Wild Crop Relatives: Genomic and Breeding Resources

Oilseeds

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Chapter 2
Brassica

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2.1 Taxonomy of the Genus

Brassica species belong to the Brassicaceae (= Cruciferae) family and some of them are widely used in human diet mainly as an important source of vegetables, condiments, and edible oils. The use of the related crops is cited in some ancient civilized regions such as in the Mediterranean and in Asia. *Brassica* taxonomic studies started since 1700 by Tournefort and were continued by Linnaeus (1753), De Candolle (1821), Hooker (1862), Baillon (1871), Prantl (1891), Schulz (1919, 1936), and Beilstein et al. (2006). The genus *Brassica* is the most economically important genus within the Brassicaceae family and belongs to the subtribe Brassicinae, one of the nine subtribes of the Brassiceae tribe that shares with other 18 tribes a wide gene pool, which over time has been utilized directly or indirectly to improve several crops. Different species of the subtribes Raphaninae and Moricandiinae seem to be closely related to Brassicinae as confirmed by a long series of investigations on the chloroplast-DNA (cp-DNA) and restriction sites (Warwick and Black 1991; Pradhan et al. 1992; Warwick et al. 1992; Warwick and Sauder 2005). These authors distinguished vertically among these three tribes of two lineages represented by Rapa/Oleracea and Nigra as suggested earlier by Erickson et al. (1983), Yanagino et al. (1987), Palmer et al. (1983), and Song et al. (1988a, b, 1990).

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Since the last century, several cytogenetic investigations were carried out to determine chromosome numbers and chromosome pairing in interspecific *Brassica* hybrids. The small sizes and absence of evident distinguishing marks on the chromosomes did not permit to clarify *Brassica* pachytene, which was recognized for 36 species (Schulz 1919, 1936). The cytogenetic relationship of the main species of the *Brassica* genus was depicted in the U triangle (U 1935; Fig. 2.1) in which *Brassica nigra* (L.) Koch ($n = 8$), *Brassica oleracea* L. ($n = 9$), and *Brassica rapa* L. ($n = 10$) represent the three diploid species in the vertices, and they developed by intercrossing to the three amphidiploid species, *Brassica carinata* A. Braun ($n = 17$), *Brassica juncea* (L.) Czern. ($n = 18$), and *Brassica napus* L. ($n = 19$). The genome A was attributed to *B. rapa L.* (= *B. campestris* in the past), the genome-B to *B. nigra* and genome-C to *B. oleracea*. The genome-A is carried by Chinese cabbage, sarson turnip, turnip greens, turnip, and turnip rape crops, which on a morphological basis are assigned, respectively, to the leafy, rapifera and oleifera types. The Chinese cabbage is economically important in Asia as salad; sarson turnip is a minor crop in Europe and in New Zealand where it is utilized for food purposes; turnip greens and turnip tops are highly used in Portugal and northern Spain for culinary uses (Padilla et al. 2005); and turnip rape is widespread in the North America for oilseed production (McNaughton 1995a). *B. campestris* has been renamed as *B. rapa* according to the International Code of Botanical Nomenclature. Oost et al. (1987) used the name since this variant has been largely adopted. The genome-B is possessed by black mustard, which is nowadays diffused in Europe as weed but was well known in the Middle Ages in Europe as condiment. The genome-C is represented by *B. oleracea,*

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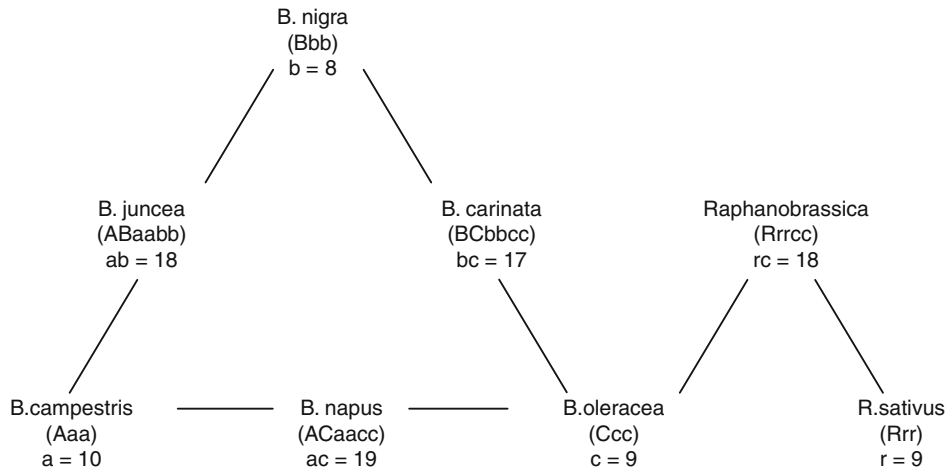

Fig. 2.1 U Triangle. In: Science, New Series, vol. 232, N. 4756, (Jun. 13, 1986), pp 1385–1389 published by American Association for the Advancement of Science

which diversified itself into several botanical varieties and related crops by domestication processes, such as var. *acephala,* var. *botrytis*, var. *capitata*, var. *gemmifera*, var. *gongylodes*, var. *italica*, and var. *sabauda*, which are represented, respectively, by kale, cauliflower, cabbage, Brussel's sprout, kohlrabi, broccoli and Savoy cabbage crops (Linnaeus 1753; Lamarck 1784; De Candolle 1821).

Among the amphidiploid species, *B. carinata* is represented by Ethiopian mustard diffused in Abyssinian Plateau derived from the union of the BB and CC-genomes; *B. juncea* by Indian mustard cultivated in Asia derived from the union of AA and BB-genomes; and *B. napus* mainly by oilseed rape grown in Asia, Europe, and North America derived from the union of the AA and CC-genomes (McNaughton 1995b). The genetic resources available for the breeding of *Brassica* crops are regulated by the genetic boundaries of their primary, secondary, and tertiary gene pools (Harlan 1975). *B. oleracea* represents the primary gene pool by itself, but several studies have been carried out to investigate the other gene pools and their potential utilization. The secondary gene pool was investigated by studies on the pachytene chromosome morphology, which permitted to identify the basic genomes of *Brassica* crops: AA ($2n = 20$) for *B. rapa*, BB ($2n = 16$) for *B. nigra*, and CC ($2n = 18$) for *B. oleracea*. Investigations on genomic libraries of *B. napus* and *B. oleracea* showed shared fragments among A, B, and C-genomes, suggesting their partial homology and the origin of the amphidiploid species *B. napus, B. carinata*, and *B. juncea* from the parental diploid ones (Hosaka et al. 1990; Slocum et al. 1990). The phylogenetic studies explain the evolution of *Brassica* and allied genera from a common ancestor with $n = 6$ through increase in the number of chromosomes and partial homology of A, B, and C-genomes (Prakash and Hinata 1980; Song et al. 1990).

Finally, the tertiary gene pool includes species and genera related to Brassica crops in 36 cytodemes capable of genetic flux, such as *Diplotaxis, Enarthrocarpus, Eruca, Erucastrum, Hirschfeldia, Rhynchosinapis, Sinapis, Sinapodendron,* and *Trachystoma* genera (Harberd 1976). These gene pools can confer favorable alleles and useful characteristics by using special methodologies. Tissue culture techniques, ovary and embryo rescue, and protoplast culture facilitated introgression of useful genes overcoming genetic boundaries.

In the last decades, a significant knowledge on pachytene studies was obtained through extensive use of molecular markers. In fact, molecular studies in *Brassica* species started with the determination of female parents of allopolyploid species using chloroplast-DNA (Palmer et al. 1983; Fukui et al. 1998) using genomic in situ hybridization (GISH) and fluorescence in situ hybridization methodology (FISH), in combination with ribosomal DNA markers (Schelfhout et al. 2004; Maluszynska and Hasterok 2005; Wang et al. 2005; Snowdon 2007). These methodologies

helped investigation on phylogenetic relationship within *Brassica* species and also with other related genera.

