

## Overview of the *Brassica oleracea* complex: their distribution and ecological specificities

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### Abstract

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The evolution and speciation within sect. *Brassica* can be characterized as allopatric with weakly developed crossing barriers. Crossing experiments show that the chromosomal pairing in species hybrids is high, indicating that all the species of the cytodeme have basically the same C genome. The relatively wide intraspecific genetical and morphological variation is probably a result of local and regional differentiation. In most regions of the distribution area the species consist of spatially well isolated populations and local adaptation has often given rise to morphologically distinct populations as in, for instance, *B. cretica*, *B. rupestris*, and *B. villosa*. Most populations are small, the reproductive capacity may fluctuate from one year to another and 'bottle-neck' situations may occur frequently. Results of crossing experiments and molecular investigations lead to the conclusion that all cultivated forms together with wild west European material belong to the same biological species, *B. oleracea*. Prospects for *in situ* conservation of wild populations are discussed.

The genus *Brassica*, section *Brassica* with the diploid chromosome number  $2n = 18$ , consists of a polymorphic aggregate of species (the *B. oleracea* cytodeme of Harberd (1972). The wild taxa occur as vicarious species in the Mediterranean region and along the Atlantic coasts (Snogerup & al. 1990). The main centers of diversity of the group are located in the East Mediterranean area and on Sicily. In the East Mediterranean region *Brassica hilarionis* Post is endemic to the Kyrenia mountains on Cyprus, while the polymorphic species *B. cretica* Lam. is represented by three subspecies which are distributed in different parts of Greece and the southwestern part of Turkey. In Sicily, populations of the widely distributed *B. incana* Ten. occur in the northeastern part of the island, while two species, *B. rupestris* Rafin. and *B. villosa* Biv., are restricted to the western parts. *B. macrocarpa* Guss. is endemic on two small islands west of Sicily (Isole Egadi). Other wild species belonging to section *Brassica* are *B. insularis* Moris.

distributed in Sardinia, Corsica and Tunisia, *B. montana* Pourret occurring in northern Italy, southern France, and northwestern parts of Spain and *B. bourgeauii* (Webb) O. Kuntze endemic to the Canary Islands. The Linnaean species *B. oleracea* comprises crop plants and wild populations distributed along the Atlantic coasts of northern Spain, France, Great Britain, and the island of Helgoland. The variation pattern in this species is complicated due to domestication – *B. oleracea* has been cultivated since ancient times. Today, different *B. oleracea* crops are cultivated throughout the world, including, for example, the vegetables kale, cabbage, cauliflower and broccoli.

### Habitat and population structure

The taxa of section *Brassica* are chasmophytic and they usually occur in maritime cliffs of limestone substrates, although occurrences on other substrates have been noted (Snogerup & al. 1990). Most *Brassica* plants are found in the so called step-crevice community of larger shelves and cracks, often in more or less steep and shady positions (Davis 1951, Snogerup 1971), but in situations protected from grazing some plants can be found below the cliffs in scree and among shrubs. *B. cretica* subsp. *aegaea* and *B. insularis* in particular, may also inhabit small, rocky islets, which are not influenced by grazing. The species *B. incana* and *B. montana* have been repeatedly found growing in habitats influenced by human activities, for instance quarries, roadsides and building grounds (see Table 1).

Table 1. Ecological preferences of species in *Brassica* sect. *Brassica*. The variation in habitat is shown for three species. n indicates number of all investigated populations.

Habitat	<i>B. cretica</i>	<i>B. incana</i>	<i>B. montana</i>	All species	
	n	n	n	n	%
Chasmophytic	47	10	7	106	72
Macchia	0	4	14	25	17
Ruderal	2	6	4	17	11
Total	49	20	25	148	100

The altitudes of the *Brassica* populations may vary from sea level up to 1 000 metres in Corsica (*B. insularis*) and Alpi Apuanae (*B. montana*). The altitude of 149 populations have been noted and 43 % of them grow in cliffs located less than 100 m above sea level, 39% between 100 to 500 m and 18 % above 500 m.

The size of the populations is very variable even within species and the number varies from single individuals up to 10 000 plants, but a considerable part of the populations are small both in area and in numbers (Table 2). On average 38 % of the populations consist of 100 plants or less.

