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Biology and conservation strategies of genetic diversity in wild Sicilian populations of *Brassica* sect. *Brassica* (*Cruciferae*)

Abstract

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The study of genetic diversity, based on allele frequency has allowed to select the Sicilian wild populations of *Brassica* deserving conservation priority. The measurement of allele richness and the presence of local alleles have been used in order to describe genetic variation patterns belonging to different hierarchical levels. The allele structure at subspecies level contributes to the total genetic diversity, the absolute contribution of a single population decreases with the increase of the number of sampled populations. Planning conservation at section level is of great advantage as regards conservation at subspecies level and can form the basis for the realization of the core collection. In order to preserve 37 alleles found in the Sicilian populations studied, priority has been given to a) *B. rupestris* subsp *hispida* (Borgetto) and of *B. incana* (Gonato) populations for their allele richness b) *B. macrocarpa* population for the presence of the rare and local allele 5 at locus *Lap-1*. c) *B. incana* (Longi) e *B. villosa* subsp *bivoniana* (Caltabellotta) for the presence of common and widely distributed alleles not captured in the populations already described in a and b. The survival percentage of the seedlings of *B. macrocarpa* in nature is very low, this fact doesn't allow the fruiting plants to produce a population able to maintain and preserve genetic diversity, for this reason *ex situ* conservation and reintroduction in the native habitat is advisable. The evaluation and monitoring of the quality of the accessions before and during the storage period, in order to maintain a collection of vigorous individuals, assume the knowledge of germination ecophysiology and of the germination testing before storage in the seed bank.

Introduction

The germplasm bank of the Botanical Garden of the University of Palermo, founded in 1993, aims at preserving the genetic diversity of rare and threatened wild species and of wild relative plants interesting for their economic potential. The method used for long term seed conservation in this gene bank (Scialabba & Raimondo 1994) shows that the storage procedure is simple, but requires multidisciplinary approaches concerning taxonomy, ecology, conservation biology, population genetics, collection management which interact in order to select the most suitable and correct strategy of conservation.The first step for the

creation of the collection is based on the knowledge of the distribution of the taxa and of the individuals from which the seeds to be preserved should be picked out. The distribution of the species, the ecology of the sites and the morphological characters of the plants should also be considered. In the laboratory the taxonomic identification based on the phenotypical characters is then undertaken.

The realization of the collection, for taxa of a particular interest, is based on the isozymatic loci allele frequency, on the knowledge of the germination ecophysiology and on the quality of seeds before their conservation. Such a procedure requires a high economical and human effort, therefore it is used for species requiring priority in conservation and for those seeds which form the core collection.The core collection idea, introduced by Frankel & Brown (1984) and by Brown (1989 a, b), insures the conservation of the maximum genetic diversity and the reduction of management costs in a genebank. The curators when forming a core subset should consider the optimal accessions number needed to retain an acceptable proportion of alleles present in a given collection, and the method used to select accessions (Crossa & al. 1993).

The optimum strategy for germplasm sampling requires the knowledge of: a) "target alleles" distribution of the examined populations. In conservation genetics the measurement of genetic diversity is the allelic richness (Marshall & Brown 1975) but we suggest that priority to local alleles should be given, as they are exclusive of a localized population in a specific site. From this point of view we need a measurement of diversity at different hierarchic levels and at population level in order to optimise the sampling of "target alleles"; b) native habitat of the taxa to be preserved in order to establish the conservation strategy (*in situ*, *ex situ*); c) seed quality, seed vigour is in fact responsible for better tolerance to environmental stresses upon storage and germination (Scialabba & al. 1999a).

Sicily represent one of the main diversification centres of the Sect. *Brassica* of the genus *Brassica*. Three species are strictly endemic (*B. villosa* Biv., *B. rupestris* L. and *B. macrocarpa* Guss.) and two (*B. incana* Ten. and *B. insularis* Moris) are distributed in the Mediterranean area (Raimondo & al. 1991). These populations belonging to the same cytodeme of *B. oleracea* L. (Harbert 1972) are wild relatives of kale crops (Heywood & Zohary 1995) and therefore represent an important genetic resource. The populations are characterized by a considerable morphological variation (Raimondo & Mazzola 1997) and show a strict genetic relationship (Geraci & al. 2002). Some of them are endangered (Raimondo & al. 1994) or threatened by antropic factors, such as hybridization with cultivated forms. The allozymatic analysis carried out on 16 populations among the known ones, concerns six populations of *B. villosa* (Scialabba & al. 2001), seven of *B. rupestris*. (Geraci & al. 1998) two of *B. incana* and one of *B. macrocarpa*. Isozyme analysis of genetic diversity in wild Sicilian populations of *Brassica* Sect. *Brassica* has shown that the levels of inter- and intra- population diversity were comparable and that populations of *B. incana* exhibit a clear separation from the *villoso-rupestris* populations; furthermore, *B. macrocarpa* forms a separate clade and is differentiated from the other Sicilian species (Geraci & al. 2002).

