat loss due to direct or indirect human intervention is affecting the size or the existence of some populations. For the first time, seed samples were collected for long-term conservation, deposited in the Albanian Genebank and publicly documented in the EURISCO catalogue. The Croatian missions were successful in monitoring already known populations of *B. incana* and collecting seed for long-term conservation and further characterization. The possible disappearance of two populations on the island of Korčula due to habitat loss was an unpleasant observation. The collected populations will be documented in the EURISCO catalogue.

The expeditions carried out in Italy also enabled monitoring of existing populations and discovering previously unknown sites, such as the case of *B. villosa* subsp. *tineoi* near Butera and to monitor the populations of the already-known sites. A few sites were observed, where the populations are endangered by human

activities. On the island of Ponza, it was significant to notice the severe reduction, close to disappearance, of *B. montana*, possibly due to a change of habitat determined by natural causes. The few seeds collected may deserve careful attention if it is confirmed that resistance to *Xanthomonas* has been found in a plant from Ponza. The very poor documentation in EURISCO of the populations conserved in Italy is improving as a result of this activity.

Overall, the activities carried out in this project have added important pieces of information on the status and trends of a few Mediterranean wild *Brassica* populations, have increased the level of collaboration across countries and obtained material which will be useful for further collaboration regarding its characterization and possible use. Given the importance and immense diversity potential of the gene pool of *B. oleracea*, which is a unique resource of the European and Mediterranean region, it is hoped that more systematic investigations with larger breadth and funding can be planned in the future (including to sort out a complicated taxonomy which is currently only based on very unstable and unreliable morphological characters). Gathered information should also be useful to raise the awareness of national and local authorities about the need to take action for *in situ* conservation of the most endangered populations.

Acknowledgements

Collecting expeditions to Sicily and Croatia were partly funded through the ECPGR Grant Scheme Activity EUBRASWILD. Collection in Ponza was funded by the ECPGR Secretariat travel funds. The Albanian missions for exploration and collecting seed samples in the Ionian coast of Vlora and Sazani Island were financed by OAA (Organic Agriculture Association). Expeditions in Croatia were partly financed by the project 'Agrobiodiversity – the basis for adapting and mitigating the consequences of climate change in agriculture (KK.05.1.1.02.0005)'. The authors wish to thank Filip Varga for the creation of the maps.

Author contributions

FB, LM, SGB and SJ contributed to the overall conception and planning of the collecting missions. These were carried out by SJ and colleagues in Albania, SGB, NJ, NI and colleagues in Croatia, LM in Italy (Ponza), FB, ST and colleagues in Italy (Sicily). The first draft of the manuscript was written by LM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

The authors have no conflicts of interest to report.

References

Anzalone, B., Iberite, M., and Lattanzi, E. (2010). La flora vascolare del Lazio. *Informatore Botanico Italiano* 42, 187–317.

Arena, D., Treccarichi, S., Bella, M. C. D., Achkar, N., Ammar, H. B., Picchi, V., Scalzo, R. L., Amari, M., and Branca, F. (2022). Evaluation of *Brassica oleracea* L. crops and wild relatives for bio-morphometric and biochemical characteristics. *Acta Hortic* 1355, 71–80. doi: https://doi.org/10.17660/ActaHortic.2022.1355.10

Baldini, R. M. (1995). Flora vascolare del Monte Argentario (Arcipelago Toscano). *Webbia* 50(1), 67–191.

Barina, Z., Pifkó, D., and Mesterházy, A. (2011). Contributions to the flora of Albania, 3. *Willdenowia* 41, 329–339. doi: http://dx.doi.org/10.3372/wi.41.41214

Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A., Ardenghi, N. M. G., Astuti, G., Bacchetta, G., Ballelli, S., Banfi, E., Barberis, G., Bernardo, L., Bouvet, D., Bovio, M., Cecchi, L., Pietro, R. D., Domina, G., Fascetti, S., Fenu, G., Festi, F., Foggi, B., Gallo, L., Gottschlich, G., Gubellini, L., Iamonico, D., Iberite, M., Jiménez-Mejías, P., Lattanzi, E., Marchetti, D., Martinetto, E., Masin, R. R., Medagli, P., Passalacqua, N. G., Peccenini, S., Pennesi, R., Pierini, B., Poldini, L., Prosser, F., Raimondo, F. M., Roma-Marzio, F., Rosati, L., Santangelo, A., Scoppola, A., Scortegagna, S., Selvaggi, A., Selvi, F., Soldano, A., Stinca, A., Wagensommer, R. P., Wilhalm, T., and Conti, F. (2018). An updated checklist of the vascular flora native to Italy. *Plant Biosystems* 152(2), 179–303. doi: https://doi.org/10.1080/11263504.2017.1419996