These studies have started to point out the potential germplasm of interest for Brassica genetic improvement that overcomes the biological boundaries. In addition, genomic investigations on *Arabidopsis thaliana* have facilitated understanding of evolution in the Brassicaceae family, especially for *Brassica* genus (*Arabidopsis* Genome Initiative 2000). These recent studies have indicated the constitution of the Brassica coenospecies formed by *Brassica* and allied taxa prefigured by Harbered (1972).

Even though the Himalayan region seems to be the primary center of diversity for Brassicaceae, from where dispersion extends to from North African and European Atlantic coasts to Saharo-Sindian phytochoreas, the southwest Mediterranean area seems to represent the secondary one, if not the primary, in the light of the recent evolutionary evidence recorded (Hedge 1976; Prakash et al. 2009).

With regard to morphological characters, high variation is evident among Brassiceae coenospecies with respect to cotyledon, adult leaf, and fruit shape (Gómez-Campo and Tortosa 1974; Prakash et al. 2009). The cotyledons are from small, slightly longer for *Diplotaxis* to wider with deeper notch for *Brassica, Raphanus, Coincya*, and *Sinapis*. Adult leaf typologies are (1) simple, entire to shallowly lobed; (2) lobed to pinnatifid; (3) pinnatisect with sinuses reaching the midnerve; (4) pinnatisect with reduced number of lateral segments (Prakash et al. 2009). The siliqua shows big variation for heteroarthocarpy, size, rib, rugosety, and wing. The evolutionary progress in *Brassica* species seems to be represented by the presence of seeds within the stylar cavity (Gómez-Campo 1999b). Half of the genera of this tribe present several types of seeded beaks showing heteroarthrocarpy. On the latter character, Gómez-Campo (1999a) formulated the "isthmus concept" of Brassiceae evolution and individuated in *Diplotaxis* genus the "bridge." Heteroarthropic siliqua with different beak size and shape is observed in *Erucastrum, Hirschfeldia, Sinapis, Coincya, Eurcaria, Trachystoma, Raphanus, Enarthrocarpus*, and *Brassica* genera. Although heteroarthrocarpy seems to represent an evolutionary crossroad, it does not support a monophylogenic evolution as showed by the chloroplast lineages distributed on both side of the "isthmus" (Prakash et al. 2009).

2.2 Conservation Initiatives

In the past decades, an important loss of natural genetic diversity of many crops has been observed due to many factors such as the introduction of new F_1 hybrids, droughts, changes in food habits and agricultural practices, and human activities such as deforestation and migration from rural to urban areas. This process is known as genetic drift. The loss of genetic variability represents not only the loss of wild germplasm but also the loss of evolved landraces resulting from the interaction of environmental selection with the genes present in both wild and cultivated populations. The *Brassica* genus has not been an exception and, in particular, conservation of wild *B. oleracea* species has been a high priority. During the 1970s, wild germplasm of *Brassica* was extensively collected and cytogenetic studies were started. Intensive efforts were made in the last decades to search and collect this material that, otherwise, would be irreversibly lost (Gómez-Campo et al. 2006, 2007). After 1970s, the introduction of the concept of biodiversity was a strong support for many improvements in ex situ and in situ conservation strategies.

Seed banks were created to maintain the genetic diversity of many crops, to minimize genetic erosion, and to supply seed material of landraces and of wild crop relatives for research. Most of the *Brassica* collections are conserved by means of seeds and, in general, they are conserved under long-term storage conditions to maintain seed viability for many years. The only exception within *Brassica* crops is a perennial kale (*B. oleracea* L. var. *ramose* DC) that can only be vegetatively propagated due to the loss of its ability to flower (Gómez-Campo 1999a).

Ex situ conservation of plant genetic resources in gene banks involves collecting traditional varieties and landraces from around the world and, in particular, from centers of genetic diversity of specific crops. The ex situ conservation also involves the selection of accessions to be conserved and the maintenance of these accessions for current and future users by regeneration. Decisions concerning both these aspects require knowledge about the distribution of genetic diversity within and between accessions sampled from the gene pool. However, they also require knowledge about changes in the variation of these samples as a result of regeneration activities.

One of the largest collections of wild *Brassica* species and allied cruciferous genera is kept by the Universidad Politécnica of Madrid (UPM), Spain. This seed bank was created in 1966 and its aim was the long-term ex situ conservation of wild taxa, thus making the accessions available for being used by researchers and breeders. The Plant Germplasm Bank from the UPM includes 600 crucifer accessions and rare and endangered species widespread in the western Mediterranean area and it is available at *http://www.etsia.upm.es/ANTIGUA/DEPARTAMENTOS/biologia/documentos/GC-2000-Int.htm*. Since 1982, several expeditions have been carried out by Professor Gómez-Campo from the UPM and his collaborators in order to rescue and collect Mediterranean populations of wild *Brassica* species. These missions were supported by the International Board for Plant Genetic Resources (IBPGR), later International Plant Genetic Research Institute (IPGRI), and now Bioversity International and were performed in the Mediterranean coast of Spain, Italy, Greece, and Tunisia and along the Atlantic coast of northern Spain, France, and the UK. As a result, different wild *B. oleracea* species with a chromosome number of $n = 9$ (including Atlantic *B. oleracea*) were collected. Four wild *B. oleracea*-related species were found in Sicily (*B. rupestris*, *B. incana*, *B. villosa*, and *B. macrocarpa*). Gómez-Campo and Gustafsson (1991) described the accessions collected in detail and the new locations found. According to the IPGRI policy, each sample was split into three parts, which were stored at the UPM (Spain), the University of Tohoku (Sendai, Japan) and also at seed banks of those countries, where the collection was done (Izmir, Turkey; Thessaloniki, Greece, Bari, Italy; Porquerolles, France; Kew, UK). Recently, two new expeditions have been carried out by the UPM team. The first one targeted the northern coast of Spain (Gómez-Campo et al. 2005) and the second one was focused on the northeastern coast of Spain in search of new localities and seeds of *B. montana* (Gómez-Campo et al. 2007).

In 1983, that collection was designated as the Global Base Collection for Wild Crucifers by the IBPGR, and in 1994, it was honored with the National Award for Environment by the Government of Spain. Recently, it has been included in the Global Biodiversity Information Facility database (*http://www.gbif.es*). The International Treaty on Plant Genetic Resources for Food and Agriculture that has recently been approved established a Multilateral System for having a facilitated access to the germplasm of a number of crops. This includes vegetables such as the Brassica complex with possible implications on the use of the diversity of these crops in the near future.

In Europe, and under the aegis of the European Cooperative Program for Crop Genetic Resources Networks (ECP/GR), a working group on Brassicas was established since 1991. One of the main efforts of this group has been to set up a European Brassica database (Bras-EDB), which was developed by the Center for Genetic Resources, Netherlands (Boukema and van Hintum 1998; *http://documents.plant.wur.nl/cgn/pgr/brasedb/*). This database includes cultivated materials as well as wild ones and contains 36 collections from 22 countries and more than 19,600 accessions. A list of wild *B. oleracea* species included in the European *Brassica* database is shown in Table 2.1. Major updates of Bras-EDB were done in 2001 and 2005, supported financially by the European Commission by means of the project RESGEN CT99 109-112: "*Brassica* collections for broadening agricultural use, including characterizing and utilizing genetic variation in *B. carinata* for its exploitation as an oilseed crop." The major aim was to create a core collection of the four *Brassica* species included in the project (*B. oleracea*, *B. rapa*, *B. napus*, and *B. carinata*). This project was an important attempt to unify efforts on Brassica germplasm within the EU and it was complementary to the activities of the ECPGR Working Group on *Brassica*.