These small populations are more or less continuously subject to genetic drift, which may lead to loss or fixation of alleles in a population. In such cases inbreeding depression may occur, although this can be counteracted by factors promoting equilibrium with permanent heterozygosity (Darlington 1958, Carson 1967).

Table 2. Population size (number of plants) in species of *Brassica* sect. *Brassica*. The variation in size is shown for three species. n indicates the number of all investigated populations.

Number of plants	<i>B. cretica</i>	<i>B. incana</i>	<i>B. montana</i>	All species	
	n	n	n	n	%
1-10	9	2	0	15	10
11-50	5	2	4	20	13
51-100	5	7	5	22	15
101-500	8	8	12	45	30
501-1000	0	1	1	6	4
> 1000	22	2	3	41	28
Total	49	22	25	149	100

### Reproductive biology

The flowers of the wild *Brassica* species are usually protandrous, with the stamens shedding their pollen a few days before the ripening of the stigma. The large anthers appear with their tips above the petal disc and produce a considerable amount of pollen. During ripening the stigma protudes beyond the anthers and the petals are usually somewhat deflexed. The inflorescences are composed of several- or many-flowered, generally ebracteate racemes. The flowering starts at the apical inflorescences, followed by flowering of the lateral branches.

Self-incompatibility regulated by S-genes is a common feature of *Brassica* species. Material of *B. cretica*, grown from seeds collected in nature, was tested for self-fertility. Buds of flowering plants were isolated and then, at anthesis, controlled pollinations were performed. Seed set was low under such conditions indicating self-sterility (Snogerup & al. 1990). In addition, male sterility by means of undeveloped anthers has been observed in several populations of *B. oleracea*. In nature pollination by wind is probably dominant, but small beetles have been noticed to creep around haphazardously in the flowers.

All the wild species are perennial and in nature the plants usually have a life span of between two and six years. Only about a half of the total number of adult plants flowers and sets seeds every year and it has been noticed in populations of, for instance, *B. cretica* and *B. rupestris* that plants with one-year-old remnants of flowering branches often do not form inflorescences the following year. Fluctuations in flowering may occur depending on climatic and edaphic conditions.

For instance, in spring 1985 there was a long period of cold weather along the Atlantic coast of France, resulting in few and late flowering plants. In two populations of *B. oleracea* at Fécamp and Le Treport, where the population sizes were estimated to be 1 100 and 10 000 plants respectively, only 50 and 75 flowering plants were observed. Thus, it can be concluded that populations of wild *Brassica* species are characterized by a large variation in size, that fluctuations in reproductive capacity from year to year are common and 'bottle-neck' situations may frequently occur.

### Morphological variation

All the wild species show a considerable intra- and interpopulational variation. In *B. oleracea* the morphology of 44 wild populations has been studied in cultivation experiments. In most vegetative and generative characters the populations differ significantly from each other. In *B. cretica* the morphological variation has been investigated in detail (Snogerup & al. 1990) and can be summarized as follows: a considerable variation is present in most traits; the largest part of the observed variation exists among populations; even adjacent populations may differ significantly in many traits; populations inhabiting small islets deviate in flower characters from those growing in steep cliffs (ecotypic differentiation). The patterns of variation in *B. insularis*, *B. villosa* and *B. rupestris* are summarized in Fig. 1. In generative characters, illustrated by the length of outer sepals and the length of filaments, the variation is small within populations of *B. rupestris*, intermediate in *B. insularis* and comparatively large in *B. villosa*. The interpopulational variation is large in all the three species, which has led some authors to recognize several taxa in *B. villosa*, for instance, *B. bioniana* Mazzola & Raimondo based on a deviating population from Castellamare del Golfo, *B. tinei* Lojac. on material from Caltanissetta and Marianopoli, and *B. drepanensis* Caruel on plants collected at Monte San Giuliano near Trapani (Sicily).