The present study aims at preserving the spectrum of genetic diversity of the Sicilian populations of *Brassica*, within the germplasm bank of botanical Garden in Palermo capturing all the different allelic forms and maintaining the genetic diversity.

Materials and Methods

The wild populations of *B. villosa* Biv. subsp *villosa*, *B. v.* subsp. *drepanensis* (Caruel) Raimondo & Mazzola, *B. v.* subsp. *bivoniana* (Mazzola & Raimondo) Raimondo & Mazzola, *B. v.* subsp. *tinei* (Lojac.) Raimondo & Mazzola, *B. rupestris* Rafin. subsp. *rupestris*, *B. r.* subsp. *brevisiliqua* Raimondo Mazzola, *B. r.* subsp. *hispida* Raimondo & Mazzola, *B. incana* Ten. and *B. macrocarpa* Guss. were monitoring the original sites identifying the number of individuals of each population (size of population) and the number of fruiting individuals (Table 1). Ten fruiting plants for each population were enclosed into a gauzed net inside which seeds were collected during the siliquae dehiescence (Fig. 1).

Table 1. Quantitative reproductive data of the *Brassica* examined populations. PF (number of fruiting plants), SP (number of seeds per plant), SS %(survival seedling percentage), PD (months of primary dormancy), TD (thermo dormancy). *missing data.

Taxa	Origin	Size (number of individuals)	PF	Seed harvest month	SP	SS (%)	PD	TD (°C)	Optimum Germination Temperature (°C)
1. *B. villosa* subsp. *villosa*	Sagana (Palermo)	250	83	june	2000	0,17	3	-	15, 25
2. *B. villosa* subsp. *bivoniana*	M. Inici (Trapani)	750	173	end of May	750	0,64	-	15, 25	15, 15/30
3. *B. villosa* subsp. *bivoniana*	Caltabellotta (Agrigento)	1000	250	June	1900	0.21	-	-	15
4. B. villosa subsp. *bivoniana*	Fontanarossa (Trapani)	30	*	*	*	*		*	*
5. *B. villosa* subsp. *drepanensis*	M.San Giuliano (Trapani)	1000	340	June	3700	0,09	-	25	15, 25, 15/30
6. B. villosa subsp. *tinei*	Marianopoli (Caltanissetta)	750	263	June	9500	0,03	-	-	15, 25, 15/30
7. B. rupestris subsp. *rupestris*	M. Pellegrino (Palermo)	1000	300	end of April	1100	0,33	1	25	15
8. *B. rupestris* subsp. *rupestris*	R. Busambra (Palermo)	1000	340	July	1050	0,31	-	-	15, 25, 15/30
9. *B. rupestris* subsp. *rupestris*	Stilo (Catanzaro)	*	*	June	*	*	2	25, 15/30	15
10. *B.rupestris* subsp. *rupestris*	San Ciro (Palermo)	75	8	end of April	2450	0,45	4	-	15, 15/30
11. *B.rupestris* subsp. *brevisiliqua*	Isolidda (Trapani)	75	25	end of April	2900	0,11	3	25, 15/30	15, 25, 15/30
12. *B.rupestris* subsp. *hispida*	M. Pizzuta (Palermo)	1000	330	July	3550	0,09	-	25	15, 25, 15/30
13. *B.rupestris* subsp. *hispida*	Borgetto (Palermo)	75	15	June	4500	0,12	2	25	15,15/30
14. *B.incana*	Gonato (Palermo)	250	83	July	2000	0,17	-	15, 25	15, 25, 15/30
15. *B. incana*	Longi (Messina)	75	21	May	2600	0,15	1	15, 25	15,25
16. B.macrocarpa	Favignana (Trapani)	750	263	end of May	4300	0,07	1	25	15, 25, 15/30