Although wild species are included in ex situ collections, most of them are very difficult to regenerate ex situ to make them readily available to users. In this case, germplasm is conserved in its natural habitat (nature reserves) by specific in situ conservation activities. A strategy for in situ conservation of wild species related to *B. oleracea* has been elaborated by Maggioni et al. (1997). The implementation of a strategy for in situ conservation of wild species of the *B. oleracea* cytodeme has been recently suggested by the ECPGR Working Group on Brassica as a complementary way of preserving the diversity of these Mediterranean relatives of cultivated *Brassica* species with $n = 9$. Priority was assigned to the Sicilian center of diversity, where the level of variability is very high and the populations of *B. incana, B. macrocarpa*, *B. rupestris*, and *B. villosa* are often threatened by

Table 2.1 List of wild $n = 9$ *Brassica* species included in the European *Brassica* database and from the U.P.M. Crucifer Seed Bank

Species	Subspecies	Number accessions (Bras-EDB)[a]	Number accessions (UPM-seed collection)[b]
Brassica albogabra		62	1
Brassica bourgeaui		5	2
Brassica cretica		34	2
Brassica cretica	*aegaea*	25	5
Brassica cretica	*cretica*	38	
Brassica cretica	*laconica*	12	2
Brassica drepanensis		5	
Brassica hilarionis		4	1
Brassica incana		39	10
Brassica insularis		25	5
Brassica macrocarpa		9	2
Brassica montana		46	8
Brassica oleracea			9
Brassica oxyrrhina		1	1
Brassica rupestris		25	6
Brassica villosa		25	8

[a]Available from *http://documents.plant.wur.nl/cgn/pgr/brasedb/*
[b]Available from *http://www.etsia.upm.es/ANTIGUA/DEPARTAMENTOS/biologia/documentos/GC-2000-Int.htm*

human activities (Figs. 2.2 and 2.3). The objective will be the evaluation of these wild species under a different point of view (DNA analysis, morphological traits, and quality aspects focused on oils and nutraceutical compounds). The role of the Working Group is seen as a contribution to highlight the usefulness of the wild germplasm for breeding purposes and to select the most appropriate accessions of the future European Genebank Integrated System (Astley et al. 2007).

Since 2007, AEGRO GENRES project founded by the European Union deals with a basic research for an Integrated European in situ management work plan to implement Genetic reserves and "on farm" concept. The case of study is related to *Avena*, *Beta*, *Brassica*, and *Prunus* with a view to develop in situ management work plans for conservation of crop wild relatives (CWR) and landraces. For case study on Brassica, the attention has been paid on a Sicilian wild *Brassica* with $n = 9$ widespread in the Island. These studies have to contribute to the development of a CWR in situ conservation strategy for Brassica in Sicily, which will form part of the European integrated work plan for management of CWR (*http://aegro.bafz.de*).

Brassica diversity conservation has been stimulated by setting up a specific core collection named Diversity Foundation Sets (DFSs), designed to represent "an informative set of genetically fixed lines representing a structured sampling of diversity across a genepool," which is under development at the Warwick HRI. These collections are based on founder accessions sourced from ex situ genetic resource collections (see *http://www.Brassica.info/diversity/diversity_sets.htm*). They are designed to represent the diversity within the *B. oleracea* crop gene pool (BolDFS) whilst the *Brassica* C-genome Diversity Fixed Foundation Set (BCgDFS) aims to fix the diversity of *B. oleracea,* which represent the C-genome with the wild *Brassica* species ($n = 9$) that could be its wild relatives.

One of the main problems that germplasm curators must face is to maintain collections in active banks in good conditions of viability to minimize the need for regeneration (Gómez-Campo et al. 2006). Regeneration of *Brassica* is very costly. Therefore, good storage conditions are essential in order to maintain the seed viability. Gómez-Campo (2002) evaluated 40 different types of containers according to their ability to exclude water vapor, by using silica gel with a cobalt indicator. Only sealed brass cans, "Kilner" jars with rubber seals, laboratory bottles normally used for liquid chemicals, or flame-sealed glass ampoules were considered to be safe for use in long-term preservation. The 36 remaining containers allowed moisture to enter within 2 or 3 years or less. Currently, Brassica seeds at the UPM are kept in flame-sealed glass vials,

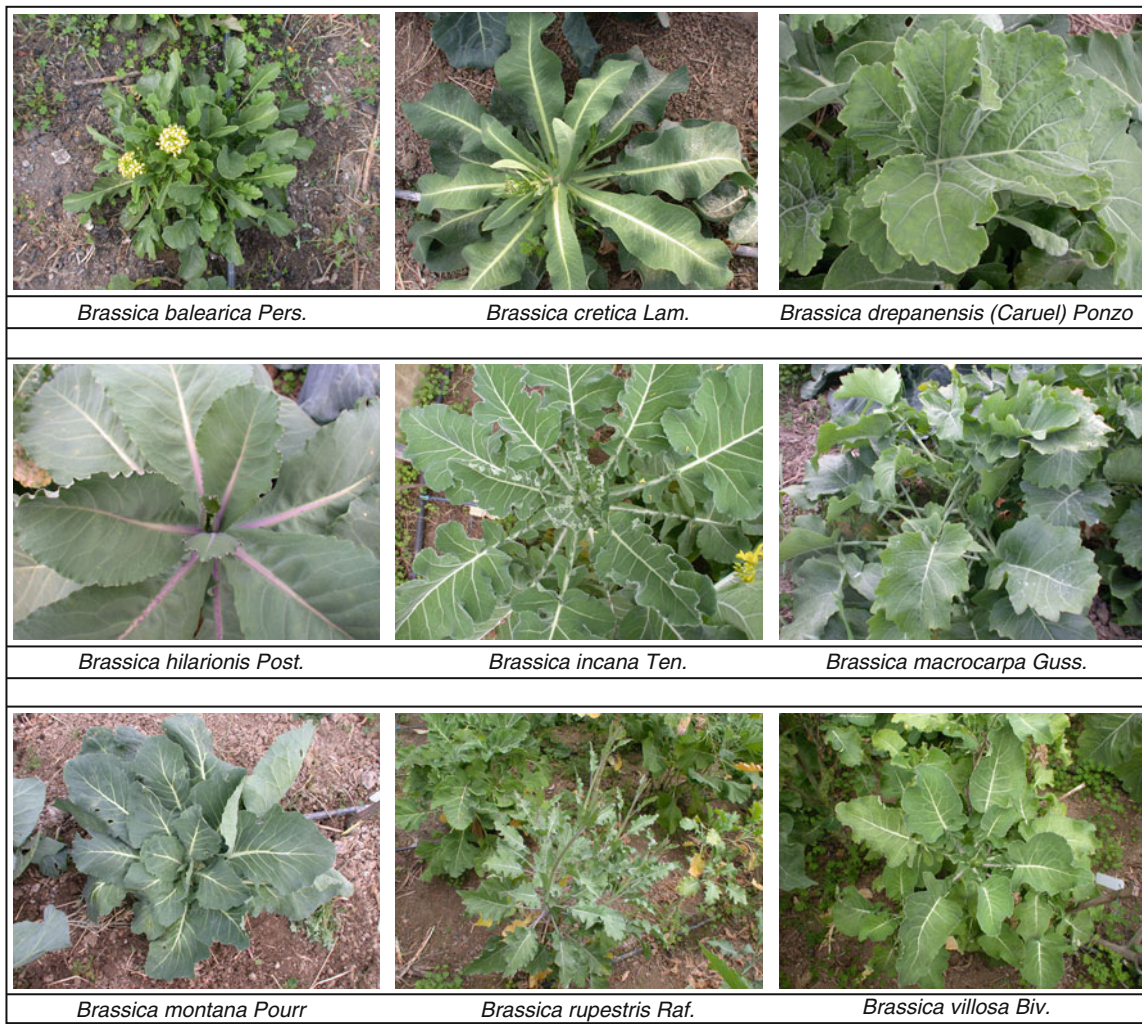

Fig. 2.2 Plant morphological diversity of Brassica wild species

having silica gel to ensure low moisture content, because this method is very convenient for small-sized seeds as those of Brassicas. Other possibilities to adapt this method to crop species have been explored (see *www.seedcontainers.net*). More recently, Pérez-García et al. (2007, 2008) concluded that the seed preservation method based on silica gel and low temperature (−5°C and −10°C) have proved to be highly efficient for Brassicaceae and other plant families and proposed the possibility of using ultra-dry methods for medium and long-term storage of orthodox seeds.