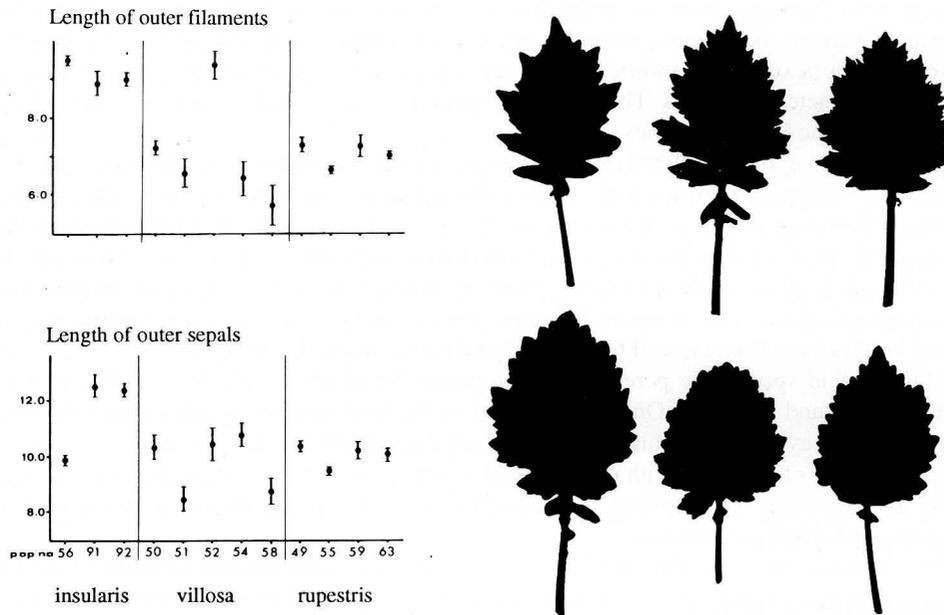


Fig. 1. To the left: variation in two floral characters (95% confidence limits) in three populations of *B. insularis*, five of *B. villosa*, and four of *B. rupestris*. The measurements were made on plants grown in a heated greenhouse with 16 h of light. To the right: variation in leaf shape of *B. rupestris* from C. Zaffarano, C. Gallo, Partinico (from left to right in the upper row), Ficuzza, Corleone and Sciacca (below).

In *B. rupestris* the variation in leaf shape (Fig. 1, to the right) is pronounced and this is also true for other characters such as leaf texture and surface (Snogerup & al. 1990).

### Genetic diversity

Diversity in natural populations of wild *Brassica oleracea* has been estimated by isozyme and RAPD analysis (Lannér-Herrera & al. 1996). Naturally occurring populations of wild *Brassica oleracea* were collected in Spain, France, and Great Britain. Allele frequencies at five isozyme loci were determined for 18 populations, while five populations were screened using five random primers to generate RAPDs. Levels of homozygosity and gene diversity,  $H$  (Nei 1973), were computed for each population using isozyme data and RAPD data when applicable. The results show that homozygosity levels tended to be higher in small populations than in larger ones, which could also be observed as increased numbers of homozygous loci in small populations. Gene diversity values based on isozymes indicated considerable within population variation regardless of population size. However, two medium sized populations, one from France and one from Great Britain deviated from the others by having considerably smaller variation. The coefficient of gene differentiation,  $G_{ST}$  (Nei 1973), for populations in each region showed that the Spanish populations were more homogeneous than the French or British ones. Clustering according to geographic region was not evident using either isozyme or RAPD data.

In all the species it is not uncommon that adjacent populations may deviate in vegetative as well as in generative traits, like the situation in *B. cretica*. A similar pattern of variation has also been obtained between populations of *B. oleracea*. Intra- and interpopulational variation in allele frequencies has been studied in five populations from Great Britain by using enzyme electrophoresis. Two of the selected populations are situated at Folkstone in Kent and three adjacent populations in Wales, of which two are located at Llandudno and one at Llandulas. In the five enzymes studied, the allele frequencies are quite different in the two populations from Folkstone as well as in the three populations from Wales (see Gustafsson 1997, this volume). For instance, the F-allele in locus PGM-1 is common in one of the Folkstone populations but not in the other. In the three populations from Wales, the A-allele in locus GPI is common in the Llandulas population, rare in one of the Llandudno populations, and not present in the other. The same situation is true for locus PGM 2, where the A-allele is fairly common in the Llandudno population but not present in the others. This and other studies indicate that local adaptation is a common differentiation pattern in the wild *Brassica* species and that gene flow between even adjacent populations, by means of seed and/or pollen dispersal, seems to be highly restricted.