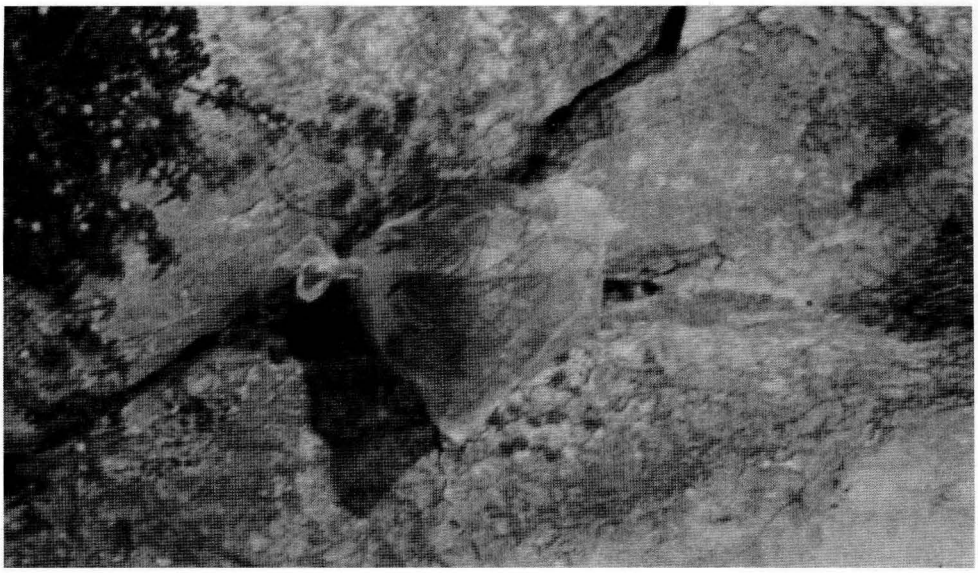

Fig 1. Fruiting individual of *B. rupestris* enclosed into a gauzed net for seed harvest.

The seeds of each individual were harvested and counted in the end period of April/July and for each population the arithmetical mean was established (Table 1). The seeds collected were stored at –20 °C, at 6% moisture content. A lot of seeds for each population were kept at room temperature for a year.

Germination test - Every month, germination tests of seeds kept at room temperature were carried out in order to know dormancy and the maximum germinative response. The germination test was carried out on four replicates of 25 seeds each placed on Petri dishes on 4 layers of filter paper previously moistened with distilled water (10 ml) at 15 °C, 25 °C and 15/30 °C in the dark. Other cultivation tests from seeds were conducted at the Botanical Garden of Palermo in order to evaluate the vigour of seeds in a controlled condition of growth.

The estimated percentage of survival of the seedling *in situ* was calculated from the ratio of the total number of individuals forming a single population and the total number of seeds produced by the population (number of fruiting plants × medium number of seeds produced by a single plant) (Table 1). The number of individuals forming a population, spread over un area less than 100 square metres, was counted in the populations with up to 250 individuals. In the populations with more than 250 individuals and scattered over a surface larger than 100 square metres, the number of individuals counted was that of those present in 20 areas of one square metre. On these the arithmetical mean was calculated and then multiplied by the total surface.

Allozyme analysis - The study of distribution of the genetic variability in the examined populations (Table 1) is based on published data, concerning the allozyme patterns obtained from 35 plants for each population (Geraci & al. 2002). The same enzymatic sys-

tems used for the genetic and nomenclatural analysis of *B. nigra*, *B. oleracea* and *B. campestris* (Chèvre & al.1995) were tested: aconitase (ACO, E.C.4.2.1.3.), leucine aminopeptidase (LAP E.C.3.4.11.1), 6 phosphogluconate dehydrogenase (6-PGD E.C.1.1.1.44), phosphogluco-isomerase (PGI E.C.5.3.1.9), phosphoglucomutase (PGM E.C.2.7.5.1). When more than one locus was observed for the same isozyme, banding patterns were recorded by numbering them sequentially starting from the fastest migrating isozyme. The first seedling leaves were crushed and electrophoresis was performed in starch gel (Kephart 1990; Shield & al. 1983).

Genetic diversity was quantified by allele frequencies. The allelic diversity of individual loci was recognised in four classes based on allele frequency and the relative distribution within the populations (Marshall & Brown 1975): a) common alleles with a frequency > 0.05; b) rare alleles with a frequency < 0.05; c) widely distributed alleles occurred in many populations; d) local distributed alleles confined to one or few populations.