Another method to preserve seed germplasm is cryopreservation. It consists of storing the material at temperatures near that of liquid nitrogen (−196°C). Under these conditions, all enzymatic processes are practically halted, and it is thought that any type of biological plant material (meristems, embryos, pollen, seeds, somatic tissues, etc.) can thus be preserved for an infinite period of time. For an efficient cryopreservation, it is fundamental to avoid the intracellular formation of ice crystals, which are highly damaging for the cell internal structures.

Pérez-García et al. (1996) evaluated the effect of cryopreservation on seeds of seven wild and cultivated *Brassica* taxa. They concluded that *Brassica* seed cryopreservation is a suitable procedure for the long-term maintenance of seed accessions of this genus (wild and cultivated species) in seed banks. Low

Fig. 2.3 Plant morphological diversity of Brassica wild species

temperature preservation is very effective not only for maintaining germplasm but also for the storage of mature pollen, vegetative stems, cell and protoplast suspensions, and microspores of many species. Microspore cryopreservation is a potentially powerful method for long-term storage of germplasm destined to in vitro embryo production in plant species. Charne et al. (1988) described the method of cryopreservation of isolated microspores of rapeseed in liquid nitrogen without loss of embryogenic capacity and proposed this approach as a useful method to increase the efficiency of the rapeseed haploid system. On the other hand, Chen and Beversdorf (1992) found that isolated microspores of *B. napus* could be stored stably for an extended period of time by using cryopreservation and proposed that this storage system can be used in the

rapeseed breeding program to produce doubled haploid lines.

In the US, the database for Germplasm Resources Information Network (GRIN) maintain at the Beltsville Agricultural Research Center the accessions held by the Regional Plant Introduction Stations. This collection is duplicated and stored for a long term at the National Seed Storage Laboratory at Fort Collins (ARS-GRIN 1997). In China, the Chinese Genetic Resources Information System – CGRIS – supports the national network of regional gene banks coordinated by the Institute of Crop Germplasm Resources, which has the responsibility for the long-term conservation of genetic resources.

2.3 Origin and Evolution of Allied Crops

The relationship among the different *Brassica* species started to be explained by Morinaga (1934) and U (1935) with the already-cited U-triangle, and according to them, the diploid species *B. rapa* (AA-genome), *B. nigra* (BB-genome), and *B. oleracea* (CC-genome) originated along the same time as the allotretrapoid species *B. juncea* (AABB), *B. napus* (AACC), and *B. carinata* (BBCC). During domestication process of each species, divergent selection enriched the diversity of the correspondent cultivars and crops. Natural hybridization events have been the basis of genome evolution of *Brassica* and interspecific crosses enabling gene exchange contributed significantly to the differentiation of the genus by generating new types or species, allowing gene exchange across boundary species. Of course, genome similarity is required to ensure chromosome pairing and genetic recombination (Leflon et al. 2006). Several studies have permitted to study the possible phylogeny of the species also by the creation of new species since 1920s when Karpechenko developed the synthetic genus *Raphanobrassica* by crossing of *Raphanus sativus* with *B. oleracea* var. *capitata* to combine their desirable traits.

Archeological evidences of the main diploid species suggest *B. rapa* (turnip rape) and *B. nigra* (black mustard) to be the first domesticated species similarly as the amphidiploid *B. juncea* (Indian mustard) that originated from crosses among the two former species. Cytogenetic evidences suggest that the evolution of the diploid species started with *B. nigra* and was followed by *B. rapa* and *B. oleracea*. Molecular studies discard the monophyletic origin and suggest *B. oleracea* and *B. rapa* to have a common origin and the same progenitor, whereas *B. nigra* evolved from another one (Namai 1976; Prakash and Hinata 1980; Song et al. 1988a; Pradhan et al. 1992; Palmer et al. 1983). This is confirmed by the two lineages "nigra" and "rapa/oleracea" of subgen. *Brassica* as suggested by several authors (Song et al. 1990; Warwick and Black 1991; Pradhan et al. 1992). The former lineage show higher affinities with the genera *Hirschfeldia* and *Sinapis* and with some species of *Diplotaxis* and *Erucastrum*, whereas the latter with all the species belonging to Sect. *Brassica,* Sect. *Rapa* and Sect. *Brassicoide,* and with the species *B. barrelieri* and *B. oxyrrhina* of the Sect. *Sinapistrum* (Gómez-Campo 1999a).

Alloploid species were originated by unidirectional natural interspecific hybridizations, whereas *B. nigra* and *B. rapa* were the cytoplasmic donors of *B. carinata* and *B. juncea,* and *B. oleracea* was the cytoplasmic donor of *B. napus* (Erickson et al. 1983; Palmer et al. 1983; Warwick and Black 1991; Pradhan et al. 1992). The evolution of the species was identified by the mitochondria and chloroplast genomes, which are co-inherited and thereby could evidence for the more recent origin of the allopolyploid species. Among them, *B. juncea* seems to be originated earlier in comparison to *B. carinata* and *B. napus*. The analysis of the cytoplasmatic genomes offered by maternal parents indicated stable genome for *B. juncea* and *B. carinata,* whereas *B. napus* seems to have a polyphyletic origin where *B. oleracea* seems to play an important role (Song and Osborn 1992; Parkin and Lodiate 1997). This fits with the hypothesis that *B. oleracea* was originated later than other *Brassica* diploid species (Quirós et al. 1985).

B. nigra (L.) Koch. is well known since the Greek civilization for its medicinal proprieties (Hippocrates 480 BC). This species is widespread in the Mediterranean basin and in some central Asian and Middle East areas and is cited as "mustard" in the New Testament for its fast growing habit. During that time, attention had been paid to similar uses of *B. juncea* and *B. carinata* in spite of *B. nigra,* which contributed as parent to the origin of both the former species. The proposed ancestor of *B. nigra* is *Sinapis arvensis,* which show high homology in terms of nuclear

DNA, cp-DNA, and protein fraction with *B. nigra* (Song et al. 1988a; Warwick and Black 1991; Pradhan et al. 1992; Poulsen et al. 1994). In addition, high degrees of pairing and similar genetic sequences were detected for the interspecific hybrids of *B. nigra* × *S. arvensis* (Mizushima 1950; Cheng and Heneen 1995). Other candidate ancestors of *nigra* lineage seem to be *Hirshfeldia incana* ($n = 7$) with some *Erucastrum* species, of which fruits are less specialized in comparison to the species, and those belonging to the Sect. *Rhynchocarpon* of *Diplotaxis* and to *Sinapidendron* genera, the latter showing seedless beak Gómez-Campo (1999a). High degree of similarity for nuclear DNA and cp-DNA has been ascertained also for *B. fruticulosa* (Takahata and Hinata 1983; Song et al. 1990; Warwick and Black 1991; Pradhan et al. 1992).

Turnip is widespread in natural habitat from Mediterranean to central Asia as a weed and probably was the first Brassica domesticated because it is very rustic, invasive, and easy to grow. In addition, it has several uses, and for that, it has been considered along the time as a multifunctional crop. Since some millennia ago, *B. rapa* (syn. *B. campestris*) was domesticated to use its roots, young flowering shoots, and seeds by several civilizations. Turnip was recovered from the Neolithic sites and was proposed in cultivation around 2500–2000 BC (De Candolle 1886; Hyams 1971). Plinius (23–79 AD) distinguished domesticated forms having flat and round roots from wild ones with big roots, whereas Columella (ca. 60 AD) mentioned types called as "Long Roman," "Round of Spain," "Syrian," "White," and "Egyptian."

Leafy vegetable forms were differentiated in western Asia from oilseed forms of *B. rapa* introduced through central Asia, and among them, pak-choi (subsp. *chinensis*) was the first to be utilized in China (Li 1982). The subsp. *pekinensis,* well known as Chinese cabbage, originated around the tenth century from the hybridization between pak-choi and turnip and that has been confirmed by restriction fragment length polymorphism (RFLP) analysis (Song et al. 1988b). The European oleiferous *B. rapa* forms were developed in the Mediterranean basin whereas the Asian forms in the central Asia and Northwest India. In the latter area are distributed the brown sarson, toria, and yellow sarson. The oldest form seems to belong to the brown sarson, which is different from toria by plant morphology and size, and growing period, whereas the yellow sarson is distinguished by yellow colored seed and self-compatibility.