### Reproductive isolation

The species are spatially isolated, although areas with overlapping distribution do exist. In the western parts of Sicily, where *B. rupestris* and *B. villosa* seem to be sympatric, each single cliff, however, is inhabited by one species only. Natural hybridization has not been observed, except for a few cases of hybridization between cultivated *Brassica oleracea* and the wild species. The development of crossing barriers have been studied in artificial

crossing experiments (Gustafsson & al. 1976, Kinanian & Quiros 1992, Bothmer & al. 1994). The results show that chromosomal pairing in meiosis usually is high, indicating that all the studied species have basically the same genome. However, a certain frequency of uni- and multivalent formation in metaphase and presence of small inversions indicate that chromosomal rearrangements are fairly common in the differentiation of the *Brassica* C-genome. In an extensive crossing program, Bothmer & al. (1994) have investigated pollen fertility in crosses including the wild species and cultivated forms of *B. oleracea* (Fig. 2). The fertility in crosses between different cultivated forms was high and very even (mean 93%). On the contrary, the fertility in crosses between the wild species and the cultivated forms of *B. oleracea* was reduced (mean 72%), where crosses with *B. macrocarpa*, *B. montana* and *B. rupestris* had the lowest fertility values. However, crosses between cultivated forms and wild populations of *B. oleracea* differed from crosses with the other wild species by having relatively high fertility values in F1 and F2 (87% in both generations). The hybrid seed set varied with the crossing combination, but it was always high enough to secure the raising of further generations. These crossing experiments show that it is rather easy to introduce genes for agronomic traits, if desired, from the wild species to cultivated *B. oleracea*.

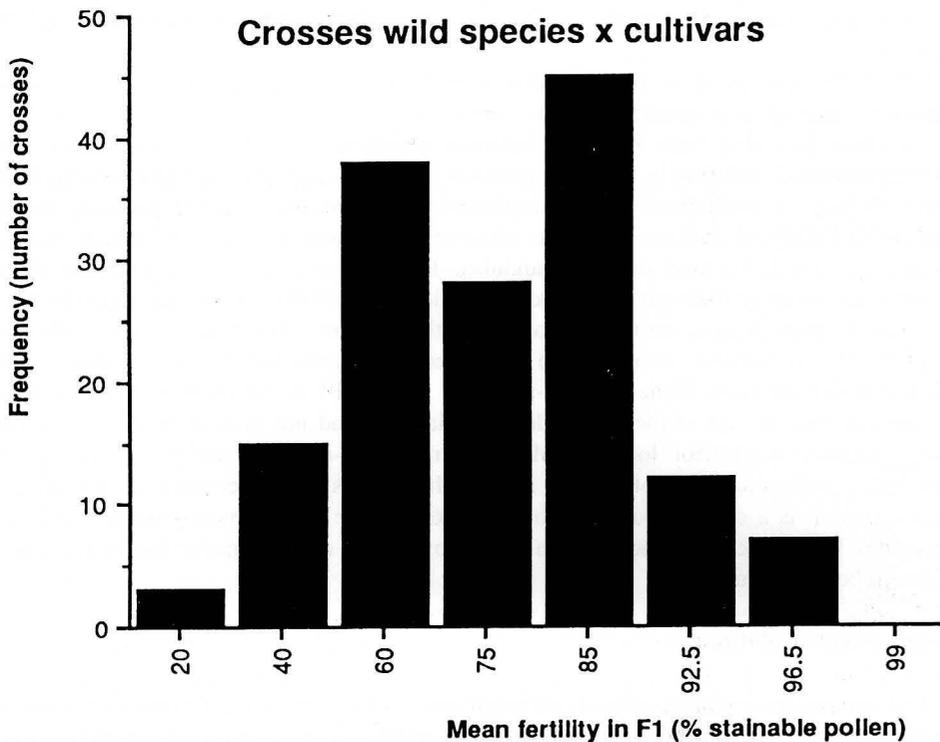


Fig. 2. Distribution of fertility in F1 hybrids in 148 crosses between all the wild species and cultivated forms of *B. oleracea*. The highest fertility values are found in crossing combinations between cultivated forms and wild *B. oleracea*.