During sampling locally common alleles deserve particular attention because they might reflect the effects of isolation by distance or they could represent adaptive variants which have been favoured by natural selection as verified by Brown (1978) reviewing the role of population genetic data, mainly derived from the analysis of allozyme, in the exploration and conservation of plant genetic resources. Locally rare alleles are exclusive of a single population and have to be sampled because they could be lost. The common widely distributed alleles can be easily sampled because they characterize more populations.

The number of plants (n) required in order to preserve at least a copy of each allele with a probability (P) of 0.999 was calculated on the basis of allelic frequency (F): $n=\log(1-P)/\log(1-F)$ for self pollinated taxa and $n=\log(1-P)/\log(1-F)^2$ for cross pollinated taxa (Lawrence & al. 1995). *Brassica* shows a mixed system of breeding in which allogamy prevails.

Results and Discussion

Conservation of genetic diversity at population, species and section level. - 37 alleles in eleven examined loci (Table 2) characterise the 16 Sicilian populations studied. Populations with greater allele richness are: 1) *B.villosa* subsp. *villosa* of Sagana, 2) *B. villosa* subsp. *bivoniana* of Mt. Inici, 3) *B. rupestris* subsp. *hispida* of Borgetto, 4) *B. incana* of Gonato (Table 2). Common and widespread alleles present in the section are captured by sampling these four populations. The first one has the alleles present in the other three populations, therefore sampling is not needed. The second has local alleles which must be protected and is sampled according to the methods recorded in Table 4. The third population has allele 1 at *Aco-3* locus which is not present in the other three populations, its frequency is 0.20, therefore following the calculation proposed by Lawrence & al. (1995) 31 plants should be sampled. The fourth population has allele 1 at *Pgm-2* locus. This is not recorded in the other three populations; its frequency is 0.143 therefore 45 plants should be sampled. Unrecorded alleles in the four populations mentioned should be distinguished into local alleles and common alleles. The first are captured during sampling of the populations as mentioned in Table 4; the second should be sampled from populations in which they show the highest frequency. Following the calculation proposed by Lawrence & al.

Table 2. Frequencies of the allozymes detected in 16 wild *Brassica* populations analyzed with 5 enzymatic system. 1-6, *B. villosa*; 7-13, *B.rupestris*; 14-15, *B.incana*; 16, *B.macrocarpa*.

System	Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
PGI	*Pgi-2*	1														0.614	1.000	
		2														**0.386**		
		3		**0.260**														
		4	1.000	0.740	1.000	1.000	1.000	1.000	1.000	0.886	1.000	1.000	1.000	1.000	1.000			
		5								0.114								1.000
PGM	*Pgm-3*	1							**0.071**									
		2	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Pgm-1	1						**0.129**										
		2		0.477			0.100	0.514		0.029			0.614	0.029				
		3	1.000	0.523	1.000	1.000	0.900	0.357	1.000	0.971	0.971	0.729	0.386	0.943	1.000	1.000	0.171	1.000
		4									0.029	0.271		0.029			0.829	
	Pgm-2	1														0.143	0.900	
		2											0.128			0.628	0.100	0.271
		3	0.957	0.900	0.574	0.486			1.000	1.000		1.000	0.329	1.000	1.000			0.700
		4	0.043	0.100	0.426	0.514	1.000	1.000			0.929		0.543			0.229		0.029
		5									**0.071**							
ACO	*Aco-1*	1	0.128						0.043						0.371			
		2	0.857	0.900	0.279	1.000	0.414	1.000	0.957	1.000	1.000	0.928	1.000	1.000	0.629	1.000	1.000	1.000
		3	0.015	0.100	0.721		0.586					0.072						
	Aco-2	1	0.971	0.286	0.147	0.571	0.129		0.271	0.571					0.286		0.029	0.757
		2	0.029	0.714	0.853	0.429	0.871	1.000	0.729	0.429	0.514	1.000	1.000	1.000	0.614	1.000	0.971	0.243
		3									0.486				0.100			
	Aco-3	1			0.441			0.186			0.429		0.343	0.057	0.200			
		1'	0.914	1.000	0.559	1.000	1.000	0.686	0.357	0.471		0.529	0.657		0.643	0.500	1.000	
		2	0.086					0.129	0.643	0.529	0.571	0.471		0.943	0.157	0.500		1.000
	Aco-4	1														0.914	1.000	0.957
		2	0.857	0.271	0.382	1.000	1.000	0.200	0.143	0.186	0.471			0.086	0.271			0.043
		3	0.143	0.729	0.618			0.800	0.857	0.814	0.529	1.000	1.000	0.914	0.729	0.086		
LAP	*Lap-1*	1		0.257								0.286						0.143
		2	0.500	0.500	0.132	1.000	0.471	0.200	0.429	0.871		0.228	0.014	0.757	0.129	0.071	0.957	0.829
		3	0.242	0.243	0.868		0.529		0.571	0.129	0.386	0.457	0.957	0.200	0.757	0.929		
		4	0.258					0.800			0.614	0.029	0.029	0.043	0.114		0.043	
		5																**0.029**
6PGD	*6Pgd-1*	1	1.000	1.000	1.000	1.000	1.000	1.000	0.400	1.000	1.000	1.000	1.000	1.000	1.000	0.429	0.700	1.000
		2							0.600							0.571	0.300	
	6Pgd-2	1														0.814	1.000	
		2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.186		1.000

