Chapter 10
Brassica

Mayank Gautam, Xian-hong Ge, and Zai-yun Li

Abstract Wild crop relatives have been playing an important role in deciphering the plant genome and genetic improvement of the crop plants both qualitatively and quantitatively. They have been used in understanding the fundamental questions related to origin, evolution, phylogenetic relationships, cytological status, and introgression of nuclear and cytoplasmic genes for the genetic improvements of their domesticated counterparts and facilitating the innovation of many novel concepts while working on them directly or by using them. Owing to their high economic importance species of *Brassica* (monogenomic diploids, *B. nigra* (B genome, $n=8$), *B. oleracea* (C genome, $n=9$), and *B. rapa* (A genome, $n=10$) and digenomics, *B. carinata* (BC, $n=17$), *B. juncea* (AB, $n=18$), and *B. napus* (AC, $n=19$)) manifest many morphological variations and research applications that have been favorite of plant breeders. Oilseed brassicas are interesting breeding material since they have a complete range of breeding systems varying from complete cross-pollination to self-pollination. Both interspecific and intergeneric hybridizations have a great potential for creating new variability. Some of them are contributing as model plants for comparative crop genetics (*Arabidopsis thaliana*, *B. rapa*, etc.). Wild allies of *Brassica* have attracted breeders due to their enormous genetic, genomic, and breeding potential which can be harnessed for crop improvement, obtaining phytomedicines and nutraceuticals, bioenergy production, soil reclamation, and the phytoremediation of ecology or environment. The sexual and somatic wide hybrids, cytoplasmic sterile line, and addition lines raised between *Brassica* crop species and interspecific/intergeneric, intersubtribal, and intertribal members have not only lead to the widening of crop gene pool but have also assisted in breeding at local, regional, and global level by introgressing desirable traits to overcome unprecedented

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environmental changes and diseases. Significant increases in seed yield have been achieved in oilseed brassicas through the development of hybrid cultivars. This chapter emphasizes upon the progenies of wide hybrids with potential agronomic traits for *Brassica* breeding as well as the achievements and impacts of alien gene transfer in *Brassica*, achieved mainly through distant hybridization and somatic hybridization.

Keywords Alien species • *Brassica* species • Cytogenetics • Wide hybridization • Somatic hybrids • Addition lines • Erucic acid

10.1 Introduction

Brassicaceae (Cruciferae) is the fifth largest monophyletic family having approximately 3,700 species in 338 genera (Gómez-Campo and Prakash 1999; Prakash 2010). Economically, it contributes to 10 % of the world's vegetable crop production and approximately 12 % of the worldwide edible oil source besides being a promising potential source of biofuel. Canola is now the world's third largest source of vegetable oil (13 %), after soybean (32 %) and palm oil (28 %). The rapeseed production has witnessed a steady upward movement during the past 25 years, and presently, it contributes about 14 % of the global vegetable oils (Gupta and Pratap 2007). One of the spectacular achievements in *Brassica* research concerns the improvement in nutritional quality of oil and meal, primarily in *B. napus* and subsequently in other species and represents a classical example of plant breeding. The *Brassica* crops are unique because every plant part has been selected and manipulated to yield different products. They provide edible oils, condiments (seeds), and vegetables (roots, leaves, stem, and inflorescence). Crop brassicas lack many desirable traits. Enriching conventional germplasm with genes from the related germplasm and widening genetic base is a highly desirable approach. Majority of the species in this germpool are wild and weedy and distributed mostly in the Mediterranean phytochorea. This germplasm referred to as *Brassica* Coenospecies (Gómez-Campo 1999a, b) has the potential to exchange genetic material with crop brassicas to confer agronomic advantages. It comprises of 14 genera from three subtribes, viz., *Brassicinae*, *Raphaninae,* and *Moricandiinae* in the tribe *Brassiceae*. Major investigations on this germplasm were initiated by Manton (1932) who determined the chromosome numbers; Mizushima (1950a, b, 1968, 1980) hybridized wild and crop species to investigate intergenomic homoeology; and Harberd (1972) classified the germplasm into cytodemes. Many investigations dealing with morphology, molecular marker-based taxonomy, and intensive hybridizations have generated a worth of vast informations since the 1950s.

Toshitaro Morinaga carried out a comprehensive genome analysis of crop species (1928–1934) and proposed the diploid nature of *B. nigra*, *B. oleracea*, and *B. rapa* and allopolyploid evolution for *B. carinata*, *B. juncea*, and *B. napus.* Korean botanist Woo Jang-choon or Nagaharu (1935) working in Japan presented the

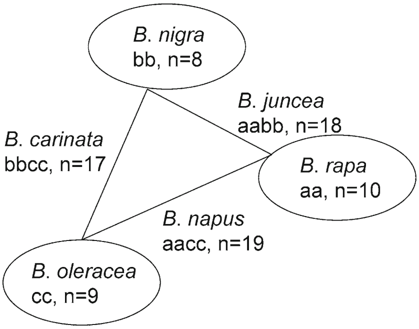


Fig. 10.1 Genomic relationship among the six cultivated *Brassica* species (1935) and showing the nucleolar dominance hierarchy in *Brassica* species bb>aa>cc

cytogenetic relationships among crop species in his famous U triangle (Fig. 10.1) consisting of three low chromosome monogenomic diploids—*B. nigra* (B genome, $n=8$), *B. oleraceae* (C genome, $n=9$), and *B. rapa* (A genome, $n=10$) and three high chromosome digenomics, i.e., *B. carinata* (BC, $n=17$), *B. juncea* (AB, $n=18$), and *B. napus* (AC, $n=19$) which evolved in nature through convergent alloploid evolution between any two diploid species and also experimentally demonstrated the allopolyploid evolution of *Brassica napus* synthesis. These relationships are further substantiated by cytogenetics, molecular analysis of nuclear and chloroplast DNA, and genomic and fluorescence in situ hybridization (Snowdon et al. 2003; Snowdon 2007), and the U triangle is now considered as a model system for investigating crop polyploidization (Lukens et al. 2006; Pires et al. 2006).