Yellow sarson is cited in the ancient Asian literature around 1500 BC (Prakash 1961; Watt 1989) and *B. rapa* seeds were identified in the stomach of a Tollund man who lived in Denmark in the fourth century BC (Renfrow 1973). Several authors agree with the comparative morphological studies of Sun (1946), which proposed two evolutionary lines of *B. rapa*, one western, in Europe and central Asia, where turnip and oilseed forms were domesticated, and the another eastern, in East Asia, in the areas of diversification of several vegetable forms. These two independent centers of origin of *B. rapa* are supported by morphological, geographical, and molecular evidences (Denford and Vaughan 1977; Song et al. 1988b).

B. oleracea, for example, is represented by several varieties, which originated several crops very different for growth habits and organ morphology. Among these, var. *acephala,* which is represented by several and diversified landraces of kale in the Mediterranean European countries, seems to be the evolutionary bridge for the other varieties of *B. oleracea.* In fact, the great variability exhibited by a core collection of European kale landraces studied by the Universities of Alnarp and Catania seems to support this hypothesis. In any case, kale landraces are often widely present in Europe in areas where the wild *Brassica* species related to *B. oleracea* are diffused and all of them are perennials. Furthermore, some wild *Brassica* species sprouts until now and are gathered and utilized in some Sicilian villages when young inflorescences start to flower to utilize the fleshy leaves and the tender shoots. Among all the varieties of *B. oleracea*, only var. *acephala* shows this characteristic, and its variants are cited in ancient Greek and Latin literature (Maggioni et al. 2010). Several authors indicate the Mediterranean basin as the center of diversity of *B. oleracea*, at least for broccoli and cauliflower, where several wild *Brassica* species ($n = 9$) are widespread and show great diversity (Gray 1982; Smith and King 2000). Recently, linguistic and literary considerations on the origin and domestication of *B. oleracea* crops suggest that its domestication process started in ancient Greek-speaking areas of the central and East Mediterranean areas (Maggioni et al. 2010). In any case, the *B. oleracea* gene pool is very rich in Sicily where *B. drepanensis, B. incana, B. macrocarpa, B. rupestris*, and *B. villosa* are

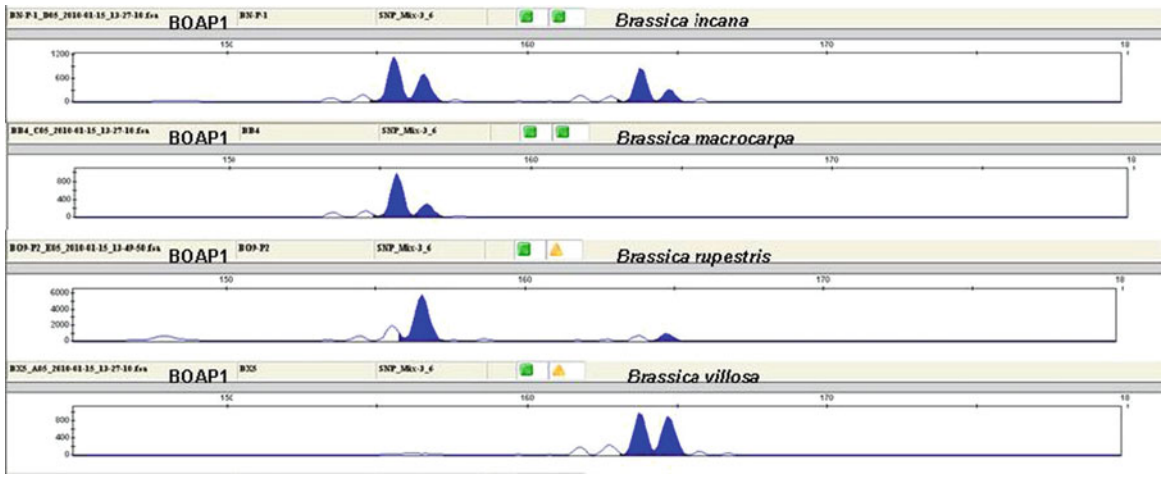

Fig. 2.4 Tetrasomy ascertained for BoAP1 SSR primer for some genotypes of crop wild relatives of Brassica widespread in Sicily

widespread, and in some areas, they are strictly associated among themselves and with *B. oleracea* crops (Branca 2008). Snogerup (1980) suggested multiple origins of several *B. oleracea* crops, which are the Atlantic coast for cabbage and the Mediterranean basin for kale, broccoli, and cauliflower. Glucosinolate profile of these wild species is also diversified in Sicily and, in some cases, is specific for each species (Song et al. 1990; Horn and Vaughan 1983; Mithen et al. 1987; Velasco and Becker 2000; Branca et al. 2002). In any case, F_1 hybrids between *B. oleracea* and wild *Brassica* are often fully fertile (Mithen et al. 1987; von Bothmer et al. 1995; Faulkner et al. 1998; Gómez-Campo 1999a).

Recent DNA analysis with molecular techniques support high similarity between Sicilian wild *Brassica* species ($n = 9$) and *B. oleracea* (Song et al. 1990; Lanner et al. 1996; Lázaro and Aguinagalde 1996; Lanner 1998; Tatout et al. 1999; Geraci et al. 2001). Recently, more similarity was observed between *B. oleracea* and the Mediterranean wild *Brassica* species than the UK *B. oleracea* wild types (Allender et al. 2007). Utilizing the simple sequence repeat (SSR) marker BoAP1, higher number of alleles were found in the wild *Brassica* species than in cabbage and cauliflower.

BoAp1-a locus located in a single genomic region on linkage group O6 chromosome of *B. oleracea* with the other ones (BoCAL, BoLFY, BoAP1-c, BoREM1, BoFULL, etc.) is related to MADs-box genes involved in flower development and evolution (Duclos and Björkman 2005). This O6 chromosomal region is strictly related to self-incompatibility controlled by the *S*-locus. This genomic region seems to be the key for selection while evolution of cauliflower that had been a subject of heavy pressure for diversification of *B. oleracea* originally utilized for its vegetative organs. Recently, characterization of Sicilian wild *Brassica* species carried out in the frame of the EU GENRES-AEGRO project, based on biomorphological, biochemical, and molecular descriptors, has started to provide important information on the genetic diversity of the *BoAP1-a* alleles of several wild populations in comparison to landraces of broccoli, cauliflower, and kale. Single nucleotide polymorphism (SNP) DNA sequence related to the BoAP1 SSR marker showed diversity of alleles for this locus. In addition, in some wild populations near villages, where *B. oleracea* crops are usually grown in several home gardens, have individuated trisonomy and tetrasomy, the signs of interspecific hybridizations with landraces (Fig. 2.4). Several studies are in progress to gain comprehensive knowledge on the genetic diversity in Sicily with a view to individuate adequate methodologies for on-farm and in situ conservation and, in the latter case, to establish genetic reserve for *Brassica*.

2.4 Role in Crop Improvement Through Traditional and Advanced Tools

Considerable progress has been accomplished in the cellular and molecular biology of *Brassica* species in the past years. The use of molecular markers in marker-assisted selection and breeding, genetic

transformation technology for the introduction of desirable traits and a comparative analysis of these traits are important components of the current research on this genus. Research priority in the *Brassica* genus was initially focused on polyploidy breeding. This original aim was later modified to the exploitation of wild allies for introgression of nuclear genes for desirable traits, cytoplasmic substitutions, and construction of chromosome maps. In the long history of the variety development of Brassica crops, genetic introgression from wild donor plants was a major approach for the introduction of valuable genes and traits.