(1995), allele 4 at *Pgm-1* locus can be captured from 4 individuals of *B. incana* population (Longi) where it shows a frequency of 0.829 and allele 3 at *Aco-1* locus can be sampled by collecting 6 individuals of *B.v.* subsp. *bivoniana* population (Caltabellotta) where it shows a frequency of 0.72.

Distribution analysis at a species level shows 4 local alleles set for *B. v.*, 7 for *B.r.*, 9 for *B.i.*, 17 for *B. m.* (Table 3). A conservation project at a single species level requires therefore the protection of 37 alleles some of which are present in 2 or more taxa, as allele 1 at *Lap-1* locus in *B. v.* subsp. *bivoniana*, *B. r.* subsp. *rupestris* and *B. macrocarpa* (Table 3). Local alleles distribution at *Brassica* section level (Table 4) shows that only 6 alleles are local and in order to preserve a representative sample of the spectrum of the local genetic variability sampling seeds from the number of plants shown in Table 4 are needed.

Table 3. Local alleles in populations of *Brassica villosa* (B.v.), *Brassica rupestris* (B.r.), *Brassica incana* and *Brassica macrocarpa*.

POPULATION	ALLELE, LOCUS	LOCALITY
B.v. subsp. *villosa*	1,*Aco-1*	Sagana (PA)
B.v. subsp. *bivoniana*	3, *Pgi-2*; 1,*Lap-1*	M. Inici (TP)
B.v. subsp. *tinei*	1,*Pgm-1*	Marianopoli (CL)
B. r. subsp. *rupestris*	1,*Pgm-3*; 2,*6Pgd-1*	M.Pellegrino (PA)
B. r. subsp. *rupestris*	5,*Pgi-2*	R.Busambra (PA)
B. r. subsp. *rupestris*	5,*Pgm-2*	Stilo (CZ)
B. r. subsp. *rupestris*	3,*Aco-1*; 1,*Lap-1*	San Ciro (PA)
B. r. subsp. *brevisiliqua*	2,*Pgm-2*	Isolidda (TP)
B. incana	4,*Pgm-1*; 1,*Aco-2*; 4,*Lap-1*	Longi (ME)
B. incana	2,*Pgi-2*; 4,*Pgm-2*; 2,*Aco-3*; 3,*Aco-4*; 3,*Lap-1*; 2,*6Pgd-2*	Gonato (PA)
B. macrocarpa	5,*Pgi-2*; 2,*Pgm-3*; 3,*Pgm-1*; 2,3,4,*Pgm-2*; 2,*Aco-1*; 1,2, *Aco-2*; 2,*Aco-3*; 1,2,*Aco-4*; 1,2,5,*Lap-1*; 1,*6Pgd-1*; 2,*6Pgd-2*	Favignana (TP)

Table 4. Distribution of local alleles (AL) in the examined *Brassica* sect. *Brassica* populations, L (loci), F (allele frequency), *Brassica villosa* (*B.v.*), *Brassica rupestris* (*B.r.*), *Brassica incana* (*B.i.*), *Brassica macrocarpa* (*B. m.*). P (protected site), NP (unprotected site), n (number of plants required to save a copy of each allele with a probability of 99% (P=0,999) in self-pollined (s1), and cross-pollined (s0) taxa).