10.2 *Brassica* Crops Are Highly Polymorphic

Figure 10.2 showed the probable geographic centers of origin and domestication of different species of *Brassica* based on genetic diversity. *Brassica oleracea*, *B. rapa*, and *B. juncea* are highly polymorphic displaying a wide range of morphotypes; *B. nigra* is exclusively cultivated for the condiment mustard. The cultivated *B. oleracea* forms exhibit enormous morphological variability in leaf, stem, and inflorescence and are collectively referred to as cole crops—a term given by Bailey (1922), the American botanist and horticulturist in 1901. All these forms are sources of popular vegetables worldwide. Forms of *B. rapa* are variously termed as turnip rape (oilseed forms of Europe and Canada), *sarson* (oil seed forms of Indian subcontinent), and leafy vegetables (China and other Southeast Asian countries). *B. carinata*, the Ethiopian mustard, has a range of uses, e.g., edible oil, spices, medicinals, and vegetables. Its cultivation is restricted primarily to Ethiopia but also extends to Kenya. *B. juncea* (Indian or brown mustard) is a major source of edible oil in Indian subcontinent and Eastern European countries, of vegetables (leaf mustard) in China, and hot mustard condiment used in mayonnaise, salad dressing, and sauces in Europe, Canada, and America. *B. napus* is a major edible oilseed crop widely grown in Europe, Canada, China, and Australia. The development of canola as a crop can

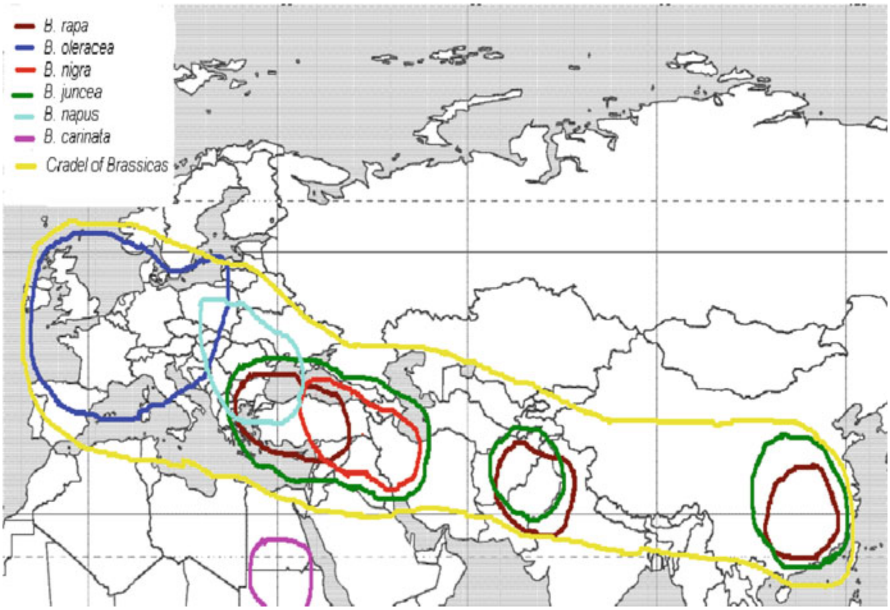
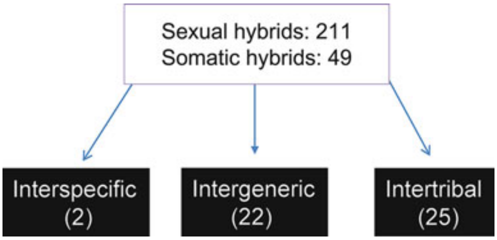


Fig. 10.2 Probable geographic centers of origin and domestication

Fig. 10.3 Hybrids obtained between wild and *Brassica* crop series



be credited to the pioneering activity of Canadian *Brassica* breeder, R.K. Downey (Rakow 2000). In the last 50 years, several new fodder and vegetable types of *B. napus* using leafy and root forming forms of *B. rapa,* viz., ssp. *chinensis*, *pekinensis*, *narinosa*, *nipposinica,* and *rapa,* have been synthesized. The brassicas are important components to the cuisine of many cultures. These represent a valuable source of vitamin C, dietary fiber, and anticancer compounds (Fahey et al. 1997).

This chapter focuses on the wide hybridization of *Brassica* spp., (interspecific/intergeneric, intertribal (Fig. 10.3), sexual and somatic hybrids), their general cytological examination, and their implications in breeding, epigenetic control and conservation of wild germplasm.

With ever-increasing world population, enhancement of food production is a major necessity. Plant breeding is the purposeful manipulation of plant species in order to create desired plant types that are better suited for cultivation, giving better yield and are disease resistant. Conventional plant breeding has been practiced for thousands of years since the beginning of human civilization; recorded evidence of plant breeding dates back to 9,000–11,000 years ago. Classical plant breeding involves crossing or hybridization of pure line, followed by artificial selection to produce plants with desirable traits of higher yield, nutrition, and resistance to diseases. With the advancement in genetics, molecular biology, and tissue culture, plant breeding is now increasingly being practiced by using molecular techniques.