Bianchi, G., Picchi, V., Tava, A., Doria, F., Walley, P. G., Dever, L., Bella, M. C. D., Arena, D., Ammar, H. B., Scalzo, R. L., and Branca, F. (2024). Insights into the phytochemical composition of selected genotypes of organic kale (*Brassica oleracea* var. *acephala*). *Journal of Food Composition and Analysis* 125. doi: https://doi.org/10.1016/j.jfca.2023.105721

Bothmer, R., Gustafsson, M., and Snogerup, S. (1995). Brassica sect. *Brassica* (Brassicaceae) II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. *Genetic Resources and Crop Evolution* 42, 165–178. doi: https://doi.org/10.1007/BF02539520

Bradburne, R., Majer, D., Magrath, R., Werner, C. P., Lewis, B., and Mithen, R. (1999). Winter oilseed rape with high levels of resistance to *Pyrenopeziza brassicae* derived from wild *Brassica* species. *Plant Pathol* 48, 550–558. doi: https://doi.org/10.1046/j.1365-3059.1999.00373.x

Branca, F. (2008). Cauliflower and broccoli. In *Vegetables I,* ed. Prohens, J. and Nuez, F., (New York: Springer), 147-182.

Branca, F. and Tribulato, A. (2011). *Brassica macrocarpa*. The IUCN Red List of Threatened Species 2011: e.T162139A5548195. url: https://dx.doi.org/10.

2305/IUCN.UK.2011-1.RLTS.T162139A5548195.en. accessed date: 2022-09

Brullo, C., Brullo, S., Galdo, G. G. D., and Ilardi, V. (2013). *Brassica trichocarpa* (Brassicaceae), a new species from Sicily. *Phytotaxa* 122(1), 45–60. doi: http://dx.doi.org/10.11646/phytotaxa.122.1.2

Castellano, G. and Bazan, G. (2009). Aspetti distributivi e fitosociologici di *Brassica incana* (Brassicaceae, Magnoliophyta) in Sicilia. *Quad. Bot. Amb. Appl* 263-268.

Coelho, P. S., Monteiro, A. A., Lopes, V. R., and Branca, F. (2018). New sources of resistance to downy mildew in a collection of wild and cultivated brassicas. *Acta Hortic* 1202, 93–100. doi: https://doi.org/10.17660/ActaHortic.2018.1202.14

Ellis, P. R., Pink, D. A. C., Barber, N. E., and Mead, A. (1999). Identification of high levels of resistance to cabbage root fly, *Delia radicum*, in wild *Brassica* species. *Euphytica* 110, 207–214.

Euro+med (2006). Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. url: http://www.europlusmed.org. accessed date: 2023-07

FAO (2009). The International Treaty on Plant Genetic Resources for Food and Agriculture. url: http://www.fao.org/3/a-i0510e.pdf.

Flora Ionica Working Group (2016). Flora Ionica - An inventory of ferns and flowering plants of the Ionian Islands (Greece). url: https://floraionica.univie.ac.at. accessed date: 2022-08

Giotta, C., Piccitto, M., and Arrigoni, P. V. (2002). Un nuovo endemismo della Sardegna: *Brassica tyrrhena* sp. nov. (Brassicaceae). *Webbia* 57, 1–5. doi: https://doi.org/10.1080/00837792.2002.10670724

Gómez-Campo, C., Gustafsson, M., Gass, T., Gustafsson, M., Astley, D., Frison, E. A., and Rome (1994). Prospects for in situ conservation of *Brassica oleracea* wild relatives. In *Report of a Working Group on* Brassica*, Second meeting, 13-15 November 1994, Lisbon, Portugal,* ed. Gass, T., Gustafsson, M., Astley, D., and Frison, E., (Rome: European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR), International Plant Genetic Resources Institute), 72-74.

Gonzalez-Benito, M. E. (2010). Status of wild *Brassica* conservation at the Universidad Politécnica de Madrid, Spain. In *Report of a Working Group on* Brassica*. Fourth Meeting, 2-4 March 2010, Linguaglossa, Catania, Italy,* ed. Maggioni, L. and Lipman, E., (Rome, Italy: Bioversity International), 5-6.

Gustafsson, M. (1982). Germplasm conservation of wild (n=9) Mediterranean *Brassica* species. *Sveriges Utsädesförenings Tidskrift* 92, 133–142.

Gustafsson, M., Gómez-Campo, C., and Zamanis, A. (1985). Germplasm conservation of wild Mediterranean *Brassica* species. Collecting missions in 1983. *Sveriges Utsädesförenings Tidskrift* 95, 137–143.