Crosses between and among *B. oleracea* and C-genome relatives are known to produce fertile or semi-fertile offspring (Kianian and Quiros 1992; Gómez-Campo 1999a). Thus, transfer of desirable genes governing qualitative and quantitative characters from wild allies into cultivated forms can be achieved both by conventional crosses and biotechnology, depending on the relatedness and crossability of the donor wild species with Brassica crops. The new technologies have facilitated breeding programs, increased the efficiency of locating desirable traits, and have opened up new opportunities for using genes that were previously inaccessible. The level of success to transfer useful genetic variation from wild sources through crosses depends on many factors such as the extent of diversity that can be accessed to introduce useful variation, the risk to introduce deleterious traits, the possibility to use a particular valuable allele in different genetic backgrounds, and the efficiency with which useful alleles can be transferred. Moreover, most of the wild species are difficult to exploit in research programs mainly because of their extended vegetative phase or due to the difficulty to obtain homozygous material (doubled haploid lines) by in vitro culture.

As the wild germplasm belongs to second and tertiary gene pools, several kinds of hybridization operate. Consequently, many of the wild species are sexually incompatible with the crop species, thus making the genes present in wild forms inaccessible. Sexual incompatibility has been overcome by advances in cellular and molecular biology, which facilitate transfer of desirable genes into plants and cloning and manipulation of genes. Several forms of manipulations have been carried out to obtain sexual hybrids as bud pollinations, grafting or mixed pollinations, and subsequent ovule or embryo rescue techniques (Inomata 1985). In addition to traditional breeding methods, interspecific and intergeneric crosses have been facilitated with various approaches, such as somatic cell genetics and recombinant DNA techniques. Interspecific and intergeneric hybrids in Brassica crops produced by sexual and asexual hybridization, embryo rescue, and genetic manipulations have been described in several reviews (Glimelius 1999; Christhey 2004).

In the recent past, the development of in vitro techniques, such as ovary and embryo culture and somatic hybridization, has increased greatly. The embryo culture technique allows overcoming the post-fertilization barriers between distant related species, while the somatic approach becomes the best method of choice to realize hybridization where pre-fertilization barriers exist. Somatic hybridization has been extensively used in the Brassicaceae with the additional merit of inducing cytoplasmic variability and recombination of cytoplasmic and nucleic genomes, which is not possible through conventional methods of sexual hybridization (reviewed by Glimelius 1999; Christhey 2004; Liu et al. 2005).

The introgression of valuable traits by interspecific hybridizations from wild Brassicas can be traced back in literature to 1950 for sexual cross and to 1979 for somatic hybridization (Prakash et al. 2009). Despite the advantages, somatic hybridization also has some drawbacks. The technique needs to be improved since only a limited number of hybrids are produced in many experiments, thus reducing the possibility of selecting usable plants among hybrids. A detailed review of the intrageneric, intergeneric, and intertribal somatic hybrids along with the traits of interest incorporated has been published by Glimelius (1999).

Regarding *B. oleracea* wild relatives, hybrids between them and their cultivated forms resulted to be at least partially fertile. Hybrids like *B. napus* plants derived from in vitro culture of embryos resulting from crosses between *B. cretica* and *B. rapa* were obtained by Inomata (1985) and hybrids obtained from this cross may be valuable in broadening the narrow genetic base of oilseed rape. Other interspecific hybrids by embryo rescue between *B. cretica, B. montana*, and *B. bourgeaui* with *B. napus* and *B. rapa* were obtained by the same author (Inomata 1986, 1987, 1993, 2002). On the other hand, Prakash and Chopra (1990) and Mithen and Herron (1991)

obtained hybrids by sexual hybridization between *B. oxyrrhina* (as female) and *B. rapa* (as male) and between *B. atlantica* (as male) and *B. rapa* (as female).

Wild *Brassica* species possess a number of useful agronomic traits. They have been widely used in plant breeding programs to broaden the genetic base in most Brassica crop species. They have also been used as sources of donor elite alleles, controlling economically important quantitative traits including crop production, disease and pest resistance, tolerance to abiotic stresses (cold, salt and drought conditions), and specialty components of quality attributes (seed oil or glucosinolates) (Ramsey and Ellis 1994). Moreover, wild relatives could be incorporated into breeding programs, including cytoplasmic and nuclear male sterility for the development of hybrid seed production. Warwick et al. (2000) published a guide to wild *Brassica* germplasm that provides information on their growth form, chromosome number, geographical distribution, and quality and agronomic traits of interest. Examples of wild relatives of *B. oleracea* as potential sources of desirable traits are shown in Table 2.2.

Regarding biotic stresses, it has been found that wild *B. oleracea* species can carry important resistance traits related to biotic stresses, and a number of potential sources of resistance are available among wild allies against various pathogens. Gene introgression from wild relatives by using different approaches (sexual hybridization, embryo rescue, protoplast fusion, and genetic transformations) can be found in the literature. For instance, it has been found that wild *B. oleracea* populations from Sicily are resistant against the flea beetle disease (Palaniswamy and Bodnaryk 1994); *B. incana* has been proved to be the best source of resistance against *Verticillium wilt* among different cultivated and wild forms of *B. oleracea* species including *B. cretica*, *B. incana*, *B. insularis*, and *B. villosa* (Happstadius et al. 2003). Because sources of resistance to this fungal disease are not found in the oilseed rape germplasm, finding of these results are of great interest. Regarding pest resistance, Ellis et al. (2000) found germplasm resistant to *Brevicoryne brassicae* within wild *B. oleracea* including *B. villosa* and *B. incana* and also identified sources of resistance to *Delia radicum* in wild species (Ellis et al. 1999). In general, satisfactory genetic control of pathogens and virus diseases has not been achieved by using wild *Brassica* species. This is primarily due to the absence of sources of resistance for the most severe pathogens of Brassica crops. Thus, there is an urgent need to develop methods for identifying resistance genes in the wild species. Informative reviews have been published on pest resistance (Earle et al. 2004) and disease resistance (Tewari and Mithen 1999).

Regarding the quality attributes related to the seed fatty acid composition, the use of *B. villosa, B. incana,* and *B. rupestris* as sources of high erucic acid, which is highly appreciated for different industrial uses, merits a special mention (Velasco et al. 1998). The potential use of several wild *B. oleracea* species, mainly *B. villosa* as a donor of beneficial glucosinolates, such as glucoiberin or glucoraphanin that are closely related to human health due to anticarcinogenic properties has also been reported (Mithen et al. 1987; Faulkner et al. 1998). Among other wild *B. oleracea* species from the $n = 9$ complex that are useful as donors of valuable genes, special attention should be paid to *B. oxyrrhima* (Prakash and Chopra 1990), which has been used as a donor species for the development of new cytoplasmic male sterile (CMS) lines and *B. hilarionis and B. macrocarpa,* which have also been identified as potential donors of resistance to pod shattering (Mithen and Herron 1991; Table 2.2).

2.5 Genomics Resources Developed

Genetic studies in Brassicaceae can be traced back to the first half of the twentieth century. However, most progress in comparative mapping was made since the beginning of the 1990s, and this was coincided with a period of rapid progress in molecular marker technologies. Molecular markers have been intensively used in *Brassica* species, and preliminary maps were constructed by employing restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and DNA fingerprinting (Quirós 2001; Quirós and Paterson 2004). The development of genetic maps in *Brassica* has been used for two purposes: first, to utilize them in genetics and breeding, and second, to analyze the genetic relationships among Brassica crops and wild related species. More precisely, wild forms of *Brassica* (including some $n = 9$ Sicilian populations) have been studied by using RAPDs (Lanner et al. 1996; Lázaro and Aguinagalde 1996, 1998), RFLPs (Song et al. 1990),