The world is entering a period of unprecedented change in climate, and plant breeders must ensure that their breeding programs contain sufficient genetic diversity to respond to potential changes in the environment. In the last 70 years, many wild species in *Brassicaceae* (Prakash et al. 2009) have been used for generating wide hybrids because of their enormous desirable agronomic traits which can be used for improvement of crop species by the breeders and plant biologists. For example, *Orychophragmus violaceus* (L.) O. E. Schulz [syn. *Moricandia sonchifolia* (Bunge) Hook Fil.] is a member of the Brassiceae tribe. This species is cultivated as an ornamental plant in China, and its wild forms occur both in China and Korea (Luo et al. 1994), having super oil quality including natural zero-erucic acid; oleic, 20.32 %; high linoleic, 53.17 %; palmic, 14.31 %; linolenic, 4.76 %; and erucic acid, 0.94 % (Li et al. 1995, 1996, 1998, 2003, 2005; Ma et al. 2006; Ma and Li 2007; Wu et al. 1997; Xu et al. 2007a, b; Zhao et al. 2007, 2008; Ge et al. 2009). *Capsella bursa-pastoris* ($2n=32$) is a traditional vegetable, medicinal plant, natural double-low (erucic acid, glucosinolates) germplasm, and highly resistant to *Alternaria*, *Sclerotinia*, and cold (Chen et al. 2007). *Capsella rubella* ($2n=16$) is being sequenced in Europe. Comparison of genomes of different crop plants with *Arabidopsis* as a model has become a routine event in plant breeding. The only donor conferring the low glucosinolate in all *B. napus* varieties was "Bronowski" from Poland and low erucic acid from "Liho" in Germany. *Isatis indigotica* ($2n=14$) is a medicinal plant. Its roots are used as raw materials for preparing medicine to cure virus—cold. Extracts of roots and leaves impart resistant to bacteria and viruses (Du et al. 2009; Tu et al. 2008, 2009, 2010). *Lesquerella fendleri* is a valuable genetic resource for the rapeseed breeding for industrial purpose as it possesses high amounts of hydroxy fatty acids. In addition, the high tolerance to drought of *L. fendleri* is also useful for the genetic improvement of rapeseed (Du et al. 2008), especially due to thick glutinous polysaccharide layer on its seed coats. With the advancement of hybridization techniques, hormonal manipulations, genetic transformation, and embryo rescue, alien gene transfer has now become a more common practice involving larger numbers of crop species.

In the early nineteenth century, Sageret (1826) obtained intersubtribal hybrid (*Raphanus sativus* × *B. oleracea*) and Herbert (1847) raised interspecific hybrid (*B. napus* × *B. Rapa*) in *Brassica*. Initially, these hybridizations were used for cytological studies for understanding genomic homoeology. Afterwards, wide hybrids were routinely obtained for widening genetic base, introgressing nuclear genes that are valuable for providing tolerance to biotic and abiotic stresses in selected combinations (Kalloo 1992; Warwick 1993; Cole 1994) or development of the alloplasmic lines by transferring the cytoplasm of the wild species to crop brassicas exhibiting cytoplasmic male sterility (CMS) (Banga 1993). Some of the alloplasmic lines have also exhibited useful agronomic traits (Downey and Rimmer 1993). Chromosome addition and substitution lines were also generated to locate genes on specific chromosomes and for constructing genetic maps. During the last three decades, in vitro techniques such as ovary culture (Inomata 1978; Bajaj et al. 1986; Chevre et al. 1994; Brown et al. 1997), ovule culture (Zenkteller 1990), embryo rescue (Harberd 1969), bridge-cross methods (Rao et al. 1996), and protoplast fusion (Primard et al. 1988) have been used vigorously to obtain a huge number of sexual and somatic hybrids.

10.3 Sexual Hybrids

Conventional breeding methods require a large number of pollinations for obtaining hybrids successfully. In many combinations the success was negligible due to the pre-fertilization barriers (hereafter PRFB) which in most of the cases occur due to the inability of pollen tubes to grow down the style to affect fertilization. A series of observations and experiments in *Brassica* intergeneric and interspecific hybridization, continued through the last three decades, have made it possible to obtain some broader information regarding the mechanism and significance of barriers in wide hybridization. PRFB operate mostly at the level of pollen germination or pollen tube entry into the stigma. In most of the crossability studies, a common feature is that they favor crossability in one direction and that too specifically when the wild species act as female parent. In a few crosses, 34 % of *Raphanus raphanistrum* pollen adhering to the stigma of *Brassica napus* germinated, but no pollen tube penetrated the pistil; however, in the reciprocal crosses, only 12 % of *B. napus* pollen germinated on the stigma of *R. raphanistrum* (Rieger et al. 2001). Cross between *Brassica tournefortii* and *B. rapa* was successful when *B. tournefortii* was used as female parent (Choudhary and Joshi 2001), while it was observed earlier that pollen grains of *B. tournefortii* did not germinate on the stigma of other species resulting in the failure of reciprocal crosses (Harberd 1976). In another investigation, pollen germination was good in those crosses where *Enarthocarpus lyratus*, a wild species, was used as female parent and crop brassicas (*B. campestris*, *B. nigra*, *B. juncea*, *B. napus*, *B. carinata*) as male (Gundimeda et al. 1992). In this study, aniline blue fluorescence of pollinated pistil in intergeneric crosses showed that in many crop brassicas pollen tube grew through the stigma, style, and ovary when *E. lyratus* was the female parent, but in reciprocal crosses, when *E. lyratus* was the pollen parent, very few pollen grains germinated and most pollen tubes failed to enter the stigmatic papillae, few of those which entered the papillae showed swelling of the tube tip and also developed callose plug. None of the pollen tube was observed in the style. In another intergeneric hybridization study between *Diplotaxis siifolia*, a wild species, and crop brassicas, the crop brassicas' pollen germination and pollen tube growth was normal on the stigma of *D. siifolia*; however, in the reciprocal cross, the *D. siifolia* pollen showed strong PRFB; although pollen grains germinated, the pollen tube failed to enter the stigma. In general, the stigma of the cultivated species inhibits pollen of the wild species, while the stigma of the wild species permits satisfactory pollen germination and the tube growth of the cultivated species (Batra et al. 1989, 1990; Gundimeda et al. 1992; Nanda Kumar and Shivanna 1993). These classical breeding results of direct and reciprocal crosses showed an inherent directional preference (hereafter DP), which ensures these wide hybridizations and overcomes the PRFB.

There are two possible hypothesis to explain the DP, viz., unilateral pollen pistil incompatibility (UI) and endosperm balance number (EBN). The mechanism of UI described in 1955 (Harrison and Darby 1955) explained that the pollen of one species rejects the pistil of another with the reciprocal direction being compatible (De Nettancourt 1977). Most commonly UI occurs with a self-incompatible (SI) species as the pistillate parent and the self-compatible (SC) species donating the pollen. Such a mechanism may be responsible for the reproductive isolation between *B. napus* and the self-incompatible species *R. raphanistrum* (Kercher and Conner 1996). However, some of the wild species including *B. fruticulosa*, *B. maurorum*, *Diplotaxis catholica*, *Erucastrum gallicum*, and *E. cardiminoides* are likely to be more efficient as male parents for developing wide crosses with cultivated *Brassica* species.