Hammer, K., Perrino, P., and Pignone, D. (1987). Segnalazioni Floristiche italiane: 464. *Brassica rupestris*. *Informatore Botanico Italiano* 19(181).

Happstadius, I., Ljungberg, A., Kristiansson, B., and Dixelius, C. (2003). Identification of *Brassica oleracea* germplasm with improved resistance to Verticillium wilt. *Plant Breed* 122, 30–34. doi: https://doi.org/10.1046/j.1439-0523.2003.00774.x

IBPGR (1980). IBPGR Secretariat Consultation on the Genetic Resources of Cruciferous Crops. AGP:IBPGR/80/100. (Rome: IBPGR Secretariat), 17-19. url: https://hdl.handle.net/10568/105100.

Mabry, M. E., Turner-Hissong, S. D., Gallagher, E. Y., Mcalvay, A. C., An, H., Edger, P. P., Moore, J. D., Pink, D. A., Teakle, G. R., Stevens, C. J., Barker, G., Labate, J., Fuller, D. Q., Allaby, R. G., Beissinger, T., Decker, J. E., Gore, M., and Pires, J. C. (2021). The evolutionary history of wild, domesticated, and feral *Brassica oleracea* (Brassicaceae). *Mol. Biol. Evol* 38(10), 4419–4434. doi: https://doi.org/10.1093/molbev/msab183

Maggioni, L. (2010). Wild brassicas in the Madrid collection - History of the agreement with IBPGR. In *Report of a Working Group on* Brassica*. Fourth Meeting, 2-4 March 2010, Linguaglossa, Catania, Italy,* ed. Maggioni, L. and Lipman, E., (Rome: Bioversity International).

Maggioni, L. (2015). Noterella 0175: *Brassica incana* Ten. *Acta Plantarum Notes* 3(114). doi: https://www.actaplantarum.org/ap_notes/pubbl/ActaPlantarum_Notes_3_marzo_2015.pdf

Maggioni, L. and Alessandrini, A. (2019). The occurrence of *Brassica montana* Pourr. (Brassicaceae) in the Italian regions of Emilia-Romagna and Marche, and in the Republic of San Marino. *Italian Botanist* 7, 1–16. doi: https://doi.org/10.3897/italianbotanist.7.31727

Maggioni, L., Bas, N., Poulsen, G., Branca, F., Ralli, P., Bothmer, R., and Lipman, E. (2013). Poster presented at the EUCARPIA genetic resources section meeting, 10-13 June 2013, Alnarp, Sweden.

Maggioni, L., Bothmer, R., Poulsen, G., Branca, F., and Jørgensen, R. B. (2014). Genetic diversity and population structure of leafy kale and *Brassica rupestris* Raf. in South Italy. *Hereditas* 151, 145–158. doi: https://doi.org/10.1111/hrd2.00058

Maggioni, L. and Domina, G. (2020). *Brassica drepanensis*. The IUCN Red List of Threatened Species: e.T137359243A137359250. url: https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T137359243A137359250.en. accessed date: 2022-09

Maggioni, L., Eastwood, A., Maggioni, L., Astley, D., Gustafsson, M., Gass, T., and Lipman, E. (1996). Monitoring of (2n=18) wild *Brassica* populations in Italy and Croatia. In *Report of a Working Group on* Brassica*, Third meeting, 27-29 November 1996, Rome, Italy,* ed. Maggioni, L., Astley, D., Gustafsson, M., Gass, T., and Lipman, E. 91-103.

Malfa, G. A., Acquaviva, R., Bucchini, A. A. E., Ragusa, S., Raimondo, F. M., and Spadaro, V. (2020). The Sicilian wild cabbages as biological resources: taxonomic update and a review on chemical constituents and biological activities. *Fl. Medit* 30, 245–260. doi: https://doi.org/10.7320/FlMedit30.245

Mei, J., Qian, L., and Disi, J. O. (2011). Identification of resistant sources against *Sclerotinia sclerotiorum* in *Brassica* species with emphasis on *B. oleracea*. *Euphytica* 177, 393–399. doi: https://doi.org/10.1007/s10681-010-0274-0

Mithen, R. F. (2014). Development and commercialisation of 'Beneforté' broccoli and potential health benefits. *Acta Hortic.* 1005, 67–70. doi: https://doi.org/10.17660/ActaHortic.2013.1005.4

Mithen, R. F. and Magrath, R. (1992). Glucosinolates and resistance to *Leptosphaeria maculans* in wild and cultivated *Brassica* species. *Plant Breeding* 108, 60–68. doi: https://doi.org/10.17660/ActaHortic.2013.1005.4