Table 2.2 Wild *Brassica oleracea* species as sources of desirable traits

Trait	Species	Reference
Agronomic traits		
Resistance to pod shattering	*Brassica macrocarpa*	Mithen and Herron (1991)
	Brassica hilarionis	Mithen and Herron (1991)
Quality traits		
Glucosinolates	Wild *Brassica oleracea* complex	Mithen et al. (1987a)
High glucoraphanin	*Brassica villosa*	Faulkner et al. (1998), Mithen et al. (2003), Sarikamis et al. (2006)
High erucic acid (> 45–50%)	*Brassica cretica*	Yaniv et al. (1991)
	Brassica villosa	Velasco et al. (1998)
	Brassica incana	Velasco et al. (1998)
	Brassica rupestris	Velasco et al. (1998)
Breeding systems		
Cytoplasmic male sterility	*Brassica oxyrrhina*	Prakash and Chopra (1990)
Biotic stress		
Disease resistance		
Blackleg – *Leptosphaeria maculans* (*Phoma lingam*)	*Brassica insularis*	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
	Brassica atlantica	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
	Brassica macrocarpa	Mithen et al. (1987b), Mithen and Herron (1991), Mithen and Magrath (1992)
Verticillium wilt	*B. incana*	Happstadius et al. (2003)
Downy mildew – *Peronospora parasitica*	*Brassica oleracea,* wild accessions	Greenhalgh and Mitchell (1976)
Insect resistance		
Cabbage aphid (*Brevicoryne brassicae*)	*B. incana*	Ellis et al. (2000)
	B. villosa	Ellis et al. (2000)
Flea beetle (*Phyllotreta cruciferae*) and *P. striolata*	*Brassica incana*	Bodnaryk (1992)
Cabbage white fly (*Aleyrodes proletella*)	*Brassica villosa*	Bodnaryk (1992)
	Brassica cretica	Ramsey and Ellis (1994)
	Brassica incana	Ramsey and Ellis (1994)
	Brassica villosa	Ramsey and Ellis (1994)
	Brassica spinosa	Ramsey and Ellis (1994)
	Brassica. insularis,	Ramsey and Ellis (1994)
Cabbage root fly – *Delia radicum*	*Brassica incana*	Ellis et al. (1999)
	Brassica villosa	Ellis et al. (1999)
	Brassica macrocarpa	Ellis et al. (1999)

and by analyzing specific sequences of chloroplast DNA (cp-DNA) (Lanner 1998).

Nowadays, Brassica databases have been developed and are being made publicly available (*http://www.brassica.info/resources.php*), managing information related to *Brassica* genetics and genomics. A set of sequence accessions, genetic maps, and markers are accessible at *http://brassica.bbsrc.ac.uk/BrassicaDB/*. In addition, this database is currently the original source of information about the "BBSRC set" of Brassica SSR markers. Among genetic resources, bacterial *E. coli* clones are widely used to isolate and characterize subsets of DNA and RNA sequences and are especially useful for characterizing complex genomes such as of *Brassica*. Genomic bacterial artificial chromosome (BAC) libraries are now available for *B. rapa*, *B. oleracea*, and *B. napus*. These databases are complemented with expressed sequence tags (ESTs) together with reference to doubled haploid mapping populations, associated linkage maps, and

public domain molecular markers (*http://www.brassica.info/ssr/SSRinfo.htm*).

The major advances in comparative genetics and molecular cytogenetics in cultivated and wild species, as well as the potential of *Arabidopsis* genomic resources for comparative studies, have been the scope of recent reviews in the last 10–15 years (Qiu et al. 2009). Species of the *Brassica* genus are closely related to *A. thaliana*, which also belongs to the Brassicaceae family. This close relationship between the two genera, *Arabidopsis* and *Brassica*, is reflected by an average identity of exon sequences at the nucleotide level, which is estimated to be 87% (Cavell et al. 1998). The completion of the *Arabidopsis* genome sequence (The *Arabidopsis* Genome Initiative 2000) has provided a valuable resource for identifying genes involved in agronomic and nutritional aspects of *Brassica* species, including the genes responsible for head formation in cauliflower and broccoli (Lan and Paterson 2000) and the genes involved in glucosinolate biosynthesis (Li and Quirós 2003). As anticipated by Lan et al. (2000), the generation of ESTs in *A. thaliana* holds an enormous potential for the cross-genomic analysis of alleles conferring specific phenotypes to Brassica. For instance, ESTs from *Arabidopsis* have been used as RFLP markers in *B. oleracea*, for comparison of the genomes of both species (Babula et al. 2003). Batley et al. (2007) demonstrated the utility of EST-SSRs for the genetic analysis of wild *Brassica* populations and commercial *Brassica* germplasm, since these markers were polymorphic and showed a consistent amplification and genome specificity. A summary of EST clones in different *Brassica* species can be found at *http://www.brassica.info/resource/clones.php*.

The genetic diversity and relationships among C-genome species have been well studied based on ecogeographic, phenotypic, and genotypic information (see Gómez-Campo 1999a). However, it is difficult to make comparisons between molecular studies, as different genetic marker systems have been used on different populations and accessions of variable or loosely defined provenance. Both nuclear and organelle-based molecular markers have been used to generate genotypic datasets. Song et al. (1990) used RFLP analysis to compare *B. oleracea* and nine C-genome wild species with *B. rapa* and found that *B. oleracea* formed a paralogous clade with its wild relatives. However, studies using other marker systems suggest different evolutionary relationships. Dendrograms based on RAPD markers (Lázaro and Aguinagalde 1996; Geraci et al. 2001) and isozyme data (Lázaro and Aguinagalde 1998) indicated that *B. oleracea* clustered with species such as *B. montana* and *B. incana*. In contrast, Tatout et al. (1999) used short interspersed nuclear element (SINE) transposons as markers and found that *B. oleracea* and *B. incana* were more similar to species such as *B. hilarionis*. A detailed study of the relationships within the *B. oleracea* cytodemes was carried out by Lanner (1998) who used non-coding sequence from the chloroplast genome to examine diversity and relationships between *B. oleracea* and nine C-genome species. More recently, Allender et al. (2007) assessed the utility of chloroplast SSRs as markers for diversity and phylogeographic studies among the *Brassica* species ($n = 9$) and found that diversity revealed by chloroplast SSRs is present in the Mediterranean wild species and is apparently almost absent from the contemporary UK natural populations of *B. oleracea* itself. This finding has implications both for the conservation of natural genetic diversity and for the search for novel sources of alleles to be used in crop improvement programs.

2.6 Scope for Domestication and Commercialization

In the past, people depended exclusively on herbal remedies or traditional medicines and used some wild plants for cosmetic and perfumery purposes. Nevertheless, in the recent years, medicinal plants have represented a primary health care source for the pharmaceutical and perfumery industries. Global trend leading to increased demand of medicinal plants for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics, and other products is an opportunity sector for wild and cultivated *Brassica* species. In that context, wild relatives of *B. oleracea* could have potential as sources of oil, condiments, and other products and, therefore, they can be used for food, medicinal purposes, (nutraceutical crops) and alternative uses as biocide crops.

Medicinal plants contain biologically active chemical substances such as coumarins, volatile oils, alkaloids, etc. In addition to these substances, plants may

contain other chemical compounds. In Brassicaceae, phytochemicals such as indole phytoalexins, phenolics, and glucosinolates are the most abundant. All of these phytochemicals contribute to the antioxidant, anticarcinogenic, and cardiovascular protective activities of Brassica vegetables (Podsedek 2007; Jahangir et al. 2009), which increase their value as therapeutic compounds to be used in medicine and as food supplements in the human diet and their value as biocides or pest deterrents in agriculture.

More specifically, and regarding glucosinolates, numerous studies have highlighted the idea that glucosinolates produced by plants may be useful as plant protection agents, as dietary supplements, and for obtaining pharmaceutical products for treating cancer, viral infections, or autoimmune diseases. After tissue damage, myrosinase (thioglucoside glucohydrolase) hydrolyzes the naturally occurring glucosinolates present, thus producing a number of end products including isothiocyanates, thiocyanate ions, nitriles, and epithionitriles according to the type of glucosinolates present and the exact hydrolysis conditions. Among these, isothiocyanates display different biological functions that allow their use as food, drugs, or fine chemicals.