AL	L	F	POPULATION	Locality	P(0,999)	
					n(s1)	n(s0)
3	*Pgi-2*	0,26	*B.v.*subsp.*bivoniana*	M. Inici (TP) – P	22	11
1	*Pgm-1*	0,12	*B.v.* subsp. *tinei*	Marianopoli (CL) –NP	54	27
1	*Pgm-3*	0,071	*B.r.* subsp. *rupestris*	M.Pellegrino(PA) – P	96	48
5	*Pgm-2*	0,071	*B.r.* subsp. *rupestris*	Stilo (CZ) - NP	96	48
2	*Pgi-2*	0,38	*B.i.*	Gonato (PA) – P	14	7
5	*Lap-1*	0,02	*B.m.*	Favignana (TP) -P	342	171

At species level, 3 rare alleles are present in *B. v.*, 9 in *B. r.*, 2 in *B. i.*, 3 in *B. m.* (Table 5). A conservation project carried out at species level requires therefore the preservation of 17 alleles, 3 of which are present in more subspecies (alleles 2 and 4 at *Pgm-1* locus and allele 4 at *Lap-1* locus) and would involve sampling from a large number of plants (Table 5). The distribution analysis of rare alleles at *Brassica* section level shows that these alleles can be easily captured from other populations in which they show frequency higher than 0.05 (Table 2). In the case of *Brassica* section rare alleles have a moderate weight but they assume great importance only when a rare allele is also local. In our case within the *Brassica* section only allele 5 at *Lap-1* locus of *B. macrocarpa* is rare (Table 5) and local (Table 4) and requires therefore the maximum protection.

Conservation strategy - Local alleles with frequency lower than 0,20 in populations which are found in unprotected sites deserve (*B. v.* subsp. *tinei*, *B. r.* subsp. *rupestris* of Stilo;

Table 5. Rare alleles (AR) of *Brassica villosa* (*B.v.*), *Brassica rupestris* (*B.r.*), *Brassica incana*, *Brassica macrocarpa*. L (loci), F (allele frequency), P (protected site), NP (unprotected site), n (number of plants required to save a copy of each allele with a probability of 99% (P=0,999) in self- pollinated (s1), and cross- pollinated (s0) taxa).

AR	L	F	POPULATION	Locality – kind of site	P(0,999)	
					n(s1)	n(s0)
4	*Pgm-2*	0,043	*B.v.* subsp. *villosa*	Sagana (PA) - NP	157	79
3	*Aco-1*	0,015	*B.v.* subsp. *villosa*	Sagana (PA) - NP	457	229
2	*Aco-2*	0,029	*B.v.* subsp. *villosa*	Sagana (PA) - NP	235	117
2	*Pgm-1*	0,029	*B. r.* subsp. *rupestris*	R. Busambra (PA) - P	235	117
2	*Pgm-1*	0,029	*B. r.* subsp. *hispida*	Pizzuta (PA) – P	235	117
4	*Pgm-1*	0,029	*B. r.* subsp. *rupestris*	Stilo (CZ) - NP	235	117
4	*Pgm-1*	0,029	*B. r.* subsp. *hispida*	Pizzuta (PA) – P	235	117
1	*Aco-1*	0,043	*B. r.* subsp. *rupestris*	M.Pellegrino (PA) – P	157	79
2	*Lap-1*	0,014	*B. r.* subsp. *brevisiliqua*	Isolidda (TP) - NP	490	245
4	*Lap-1*	0,029	*B. r.* subsp. *brevisiliqua*	Isolidda (TP) - NP	235	117
4	*Lap-1*	0,029	*B. r.* subsp. *rupestris*	San Ciro (PA) - P	235	117
4	*Lap-1*	0,043	*B. r.* subsp. *hispida*	Pizzuta (PA) – P	157	79
1	*Aco-2*	0,029	*B. incana.*	Longi (ME) - P	235	117
4	*Lap-1*	0.043	*B. incana*	Longi (ME) - P	157	79
4	*Pgm-2*	0,029	*B. macrocarpa*	Favignana (TP) – NP	235	117
2	*Aco-4*	0,043	*B. macrocarpa*	Favignana (TP) - NP	157	79
5	*Lap-1*	0,029	*B. macrocarpa*	Favignana (TP) - NP	235	117

Fig. 2. *B. macrocarpa* growing in field under standard conditions after two months (a) and after four months (b) from the germination.

Fig. 3. Seedlings of *B. villosa* subsp. *bivoniana* (arrow) in their natural habitat of Mt.Inici.The competition with the other species is visible.

Table 4) an integrated conservation program *ex* and *in situ* with particular attention for the conservation *ex situ*. Local alleles with a frequency higher than 0.20 which are located in protected sites (*B. v.* subsp. *bivoniana* of Mt. Inici and *B. incana* of Gonato) can be preserved with an integrated program which requires both conservation *ex situ* and *in situ*.