Majority of the crosses which do not show PRFB, invariably show postfertilization barriers (PSFB), usually observed as lack of functional endosperm or its early degeneration. The PSFB can be explained by many mechanisms like negative interaction between diverged sequences, global genome rearrangements, widespread epigenetic reprogramming, and imbalance of paternally and maternally imprinted genes in the endosperm. The effects of PSFB are embryo abortion at early globular stage, and lack of functional endosperm (Nanda Kumar and Shivanna 1990). There are many models to explain the operation of PSFB. Dobzhansky–Muller model explains that there is a negative interaction between the genes of two different crossing species, which leads to inviability or sterility in the hybrid offspring (reviewed by Coyne and Orr 1998; Rieseberg and Carney 1998). Another important model is "genomic shock" (allelic incongruity) that causes extensive preprogrammed changes to genomic structure namely, changes in chromosomal organization and repetitive sequences (McClintock 1984).

In most of the interploidy crosses within and between the species, endosperm breakdown is observed as the primary reason for failure of seed development (Watkins 1932; Brink and Cooper 1947; Stebbins 1958; Haig and Westoby 1991). During seed development three different tissues are in intimate contact, viz., embryo, endosperm, and the surrounding somatic tissue of the mother plant. In normal condition the relation between these tissues is 2:3:2. If the uniting gametes have different chromosome numbers, this relation would be altered which would result in poor seed development or complete abortion of seeds (Sikka 1940). Recently the imprinted genes in the endosperm are one of the viable reasons for the PSFB. Endosperm disruption depends upon 2m:1p (maternal:paternal) which is the EBN mentioned above. If maternal genome is in excess (a ratio of >2m:1p), endosperm proliferation inhibits, and if paternal genome is excess (a ratio of <2m:1p), it results in endosperm proliferation (Haig and Westoby 1991; Scott et al. 1998). The mechanism controlling the parental genomic ratio in the endosperm is called parental imprinting. Parental imprinting is the result of complex theories occurring in endosperm, and one of them is parental conflicting theory. Parental conflicting theory is the fundamental theory which explains the struggle between maternally and paternally derived genomes over resource allocation from mother to offspring (Haig and Westoby 1989, 1991). According to this model the growth promoters essential for endosperm development are expressed when inherited from the father but silenced when they are inherited from the mother, where as growth inhibitors were silenced when inherited from the father and expressed when inherited from the mother. The extra doses of maternal genome provide extra copies of growth inhibitors which lead to small endosperm and seeds. However, extra paternal genome leads to the expression of growth activators which lead to proliferation of endosperm. Both these situations are the result of deviation from normal EBN which leads to an abnormal growth of endosperm (either undergrowth or proliferation). Furthermore, the role of hypomethylation in the parental imprinting was also estimated by antisense *MET1* gene (Adams et al. 2000). These studies were carried out on *Arabidopsis* hybrids, and since *Arabidopsis* is the member of *Brassica*ceae, these studies could be correlated with hybrids of *Brassica*; nevertheless, it is not sufficient to draw a definite conclusion.

In addition to PRFB and PSFB, the genotypes also control the crossability of wide hybrid combinations. The success of interspecific crosses depends not only on the species and direction of cross but also on the genotype and ecotype of species involved in the hybridization (Bozorgipour and Snape 1990). This indeed indicates that a wide range of variation among the different *Brassica* genotypes with respect to their difference in genotypes can lead to difference in pollen fertility in the hybrids. There are several reports on wide crossing which clearly mention about the influence of genotypes and ecotypes on the crossability. For example, the crossability of different *B. napus* cultivars was carried out with *Orychophragmus violaceus*, and it was observed that the crossability of *O. violaceus* was successful with *B. napus* cultivars “Oro,” “Huayou No. 8,” and “GR 144–149” when *B. napus* was used as female parent. However, no hybrid plants were obtained in the crosses using the cultivars “Canadian twinlow,” “Atlex,” “81008,” and “Senli” (Li et al. 1995). In an another study, two genotypes of *B. tournefortii* ecotypes crossed with the three ecotypes of *B. rapa* i.e *B. rapa* var *trilocularis* (yellow sarsoon), *B. rapa* ssp. *Sarson* (Brown Sarsoon), *B. rapa* var *dichotoma* (toria) showed difference in crossability of different ecotypes (Choudhary and Joshi 2001). It appeared from these studies that genotype in addition with PRFB and PSFB controls the crossability. The presence of strong PRFB and PSFB can be overcome by using tissue culture procedures such as ovary/ovule culture, embryo culture, sequential culture (Inomata 1976; Nanda Kumar et al. 1988a, b; Batra et al. 1990; Gundimeda et al. 1992; Vyas et al. 1990) which involves successive culture of ovaries, ovules, and seeds/embryos and is more effective than simple ovary or ovule culture. Certain other techniques namely grafting, mixed pollination, bud pollination, stump pollination (Hosoda et al. 1963; Sarashima 1964) are also found to be effective in overcoming the barriers.