Many wild members of the *B. oleracea* species complex have high levels of individual aliphatic glucosinolates (Mithen et al. 1987). The first studies about leaf and seed glucosinolates content in wild *B. oleracea* relatives can be found in the eighties. Mithen et al. (1987) analyzed leaf glucosinolates in 18 *B. oleracea* populations, including wild and cultivated crops, and found a great variability among species in glucosinolate content and profile. The major glucosinolate found in *B. montana, B. incana*, and *B. cretica* subsp. *cretica* was gluconapin, whereas *B. rupestris* does not contain gluconapin but contains glucoiberin; *B. drepanensis* contains glucoiberin and glucoiberverin, whereas *B. macrocarpa*, *B. insularis*, and *B. cretica* subsp. *laconica* showed a very high content of sinigrin. In the same study, wild populations showed a higher total glucosinolate content than the cultivars. Mithen (2001) provided further details about the range of glucosinolate contents found within wild and cultivated *Brassica* species, and environmental factors that influence glucosinolate expression. Afterwards, Branca et al. (2002) evaluated the content and profile of glucosinolates in the Sicilian *B. oleracea* germplasm including wild species related to it. They also found a great level of variability in glucosinolate content in most crops and wild species, and wild types showed low contents of glucoiberin and progoitrin.

Horn and Vaughan (1983) evaluated seed glucosinolates of 14 wild *Brassica* species including *B. insularis*, *B. incana, and B. oxyrrhina*. Neither *B. insularis* nor *B. incana* showed sinigrin in their seeds, while *B. incana* gave a high level of gluconapin. *B. insularis* showed an unusual pattern of glucosinolates, having low levels of progoitrin and high levels of gluconasturtiin and some benzyl glucosinolates. More recently, Velasco and Becker (2000) evaluated a germplasm collection of 20 *Brassica* species (including accessions of *B. incana, B. montana, B. oxyrrhina*, *B. rupestris*, and *B. villosa*). In that work, *B. montana* had the highest glucosinolate content, and authors concluded that the detected variability in this species might be useful for the development of *Brassica* crops containing high glucosinolate content and specific glucosinolate profiles.

Studies on the glucosinolate genetics in these taxa have been essential for elucidating the genetic pathway for glucosinolate biosynthesis. It is evident that certain species in this group could be valuable in *Brassica* breeding programs designed to specifically enhance glucoraphanin and/or glucoiberin and, by so doing, to enhance the anticarcinogenic potential of the plant. Several recent research programs indicate that isothiocyanates derived from the hydrolysis of glucoiberin and glucoraphanin glucosinolates may be important for human diet in preventing the development of cancer. Glucoiberin shows to have phase 2 enzyme induction activity, and glucoraphanin is the precursor of the anticarcinogenic isothiocyanate sulforaphane (Mithen 2001). The possible chemoprotective effect of these compounds has led to the interest in the dietary intake of glucosinolates and isothiocyanates in broccoli and other cruciferous vegetables. In an effort to obtain higher levels of these glucosinolates, formulated foods containing designed glucosinolates are being developed. The idea is to provide an elevated level of certain isothiocyanates to the consumer, particularly those that have been found to have health benefits.

The major finding from studies about glucosinolates in a wild *B. oleracea* complex was that they are members of the *B. villosa-rupestris* complex from Sicily, which possesses a nonfunctional *GSL-ALK* allele that turns these populations into useful donors of beneficial glucosinolates and into wild progenitors

of cultivated broccoli. Faulkner et al. (1998) described the use of *B. villosa* and other members of the *B. oleracea* complex as progenitors of cultivated broccoli and showed that F_1 hybrids, which had high levels of glucoraphanin and enhanced ability to induce quinone reductase in cell cultures. Hybrids between commercial broccoli cultivars and three wild members of the *B. oleracea* complex, *B. drepanensis*, *B. villosa*, and *B. atlantica,* resulted to be fully fertile and backcrossed populations were developed. In F_1 hybrids with *B. drepanensis* and *B. villosa*, the major glucosinolates were glucoiberin and glucoraphanin. This is similar to the profile found in broccoli, whereas in hybrids with *B. atlantica*, the major glucosinolates were sinigrin, gluconapin, and progoitrin (Faulkner et al. 1998). The different glucosinolate profiles in these hybrids resulted from the interaction of the genes in their respective parents.

Following with the interest in developing broccoli that can deliver high levels of sulforaphane, Mithen et al. (2003) described the use of these hybrids to develop broccoli breeding lines and experimental F_1 hybrids having enhanced levels of glucoiberin and glucoraphanin. Experimental hybrids were obtained through conventional breeding by the introgression of small segments of the *B. villosa* genome that express high glucoraphanin levels. Hence, it is feasible to develop broccoli lines with enhanced levels of glucoraphanin that may be valuable for experimental purposes in dietary intervention studies and for commercialization for specific purposes. For example, lines having a high glucoraphanin content for functional food development (cancer protection) and lines having a high sinigrin content for biological pest control (nematodes and fungal pathogens) may be produced. Sarikamis et al. (2006) described the development of ITC-enriched broccoli through the introgression of three small segments of the genome of *B. villosa*, each one containing a quantitative trait loci (QTL), into a commercial broccoli via marker-assisted selection and analysis of glucosinolates in the florets of backcross populations. An interesting feature to point out here is that the use of wild allied plants for improving quality in vegetable crops is a difficult approach, since commercial appearance is a major trait to ensure commercial success. Thus, it is important to define the minimum number of introgressed segments (from *B. villosa* to commercial broccoli) required to increase glucosinolate content sufficiently to achieve the health benefits.

As a conclusion, significant qualitative variations in the glucosinolate profiles of wild *B. oleracea* species suggest differences in the health-promoting properties among them. Leaves and seeds of Brassica may, therefore, be used as sources of glucosinolates and isothiocyanates in the diet, especially in formulated foods.

Other crucial metabolites because of their therapeutic value in Brassica crops are phenolic compounds, especially flavonoids. The main important biological effects derived from these compounds are the antioxidant activity, the capillary protective effect, and the inhibitory effects elicited in the various stages of a tumor. In many in vitro studies, phenolic compounds demonstrated to have a higher antioxidant activity than antioxidant vitamins and carotenoids (Podsedek 2007). Studies have been mainly focused on *B. oleracea* (Vallejo et al. 2004) and *B. rapa* (Fernandes et al. 2007) and very little studies have been focused on wild *B. oleracea* species. Only works of Aguinagalde et al. (1992) and Aguinagalde (1993) used these flavonoids as biochemical markers to study the interspecific variability among a set of wild *Brassica* populations and between wild and cultivated forms of *B. oleracea*. No differences were found between wild and cultivated *B. oleracea* accessions in that study; *B. bourgeaui* closely resembled the group formed by *B. oleracea* and *B. montana* (all lacking isorhamnetin) and a high diversity was found in *B. cretica*.

Among the alternative uses to which species of *B. oleracea* complex can be devoted, it is interesting to emphasize on biofumigation. This is an agronomic technique that is an alternative to chemical fumigants in order to manage soil-borne pests and diseases in an integrated way. Rotation with Brassica crops and incorporation of Brassica residues into soil have been reported to suppress a variety of pest and disease organisms, including fungi, nematodes, insects, bacteria, and weeds (Brown and Morra 1997). Plants from Brassicaceae have been recognized as having a potential use on biofumigation practices, based on production of active volatiles released after enzyme hydrolysis. The most common volatile compounds produced during the breakdown of Brassicas are isothiocyanates. In particular, the breakdown product of sinigrin seems to protect the plant against certain pests and possibly soil pathogenic fungi and nematodes. Numerous studies have been carried out with mustard

species (*B. juncea, B. nigra, B. carinata*) and with species within the *Sinapis* genus (*S. alba* and *S. arvensis*) with this aim. The major glucosinolate types in these species (allyl and *p*-hydroxybenzyl) have shown greater allelopathic effects compared to other glucosinolate types.

Regarding the wild *B. oleracea* complex, Branca (2004) evaluated the possible biofumigant activity of *B. macrocarpa* for the control of knot-root nematodes on cherry tomato crops. In a previous study carried out by the same author (Branca et al. 2002) with Sicilian wild *B. oleracea* species, *B. macrocarpa* resulted to have the highest glucosinolate content in leaves, of which about 90% are represented by sinigrin. As a conclusion, the insertion of *B. macrocarpa* dry biomass into the soil permitted to reduce the attack caused by soil nematodes. Nowadays, some chemical synthetic isothiocyanates are already utilized as nematocides for controlling nematodes in several commercial fumigant products.

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