Ex situ conservation is realizable since the seed production for plant in the examined populations is high (Table 1). Seeds are of high quality because they give a germinative response of 90-100 % (un reported data) at an optimum germination temperature (Table 1). In the field, under uniform environmental conditions, the seedling development in a adult plant is 95% (Fig. 2 a-b) while in the original sites the seedling formation (Fig. 3) is not followed by the development in an adult plant. The 0.03-0.64 % of seeds produced by a population could give origin to adult individuals (Table 1) indicating that both the competition with other plant species and the cliff habitat (Fig. 4 a-b) do not allow big sized populations to occur. Therefore conservation *in situ* could not insure the protection of examined taxa, while conservation of seeds in a gene bank can provide a useful support for a possible reintroduction in the original sites. The genetic diversity for *B. macrocarpa* population could be preserved sampling from 342 plants (Table 4). Since fruiting plants are 263 (Table 1) it is necessary to repeat sampling for several years.

Maintenance of genetic diversity through time - Seed collection in a gene bank has the important role of preserving genetic diversity of populations. High seed vigour is an essen-

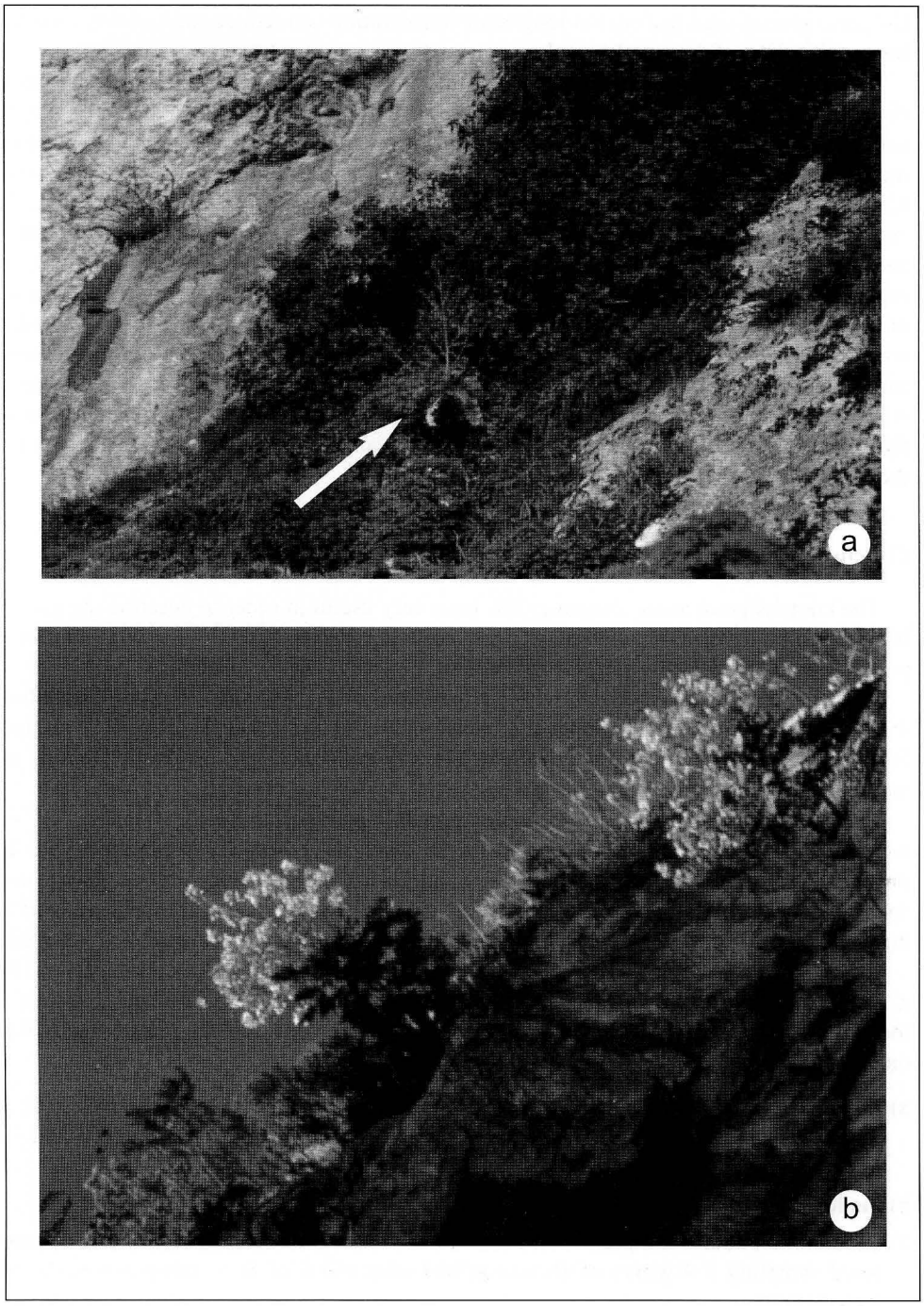

Fig. 4. The competition between *Brassica* and other species in the limestone cliffs habitat. Fruiting plants of *B. rupestris* in R. Busambra (a) and flowering plants of *B. v.* subsp *drepanensis* (b).

tial parameter of seed quality but long-term conservation can reduce seed quality causing the modification of the genetic pool.

The germination percentage and the mean germination time of *Brassica* seeds (Scialabba & al. 1999 b) allow to monitor the seed vigour through time. Monthly germination tests conducted on *Brassica* seeds at different temperatures has shown that the optimum germination temperature is at 15 °C and/or 25 °C and/or 15/30 °C and that some populations are dormant for one month (*B. rupestris* subsp *rupestris* Monte Pellegrino, *B. incana* di Longi and *B. macrocarpa*) some for two months (*B. rupestris* subsp. *rupestris* and *B. r.* subsp. *hispida*) some others for three months (*B. r.* subsp *brevisiliqua*) and for four months (*B. r.* subsp. *rupestris* of San Ciro) other are thermodormient at different temperatures (Table 1). The knowledge of the germinative pattern has allowed to know under which conditions of dormancy or thermodormancy seeds from single accessions have been stored, and to establish the seed quality at the time of conservation. These parameters, which integrate the passport data, are the initial reference in order to monitor the seed quality during conservation in the following years, to evaluate the collection status and to fix the times for the regeneration of accessions.

Conclusions