Nikolić, T. (2005). Flora Croatica Database. University of Zagreb, Faculty of Science, Department of Botany, Zagreb. url: https://hirc.botanic.hr/fcd. accessed date: 2022-05

Palaniswamy, P. and Bodnaryk, R. P. (1994). A wild *Brassica* from Sicily provides trichome-based resistance against flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *The Canadian Entomologist* 126, 1119–1130. doi: https://doi.org/10.4039/Ent1261119-5

Paparisto, K. and Qosja, X. (1976). Kontribut për florën e R. P. të Shqipërisë. *Bul. Shkencavet Nat* 30(2), 85–98.

Pelgrom, K. T. B., Broekgaarden, C., Voorrips, R. E., Bas, N., Visser, R. G. F., and Vosman, B. (2015). Host plant resistance towards the cabbage whitefly in *Brassica oleracea* and its wild relatives. *Euphythica* 202, 297–306. doi: https://doi.org/10.1007/s10681-014-1306-y

Picchi, V., Scalzo, R. L., Tava, A., Doria, F., Argento, S., Toscano, S., Treccarichi, S., and Branca, F. (2020). Phytochemical characterization and in vitro antioxidant properties of four *Brassica* wild species from Italy. *Molecules* 25(15), 3495–3495. doi: https://doi.org/10.3390/molecules25153495

Qosja, X., Paparisto, K., Vangjeli, J., and Ruci, B. (1996). Flore de l'Albanie (Flora e Shqipërisë) volume 3. (Tiranë: Akademia e Shkencave e Republikës së Shqipërisë. Instituti i Kërkimeve Biologjike), 331p.

Raimondo, F. M., Mazzola, P., and Ottonello, D. (1991). On the taxonomy and distribution of *Brassica* sect. *Brassica* (Cruciferae) in Sicily. *Flora Mediterranea* 1, 63–86. url: https://www.herbmedit.org/flora/01-063.pdf

Sheng, X. G., Branca, F., Zhao, Z. Q., Wang, J. S., Yu, H. F., Shen, Y. S., and Gu, H. H. (2020). Identification of black rot resistance in a wild *Brassica* species and its potential transferability to cauliflower. *Agronomy* 10(1400). doi: https://doi.org/10.3390/agronomy10091400

Snogerup, S., Gustafsson, M., Bothmer, R., and Von (1990). *Brassica* sect. *Brassica* (Brassicaceae). I. Taxonomy and variation. *Willdenowia* 19, 271–365. doi: https://doi.org/10.1007/BF02539520

Stanisci, A., Feola, S., and Blasi, C. (2005). Map of vegetation series of Ponza island (central Italy). *Lazaroa* 26, 93–113.

Taylor, A., Rana, K., Handy, C., and Clarkson, J. P. (2018). Resistance to *Sclerotinia sclerotiorum* in wild *Brassica* species and the importance of *Sclerotinia subarctica* as a *Brassica* pathogen. *Plant Pathology* 67, 433–444. doi: https://doi.org/10.1111/ppa.12745

Treccarichi, S., Ammar, H. B., Amari, M., Cali, R., Tribulato, A., and Branca, F. (2023). 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*). *Plants* 12(2), 407–407. doi: https://doi.org/10.3390/plants12020407

Vosman, B., Pelgrom, K., Sharma, G., Voorrips, R., Broekgaarden, C., Pritchard, J., May, S., Adobor, S., Castellanos-Uribe, M., Kaauwen, M., Van, Janssen, B., Workum, W., and Ford-Lloyd, B. (2015). Phenomics and genomics tools for facilitating brassica crop improvement. *Crop wild relative* 10, 12–14. url: http://www.cropwildrelatives.org/fileadmin/templates/cropwildrelatives.org/upload/documents/CWR_newsletter_10.pdf.

Wiersema, J. H. and Schori, M. (2022). Taxonomic Information on Cultivated Plants in GRIN-Global. url: https://npgsweb.ars-grin.gov/gringlobal/taxon/abouttaxonomy. accessed date: 2022-09

Zhang, X., Liu, Y., Fang, Z., Li, Z., Yang, L., Zhuang, M., Zhang, Y., and Lv, H. (2016). Comparative transcriptome analysis between broccoli (*Brassica oleracea* var. *italica*) and wild cabbage (*Brassica macrocarpa* Guss.) in response to *Plasmodiophora brassicae* during different infection stages. *Frontiers in Plant Science* 7. doi: https://doi.org/10.3389/fpls.2016.01929