### Phylogenetic relationships

In an RFLP study of the *B. oleracea* group Song & al. (1990) found that cultivated forms of *Brassica* with  $2n = 18$  clustered well together and together with wild *B. oleracea* and *B. alboglabra* (treated by them as a separate species). A second group was formed by the other wild species of the *B. oleracea* cytodeme. Hosaka & al. (1990) found a close evolutionary proximity between cultivated and wild forms of *B. oleracea*. Dias & al. (1993) found in an RFLP study that the 48 studied accessions clustered into five major groups, of which four groups consisted of landraces and cultivars of *B. oleracea* and the fifth group of *B. cretica* and *B. insularis*. From crossing results and molecular studies it is evident that all cultivated forms are closely related, and that there are no or weak crossing barriers between them. *B. alboglabra*, the Chinese endemic, cultivated form was first described as a separate species by Bailey (1922), who considered it to be an isolated ancient Asiatic taxon of the cytodeme. However, the crossing data show that *B. alboglabra* is completely interfertile with other cultivated forms (Kinian and Quiros 1992, Bothmer & al. 1994). *B. alboglabra* also gives the same pattern of crossability in combinations with wild *Brassica* species as all the other cultivated forms. These results are also supported by e.g. Warwick and Black (1991) based on cpDNA, Song & al. (1990) and Dias & al. (1992) on RFLPs. All these results lead to the conclusion that all the cultivated forms including "*alboglabra*" should be kept together with the wild west European material within a single species, *B. oleracea*.

### Conservation aspects

During a series of IPGRI-supported missions, with the aim of collecting germplasm of wild relatives to cultivated *B. oleracea*, about 200 localities throughout the entire distribution area have been visited. Apart from seed collecting, additional information was documented. Thus, the field surveys included information about companion species, ecological associations, population structure, risk of endangerment, and other data of relevance to conservation.

#### *Threats to the wild populations*

Actual and potential threats have been observed in about half of the investigated populations, but the situation varies in different parts of the Mediterranean region. Overgrazing by goats was found to be a real threat in Greece in particular, where goats are abundant and often have free access to natural habitats. Quarrying has been recorded in many places and e.g. at Montagnagrande in the western part of Sicily, where a population of *B. villosa* is growing, an important portion of the cliff has been sliced off. Building activities and other human influences in the proximity of *Brassica* localities have been observed in several cases. For instance, in Campania (Italy) terraces of houses were relatively often situated close to cliffs inhabited by plants of *Brassica incana*. Many populations of *B. montana* along the Mediterranean Riviera, Côte d'Azur and Costa Brava and *B. oleracea* along the Atlantic coasts are now located in or near townships and settlements, so they permanently risk extinction by human activities and/or introgression from cultivars kept in adjacent gardens. So far, natural hybridization has been recorded

only occasionally and exclusively between cultivated and wild material. Fire is probably a natural factor in Mediterranean ecosystems, but its frequency in the past few years is raising concern. On several occasions, signs of fire have been observed not only in grasslands below cliffs but also in the true cliffs, where chasmophytic plants have been affected or destroyed. In certain cases invasion of cliff crevices by weedy aliens has been noticed. Thus, the species *Agave americana* and *Opuntia ficus-indica* are widely spread and aggressive in Sicilian cliffs and can be a potential threat to the *Brassica* plants.

#### Ex situ conservation

Large efforts for collection and conservation of *Brassica* germplasm, mainly initiated by IPGRI (formerly IBPGR, International Board for Plant Genetic Resources), have been made over the last decade (Gustafsson & al. 1983, 1985). A substantial stock of seeds of all the species is now available for breeding and research purposes at UPM, run by professor C. Gómez-Campo, Universidad Politécnica, Madrid. A database of all collected information has also been set up.

#### In situ conservation

Prospects for *in situ* conservation of the ( $n = 9$ ) Mediterranean *Brassica* species have been discussed by Gómez-Campo & Gustafsson (1994). During a discussion within the ECP/Gr Brassica group of IPGRI they have stressed a number of aspects. To conserve the ( $n = 9$ ) *Brassica* species is not an especially difficult task in comparison with other plant conservation problems. Many of the sites are already protected either by their own structures (maritime cliffs) or because they are situated in relatively remote areas (massifs, capes, islands). Legal protection of as many *Brassica* sites as possible should be a necessary first step. However, diverse types of action are necessary, because completely passive conservation has not been shown to be a good policy in all cases. Legal protection should be accompanied by the effective prevention of threats and by periodic monitoring of the status of the flora, including present populations of *Brassica*. It has been pointed out that there is an urgent need to work out a priority list of possible target areas. According to our opinion the main centre of diversity of the group is located on Sicily, where the cytodeme is represented by several, endemic species (Fig. 3).