The first test-tube fertilization in plant was carried out in poppy (Kanta et al. 1962). Test-tube fertilization of excised ovules in *Brassica* is a practical technique for overcoming the PRFB (Kameya and Hinata 1970). The method of in vitro fertilization of ovules can be successfully applied to various species of Brassicaceae. Mature embryos and plants were obtained after in vitro pollination of the ovules of *Arabis caucasica*, *B. napus*, *B. oleracea* var. *sabellica* (kale), *B. oleracea* var. *italica* (broccoli), *Diplotaxis tenuifolia*, *Moricandia arvensis*, and *Sisymbrium loeselii.* In the case of *Sinapis alba*, fertilization and embryo development did not occur. Placental pollination has been successfully used for obtaining hybrid immature embryos at different stages of development from crosses between *B. napus* × *D. tenuifolia*, *B. napus* × *M. arvensis*, *B. oleracea* var. *italica* × *D. tenuifolia*, *D. tenuifolia* × *B. napus*, *D. tenuifolia* × *M. arvensis*, and *D. tenuifolia* × *S. loeselii.* These findings show that in vitro pollination of ovules of various species of *Brassicaceae* makes it possible to perform the whole process of fertilization and embryogenesis and obtain intergeneric hybrid embryos (Zenkteller 1990). Embryo rescue technique is useful as a means for the progress of the study on the interspecific and intergeneric hybridization of crucifer vegetables, where hybrid embryos abort at early stages of development (Nishi et al. 1959; Zhang et al. 2003, 2004; Wen et al. 2008). Mizushima (1950a, b, 1968) carried out the pioneer work of hybridizing species from secondary and tertiary gene pools.

Sexual hybrids show aberrant meiotic chromosome behavior when both the parents are diploid. Chromosome homology between various genomes in *Brassica* has been thoroughly investigated (reviewed in Prakash and Hinata 1980; Prakash et al. 2009 and Prakash 2010). The occurrence of unreduced male as well as female gamete is quite common in *Brassica*ceae (Ripley and Arnision 1990). Cytologically, the hybrids predominant show the presence of univalents, a small proportion of bivalents and even higher associations (tri-, tetra-, pentavalents). The primary reason for the formation of univalents in the wide hybrids is the absence of a homologous partner, relatively less bivalents due to occasional pairing which would bring about low frequency of chiasma formation and ultimate non-conjugation. Bivalents, when they occur, are mostly rod shape monochiasmatic and rarely ring shaped with multiple chiasmata. Multivalents in diploid hybrids occur only rarely. Nevertheless, a variable number of bivalents and frequent trivalents as well as quadrivalents are formed in triploid (tetraploid × diploid) and tetraploid (tetraploid × tetraploid) species combinations.

Wide hybrids have meiotic irregularities such as numerous disjunctional abnormalities including laggards and segregational anomalies (Stebbins 1966) resulting in pollen sterility. The anthers are generally small, flaccid, and empty. The bridge fragment configuration at anaphase I is observed rarely in the wide hybrids, resulted from chiasma formation within a heterozygous inversion. The very rare formation of the bridge occurs, because the length of the inverted segment is so small and does not permit frequent crossover in that region (Sikka 1940).

Among the diploid hybrids, high chromosome pairing has been observed in several combination: *Sinapis arvensis* × *B. nigra* ($2n = 17$, 8II, Mizushima 1952), *Diplotaxis erucoides* × *B. nigra* ($2n = 15$, 6II, Quiros et al. 1988), *B. fruticulosa* × *B. nigra*

(2*n* = 16, 7ii Mizushima 1968), *B. nigra* × *Hirschfeldia incana* (2*n* = 15, III+5II, Quiros et al. 1988), *Erucastrum canariense* × *B. oleracea* (2*n* = 18, 8II, Harberd and McArthur 1980), and *E. cardaminoides* × *B. oleracea* (2*n* = 18 IIV+1III+1II, Mohanty 1996). The triploid and tetraploid hybrids where higher associations have been observed include *B. juncea* × *Diplotaxis virgata* (IIV/2III; Inomata 2003), *B. napus* × *H. incana* (IIV, Kerlan et al. 1993), and *D. viminea* × *B. napus* (2IV, Mohanty 1996). Triploids and tetraploids bivalents and higher associations may result from the homology within the chromosome of the same genome (autosyndesis) or because of intergenomic homology (allosyndesis). In the past, it was difficult to draw conclusions in terms of autosyndesis and allosyndesis. However, it can be stated now with further experimentations that intergeneric homology is always higher than intrageneric homology. Cytogenetics has played an important role in determining the chromosome number, genome analysis, resolving taxonomic status and phylogenetic relationships, genome manipulation, chromosome addition lines for locating genes and introgression, and in situ hybridization for chromosome identification (Chen et al. 2007; Du et al. 2008, 2009). Recent use of genomic in situ hybridization (GISH) (Li et al. 2007; Tu et al. 2009; Ge et al. 2009) and fluorescence in situ hybridization (FISH) enable us to precisely ascertain the degree of autosyndesis and allosyndesis. GISH and FISH assisted in characterization of individual chromosomes, construction of karyotypes, determination of the genomic component of allopolyploid species, analysis of meiotic behavior in hybrids, integration of genetic and physical maps, and studying the genome evolution by FISH mapping. Hybrids between *Brassica* spp and *Orychophragmus violaceus* (2*n* = 24) (an ornamental plant in China) are extensively used to study meiotic and mitotic behavior for introgression of the nuclear genes of interest. All the hybrid combinations were generated when *Brassica* crop species was crossed as female parent with *Orychophragmus violaceus* as the pollen donor (Li et al. 1995, 1996, 1998, 2003; Li and Hensen 1999; Hua and Li 2006; Ge et al. 2006, 2009).

10.4 Somatic Hybrids

Somatic hybridization is an effective technique to overcome barriers to sexual reproduction. Protoplast fusion has been very successful in Brassicaceae (see reviews by Glimelius 1999; Christey 2004; Navarátilova 2004; Li et al. 2005; Fig. 10.4). Biotechnological tools such as embryo rescue and protoplast fusion have made it possible to overcome not only intergeneric but also intertribal incompatibilities. As a consequence 44 somatic hybrids between crop and wild species have been obtained. These represent interspecific/intergeneric [*Brassica spinescens* (Kirti et al. 1991), *Brassica tournefortii* (Stiewe and Robbelen 1994), *Diplotaxis catholica* (Kirti et al. 1995), *Diplotaxis harra* (Begum et al. 1995), *Diplotaxis muralis*, *Eruca sativa* (Fahleson et al. 1997), *Moricandia arvensis*, *Moricandia nitens* (Meng et al. 1999), *Raphanus sativus* (Wang et al. 2006a, b), *Sinapis arvensis*, *Sinapis alba* (Wang et al. 2005), and *Trachystoma ballii*], intersubtribal, and a