The knowledge of allele frequency has been very useful in order to describe the genetic variation patterns in populations of *Brassica* wild relatives and will allow to monitor a possible genetic erosion *in situ*, for rare alleles.

The genetic diversity spectrum within populations of *Brassica* has allowed to establish that the section is the hierarchic level at which conservation should be done. The section level conservation preserves "target alleles" in core collection and avoids the creation of numerous accessions.

The analysis shows in the 16 examined populations the presence of 37 alleles, 17 of which are rare and 37 are local. The distribution analysis at *Brassica* section level points out that there is one rare allele and that local alleles are 6. The allele composition at subspecies level therefore contributes to the total genetic diversity and that the whole contribution of a population decreases with the increase of the number of populations sampled.

Local alleles which are found in unprotected sites (*B. v.* subsp. *tinei* of Marianopoli, *B. r.* subsp. *rupestris* of Stilo) or in populations where they are also rare (allele *5* at *Lap-1* locus of *B. macrocarpa*) deserve priority. The field collector in order to preserve at least one copy for each one of the different alleles should sample:

a) two of the four populations with highest allele richness in order to capture the higher quantity of common alleles (31 plants of *B. rupestris* subsp. *hispida* of Borgetto and 45 plants of *B. incana* of Gonato);
b) the local alleles recorded in Table 4;
c) the common alleles not present in the populations mentioned in a) and b) can be captured sampling e 4 plants of *B. incana* of Longi and 6 of *B. v.* subsp *bivoniana* of Caltabellotta.

The *ex situ* conservation is the most qualified for the preservation of populations placed

in unprotected sites within a strategy of integrated conservation. For this purposes sampling the number of plants shown in Table 4 is necessary. The germplasm produced by the studied populations is of high viability at the harvest and can provide vigorous individuals which can be used for reintroduction in the original sites. Long-term monitoring of seed quality in the collection will be conducted following a germination testing programme. Seeds stored on the basis of their viability can be monitored in the following years by the use of appropriate conditions.

Conservation science is forced to treat distinct aspects of biological science and to balance them with economic and social aspects (Simberloff 1988). The experiences carried out on *Brassica* suggest that a bank can preserve successfully only limited groups of plants due to the care required and to different efforts in the knowledge of the area, the laboratory work, the elaboration of the conservation strategy and the management of a gene bank.

The present work is an attempt to optimise population sampling and to plan conservation strategy for populations restricted to small area.

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