A programme for *in situ* conservation of wild *Brassica* species should give the preservation of the genetic resources of the Sicilian species a high priority for the following reasons:

- The area is limited and some of the cliff habitats are already protected.
- It will be fairly easy to define target populations.
- A high level of diversity can be expected to be found.
- A high proportion of other endemic plant species/taxa is located at the same sites and habitat.
- At present, many of the *Brassica* populations are influenced by human activities and need some protection in order not to risk extinction.

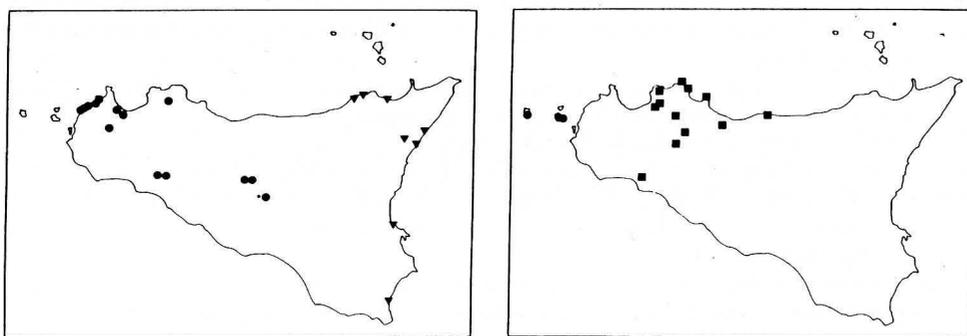


Fig. 3. Distribution of *Brassica* species in Sicily. To the left: known localities for *B. villosa* (dots) and *B. incana* (triangles). To the right: *B. macrocarpa* (dots) and *B. rupestris* (squares).

*In situ* conservation is dynamic with respect to the populations conserved. The target species will certainly continue to evolve and the populations will change in many specific attributes with time. A major obstacle for the conservation and management of wild relatives of cultivated plants is the timing for phasing out research and initiating conservation and management practices (Safriel & al.1997, this volume). Past experience indicates that there is a tendency for research to continue for years and that conservation does not start. Therefore, a preliminary framework program for wild *Brassica* species should include plans for dynamic conservation and management from the beginning. Such a conservation and management plan may consist of the following phases:

1. Designation of potential target populations and distribution of existing reserves.
2. Survey of potential populations, which may include information about
  - habitat (rock and soil types, exposure, climate);
  - ecosystem (frequency of endemic species/taxa, companions etc.);
  - population structure (size, dispersion, fluctuations, gene flow);
  - reproductive capacity (flowering, pollination, seed set);
  - genetic diversity;
  - threats to the population.
3. Selection of target populations.
4. Collection of the available information into a database.
5. Detailed formulation of management and monitoring plans.
6. Preparation of a programme for public information.

Although legal protection and actual conservation of areas or habitats are under the jurisdiction of national governments, international organisations could play an important role by stimulating and coordinating the activities in those countries where the wild *Brassica* species grow.

#### References

- Bailey, L. H. 1922: The cultivated Brassicas. — *Gentes Herbarium* **1/2**: 53-108.