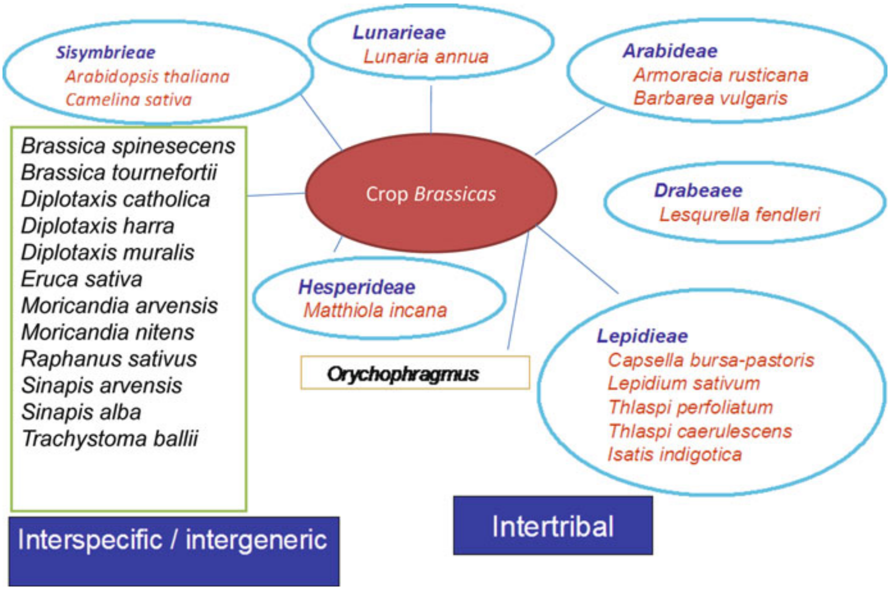


Fig. 10.4 Somatic hybridization involving crop brassicas

substantial number of intertribal combinations from six different tribes, viz., *Sisymbrieae* [*Arabidopsis thaliana* (Siemens and Sacristan 1995), *Camelina sativa* (Narasimhulu et al. 1994)], *Arabidea* [*Armoracia rusticana* (Navarátilova et al. 1997), *Barbarea vulgaris* (Ryschka et al. 1999)], *Drabeaa* [*Lesquerella fendleri* (Nitovskaya et al. 2006)], *Lepidieae* [*Capsella bursa-pastoris* (Nitovskaya and Shakhovskii 1998), *Lepidium sativum*, and *Thlaspi perfoliatum* (Fahleson et al. 1994a, b), *Thlaspi caerulescens,* and *Isatis indigotica* (Du et al. 2009)], *Lunarieae* [*Lunaria annua* (Craig and Millam 1995)], *Hesperiidae* [*Matthiola incana* (Sheng et al. 2008)], and *Orychophragmus* (Li et al. 1995, 1996, 1998, 2003). In many instances, desirable characters have been observed in hybrids. However, the introgression has not been largely possible because of high degree of sterility and lack of sufficient intergenomic chromosome pairing. Nevertheless, the results are not very discouraging and few characters have been incorporated. Examples include somatic hybrids in *Camelina sativa* + *B. carinata* (Narasimhulu et al. 1994) and *Camelina sativa* + *B. oleracea* (Hansen 1998) which were, however, not established as viable field plant due to large phylogenetic distance between the partaking genomes which severely affected the vegetative growth and development of normal plant parts, particularly floral organs. The hybrids are generally intermediate to the respective parents in most of the quantitative characters. Morphologically, they resemble largely the female parent although they also posses distinct male characters, such as 6–8 petals and multiple carpel-like structures in *A. thaliana* + *B. napus* (keller et al. 1993; Bauer-Weston et al. 1993) and 1 or 2 petals in *Thlaspi perfoliatum* + *B. napus* (Fahleson et al. 1994a, b).

A majority of somatic hybrids are seed sterile, if selfed. Generally, with the decrease in the number of alien chromosomes, the fertility increases, and the plants possessing the entire alien chromosome are completely sterile. Somatic hybrids have different possibilities regarding the cytoplasmic genomes; either the parental genomes segregate completely during cell division or both the parental genomes occur as mixed population, and it further leads to recombination in the parental genome (see review by Prakash et al. 2009). The mitochondrial and chloroplast genomes segregate independent of each other. Mitochondrial recombination has been reported to occur frequently in *Brassica* (Glimelius 1999). On the contrary, intergenomic chloroplast recombination is rare. In hybridization involving wild species, generally the chloroplast from the crop parent is favored and the higher the ploidy of the partaking genome is, the more is the contribution of the chloroplast per cell (Butterfass 1989). Mostly, *B. napus* or *B. juncea* allopolyploids take part in hybridization with diploid wild parents; therefore, the chance of crop species chloroplast is more. In comparison to the symmetrical fusion, the asymmetrical fusions (irradiating donor (wild) protoplast to induce double-strand break) are having more chances of survival and adaptability because only a fraction of alien genetic content is present.

10.5 CMS Systems Originated from Wild Taxa

The most rewarding utilization of wild species in crop brassicas has been in synthesizing alloplasmic lines of crop species exhibiting male sterility with the wild species being as cytoplasm donors (*Arabidopsis thaliana*, *B. oxyrrhina*, *B. tournefortii*, *Diplotaxis muralis*, *D. erucoides*, *D. berthautii*, *D. catholica*, *D. siifolia*, *Eruca sativa*, *Erucastrum canariense*, *Enarthocarpus lyratus*, *Moricandia arvensis*, *Orychophragmus*, *Raphanus sativus*, *Sinapis arvensis*, *Trachystoma ballii*) and introgression of male fertility restoration genes. Out of these CMS sources, fertility restoration has been identified in *Raphanus*-based Ogura CMS and Polima CMS in the western countries, and it has been detected in the CMS-based crosses in *B. tournefortii*, *B. juncea* CMS, *Polima* CMS, and *Siifolia* CMS in India (Rai et al. 2007). Heterotic *B. napus* hybrids based on *Raphanus/Ogu* system in Europe and Canada and *B. juncea* hybrids on *Moricandia arvensis* (Prakash et al. 1998) system in India have been developed.

10.6 Monosomic and Disomic Addition Lines

Wild germplasm needs to be thoroughly characterized for different traits. Dissecting their genomes and developing chromosome addition lines to locate gene(s) of importance will accelerate the map-based cloning of these genes. Monosomic addition lines are used for dissecting several *Brassica* and related genomes, viz., *B. nigra*, *B. oleracea*, *B. rapa*, *B. oxyrrhina*, *Diplotaxis erucoides*, *Raphanus sativus*, *Sinapis alba*, *S. arvensis*; and several disomic addition lines have been developed including *A. thaliana–B. napus* (Leino et al. 2004), *B. napus–S. alba* (Wang et al. 2005), and *B. napus–C. abyssinica* (Wang et al. 2006a, b). A full set of nine disomic *B. napus–R. sativus* addition lines developed by Budahn et al. (2008) is the first disomic addition line series in Brassicaceae.

10.7 Introgression of Nuclear Genes from Wild *Brassica* Species for Breeding

Nuclear genes for abiotic and biotic stress tolerance have been successfully introgressed in the crop species to generate progenies of wide hybrids with additional useful traits for breeding. The agronomic characters in wild species are an attraction to breeders including resistance to various biotic stresses such as beet cyst nematode, alternaria blight [*Diplotaxis erucoides* (Klewer et al. 2003), *Camelina sativa*, *Capsella bursa-pastoris* + *B. napus*, *Coincya* spp.], blackleg [*Arabidopsis thaliana* + *B. napus* (Saal et al. 2004), *Sinapis arvensis*)], flea beetle (*Crambe abyssinica*), and other traits such as C3–C4 intermediate photosynthesis [*Moricandia* spp. (Bang et al. 2003), *Diplotaxis tenuifolia*]; drought tolerance (*Brassica tournefortii*, *Diplotaxis acris*, *Eruca* spp., *Lesquerella* spp.); cold tolerance (*Coincya richeri*, *Erucastrum abyssinicum*); high erucic acid content [*Barbarea* spp., *Cardamine*, *Lepidium* (Hu et al. 2002)]; increased level of palmitic and linolenic acids from *Orychophragmus* (Wang et al. 2003); greater amount of erucic acid from *Crambe abyssinica* + *B. napus* (Schroder-Pontoppidan et al. 1999); high amount of lesquerolic acid from *Lesquerella fendleri* + *B. napus*; fertility restoration from *R. sativus Diplotaxis catholica Moricandia*, *Trachystoma balli*, and *Sinapis arvensis* (see review Prakash et al. 2009); zinc and cadmium accumulation (*Thlaspi caerulescens* + *B. napus*); and high nervonic acid content (Fahleson et al. 1994a, b, *Thlaspi perfoliatum* + *B. napus*). Breeding for improvement of fatty acid composition in rapeseed has been emphasized for high-quality rapeseed breeding. Most of the Asian varieties have high oil content, but lower oleic and higher erucic acid content (Fig. 10.5) than the European varieties. The variation in the oleic and erucic acid content in the European varieties were larger than in the Asian varieties. *Brassica-related* wild germplasm will have an increasingly important role to develop abiotic and biotic stress free and better adapted cultivars in the future. Their utilization will be more successful using cellular and molecular biotechnological tools.