- Bothmer, R. von, Gustafsson, M. & 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.
- Carson, H. L. 1967: Permanent heterozygosity. — Pp. 143-168 in: Dobzhansky, T., Hecht, M. K. & Steeve, W. C. (ed.), *Evolutionary biology I*. — Amsterdam.
- Darlington, C. D. 1939: *The evolution of genetic systems*. — New York.
- Davis, P. H. 1951: Cliff vegetation in the eastern Mediterranean. — *Journal of Ecology* **39**: 63-93.
- Dias, J. S., Lima, M. B., Song, K. M., Monteiro, A. A., Williams, P. H. & Osborn, T. C. 1992: Molecular taxonomy of Portuguese tronchuda cabbage and kale landraces using nuclear RFLPs. — *Euphytica* **58**: 221-229.
- Gómez-Campo, C. & Gustafsson, M. 1995: Prospect for in situ conservation of *Brassica oleracea* wild relatives. — Pp. 72-74 in: Gass, T., Gustafsson, M., Astley, D. & Frison, E. (Compilers), Report of a working group on *Brassica*. — Second meeting, 13-15 November 1994, Lisbon. ECP/GR. International Plant Genetic Resources Institute, Rome.
- Gustafsson, M. 1979: Biosystematics of the *Brassica oleracea* group. — Eucarpia "Cruciferae 1979" Conference. Wageningen.
- 1982: Germplasm conservation of wild ( $n = 9$ ) Mediterranean *Brassica* species. — *Sveriges Utsädesförenings Tidsskrift* **92**: 133-142.
- 1995: A strategy for in situ conservation of wild *Brassica*. — Pp. 75-76 in: Gass, T., Gustafsson, M., Astley, D. & Frison, E. (Compilers), Report of a working group on *Brassica*. — Second meeting, 13-15 November 1994, Lisbon. ECP/GR. International Plant Genetic Resources Institute, Rome.
- , Gómez-Campo, C. & Zamannis, A. 1983: Report from the first *Brassica* germplasm exploration in Greece 1982. — *Sveriges Utsädesförenings Tidsskrift* **93**: 151-160.
- , — & — 1985: Germplasm conservation of wild Mediterranean *Brassica* species. Collecting missions in 1983. — *Sveriges Utsädesförenings Tidsskrift* **95**: 137-143.
- , Bentzer, B., Bothmer Von, R. & Snogerup, S. 1976: Meiosis in Greek Brassicas of the *oleracea* group. — *Botaniska Notiser* **129**: 73-84.
- Harberd, D. J. 1972: A contribution to the cyto-taxonomy of *Brassica* (*Cruciferae*) and its allies. — *Botanical Journal of Linnean Society* **65**: 1-23.
- Hosaka, K., Kinaian, S. F., Mcgrath, J. M. & Quiros, C. F. 1990: Development and chromosomal localization of genome-specific DNA markers of *Brassica* and the evolution of amphiploids and  $n = 9$  diploid species. — *Genome* **33**: 131-142.
- Kinanian, S. F. & Quiros, C. F. 1992: Trait inheritance, fertility, and genomic relationships of some  $n = 9$  *Brassica* species. — *Genetic Resources and Crop Evolution* **39**: 165-175.
- Lannér-Herrera, C., Gustafsson, M., Fält, A.-S. & Bryngelsson, T. 1996: Diversity in natural populations of wild *Brassica oleracea* as estimated by isozyme and RAPD analysis. — *Genetic Resources and Crop Evolution* **43** (in press).
- Nei, M. 1973: Analysis of gene diversity in subdivided populations. — *Proceedings of Natural Academy of Science U.S.A.* **70**: 3321-3323.
- Snogerup, S. 1971: Evolutionary and plant geographical aspects of chasmophytic plant communities. — Pp. 157-170 in: Davis, P. H. (ed.), *Plant life of South-West Asia*. — Edinburgh.
- 1980: The wild forms of the *Brassica oleracea* group ( $2n = 18$ ) and their possible relations to the cultivated ones. — Pp. 121-132 in: Tsunoda, S., Hinata, K. & Gómez-Campo, C. (ed.), *Brassica crops and wild allies*. — Tokyo.
- , Gustafsson, M. & Bothmer von, R. 1990: *Brassica* sect. *Brassica* (*Brassicaceae*). 1. Taxonomy and variation. — *Willdenowia* **19**: 271-365.
- Song, K., Osborn, T. C. & Williams, P. H. 1990: *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs): 3. Genome relationships in *Brassica* and related

- genera and the origin of *B. oleracea* and *B. rapa* (syn. *B. campestris*). — Theoretical and Applied Genetics **79**: 497-506.
- Safriel, U. N., Anikster, Y. & Valdman, M. 1997: Management of nature reserves for conservation of wild relatives and the significance of marginal populations. — *Bocconeia* **7**: 233-239.
- Warwick, S. I. & Black, L. D. 1991: Molecular systematics of *Brassica* and allied genera (subtribe *Brassicinae*, *Brassicaceae*) - chloroplast genome and cytodeme congruence. — Theoretical and Applied Genetics **82**: 81-92.

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