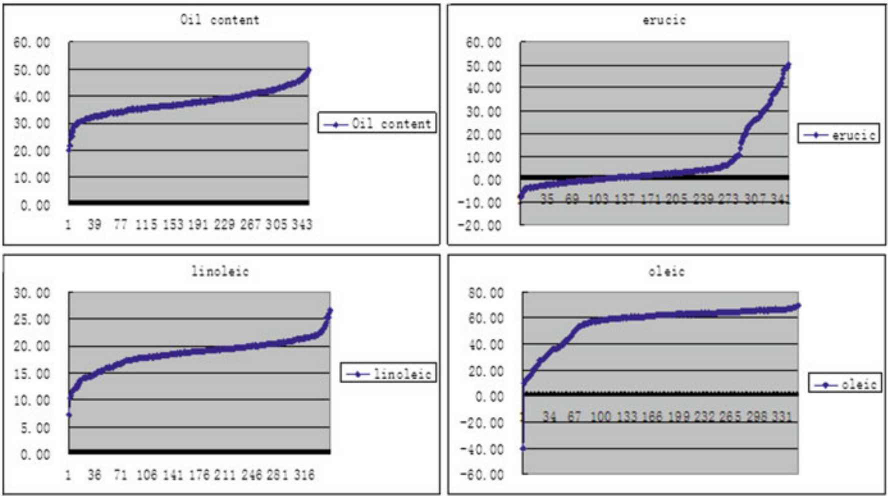


Fig. 10.5 Useful traits incorporated in *Brassica* crops for breeding

10.8 Epigenetics of Interspecific and Intergeneric Hybrids: Nucleolar Dominance

In distant hybrids, it is often observed that the ribosomal genes of one species (one genus) are transcriptionally dominant over the ribosomal genes of other species (other genus). This phenomenon is known as Nucleolar Dominance (hereafter ND) (see review by Pikaard 1999, 2000, 2001). It occurs both in the hybrids of animal and plant kingdom like *Xenopus*, *Drosophila*, *Crepis*, *Salix*, *Ribes*, *Solanum*, *Hordeum*, *Avena*, *Agropyron*, *Triticum*, Zea, *Triticale*, *Brassica*, and mammalian cells. It is an epigenetic effect and is second only to inactivation of one X chromosome that occurs in the somatic cells of female mammals. However, unlike X inactivation, the choice of which set of rRNA genes to silence is not random, rather there is dominant and underdominant rDNA, playing a crucial cross talk in ND (wheat–rye addition line). ND is independent of maternal and paternal imprint. ND studies on plants is having a history of more than seven decades, and it was for the first time described by M. Navashin, a Russian cytogenetist, who began a series of caryological investigation on the plant genus *Crepis*. In 1928, he found that there is a reversible change in the chromosome morphology. Navashin used the term “amphiplasty” to describe the ability of metaphase chromosome to adapt new forms. Navashin stated that “It was a great surprise to find that the chromosomes of two or more different species brought together by hybridization in certain specific combination suffer striking alterations of their individuality (Navashin 1934).” In his study out of 21 different hybrid combinations, eight hybrid combinations have both the D chromosome (earlier the chromosome were designated with alphabets) of the parent at metaphase. In other 13 hybrid combinations, one progenitor had retracted its satellite. NORs include active rRNA genes which give rise to secondary constriction of metaphase chromosomes and silent rRNA genes which are often highly compacted in dense heterochromatin. Each rRNA genes at NOR is nearly identical in sequence. Differences in the number of repeated DNA elements occur commonly in the intergenic spacer region. These unparalleled investigations done by pioneer researcher have paved the way for the study of this amphiplastic (ND) phenomenon. Hierarchy of rRNA gene transcriptional dominance in *Brassica* is *B. nigra*, BB>*B. rapa*, AA>*B. oleraceae*, CC (Chen et al. 1997) (Fig. 10.1). So, the size of repetitive region and the number of repetitive elements of NORs are not the determining factors for ND in crop *Brassica*. In the last decade a lot of studies were carried out on epigenetics, and it was emphasized that the ND is an epigenetic phenomenon, and DNA methylation and histone modification play a crucial role in maintenance of nucleolar dominance in allotetraploids of *Brassica*. Hypermethylation and hypoacetylation lead to transcriptional silencing of rDNA of one of the parents in the hybrid. A role of methylation and acetylation was dramatically revealed in allotetraploid *Brassica* by using inhibitors of cytosine methylation (like 5-aza 2′-deoxycytidine) and histone deacetylase (HDAC) inhibitors like sodium butyrate and trichostatin A (TSA).

10.9 Conservation of Wild Germplasm

Collection and conservation of all the different wild relatives, species, and relative of the cultivated species (followed by the evolution of their characteristics) are a prerequisite for the effective exploitation of the natural genes available in the populations. During the 1970s wild germplasm of *Brassica* were extensively collected and cytogenetic studies were started. In many crops preexisting genetic variability is available from wild relatives of crops. The vast collection of wild crucifers was carried out by the expeditions to the Mediterranean region (1970–1975) by Spanish researcher Prof. Cesar Gómez-Campo and Japanese researchers U. Mizushima, S. Tsunoda, and K. Hinata. Mizushima initiated investigations on wild germplasm in the early 1970s, executed hybridizations between wild and crop species, and studied observations on chromosome pairing and interpreted genome homoeology (Gómez-Campo and Gustafsson 1991). Harberd (1972, 1976) classified germplasm referred to *Brassica* coenospecies into cytodemes (crossing groups) and studied the chromosome pairing in a large number of interspecific and intergeneric hybrids. Intensive efforts have been made in the last decade to search and collect this material; otherwise, it would have been invariably lost. Most of the *Brassica* collections are conserved by means of seeds, and in general, they are conserved under long-term storage condition to maintain seed variability for many years. The only exception within the *Brassica* crop is perennial Kale that is vegetatively propagated (Gómez-Campo 1999a, b). Ex situ conservation of plant genetic resources in gene bank 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 conservation and maintenance of these accessions for current and future users for regeneration activities.

Climate, plant diseases, insect pests, and market demands for new quality traits are included in the broad definition of environment. Wide hybridization is an important tool to introduce alien variation into the cultivated crops. Alien gene transfer has played an important role in creating additional genetic variability in crop species; introgression of newer, useful, and desirable alleles; and devising several innovative and advanced techniques like preferential chromosome elimination leading to techniques like doubled-haploidy breeding. Somaclonal variation during the culture phase of hybrid embryos developed through distant hybridization generates additional avenues of variability in several crop plants. The impact of alien gene introgression has been well seen during the "Green Revolution" and also after that in the development of improved plant varieties in a spectrum of crops including cereals, pulses, oilseeds, and vegetables and ornamental and horticultural crops. Wild species are a rich reservoir of several useful alien genes which are no longer available within the cultivated gene pools. Continuous efforts have been underway to collect and conserve wild relatives of various crops in national and international gene banks and use them for alien gene transfer into the cultivated background.

10.10 Conclusions and Future Prospects

Distant hybridizations in crop brassicas through sexual cross can be traced back to the 1950s and through somatic hybridization to the late 1970s. The sexual hybrids and their progenies recovered by the aid of special efforts such as repeated pollination, embryo rescue, and sequential culture have contributed immensely in widening the genetic base of cultivated brassicas as well as generating newer genetic variability providing additional avenues of selection. This has resulted in the transfer of several desirable genes into cultivated background from wild species including those for disease and insect-pest resistance, improved oil quality, fatty acid composition, male sterility and fertility restoration systems, and, of late, tolerance to a few abiotic stresses. Nevertheless, pre- and postfertilization barriers in producing viable hybrids and their progenies have slowed down the progress in transferring useful alien genes. In such case somatic hybridization has played a great role, and among all crop plants, *Brassica* has been one of the most cited examples where somatic hybridization has witnessed great success.

The vast knowledge of molecular markers has also greatly aided in *Brassica* improvement, and it has helped in mapping important traits. The sequencing of *Arabidopsis* genome has proven to be of great use in marker-assisted breeding. This knowledge further needs to be integrated with conventional breeding or transferring alien genes through AB-QTL approach. Further strides are required in genetic transformation and intragenesis and cisgenesis to deploy alien genes from across the genome boundaries for the genetic improvement of crop brassicas. At the same time, further improvements are required in somatic hybridization and tissue culture protocols, especially to solve the problems of low regeneration rate and genotype dependency. For a direct use of wild species in genetic improvement of crop brassicas, chromosome fragmentation and integration of only a specific fragment conferring a useful trait will be a more practical approach in improving *Brassica* utilizing wild species.

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