Chapter 3
Brassicas

S.K. Gupta

Abstract Oleiferous brassicas are interesting breeding material since they have a complete range of breeding systems ranging from complete range of cross-pollination to self-pollination. Besides improvement in production and productivity of various economically important brassicas, improvement in the nutritional profile of their oil and defatted meal, and development of traits like herbicide tolerance, male sterility, disease and insect-pest resistance, and development of hybrid cultivars remain the prime objectives for their genetic improvement. To achieve these goals, conventional breeding efforts in conjunction with modern biotechnological tools such as molecular marker-assisted selection, doubled haploidy breeding, in vitro mutagenesis, and transgenic technology offer a great promise. The doubled haploidy (DH) technology in combination with other biotechnological and conventional breeding tools has resulted in improvements in many yield and quality attributes in Brassicaceae. Interspecific and even intergeneric hybridizations have greatly helped in generating additional variability through the recovery of distant hybrids. Further, in vitro technologies such as microspore culture, and embryo and ovary rescue coupled with in vitro mutagenesis can also generate additional selection avenues by creating variability through gemetoclonal and somaclonal variation. This review focuses on breeding methods, which individually or in combination could be deployed for solving the pressing problems of male sterility and fertility restoration mechanisms for hybrid seed production in crop brassicas, their crossability improvement and generation of variability and quality improvement.

Keywords Brassicaceae • Origin and evolution • Double haploidy • Molecular-assisted selection • Transgenic technology • Male sterility and fertility restoration • In vitro mutagenesis

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1 Introduction

Rapeseed–mustard is the most important source of vegetable oils after palm and soybean (Beckman 2005). The rapeseed production has witnessed a steady upward movement during the past 25 years. More recently, the introduction of low erucic acid varieties enhanced its value as edible oil, particularly among the health conscious consumers and varieties with low glucosinolates increased the value of its defatted meal for use as a feed for livestock. The development of double low varieties (canola) (Downey and Rakow 1987) has made rapeseed one of the major plant oil sources at the global level, and now there is a constant tendency to increase its share in the production of oilseeds (Bartkowiak-Broda et al. 2005). Oleiferous Brassicas are generally derived from two species, *Brassica napus* L. and *B. campestris* L. syn. *B. rapa* L. *B. campestris* is also referred to as toria, sarson, summer turnip rape, Polish rape, and so on. Similarly, different names are also given to *B. napus* such as Argentine rape, Swede rape, and colza. The name rape is derived from the Latin word "rapum," which means turnip. All the rapeseed contributing cultivated *Brassica* species are highly polymorphic including oilseed crops, root crops, and vegetables such as Chinese cabbage, broccoli, and Brussels sprouts. However, a few of them are cultivated as salad, vegetable, and condiment crops as well.

B. juncea is of much importance in Asia and *B. napus* in Europe and Canada. Under European and Canadian conditions, both winter and summer (spring planted) forms of *B. campestris* (syn. *B. rapa*) and *B. napus* are being grown but in *B. juncea*, only spring form has evolved. Winter types of *B. napus* are largely grown under north-European, Chinese, and Canadian conditions (Rai et al. 2007). However, spring types of *B. campestris* are usually preferred and are largely grown in Sweden, Finland and some parts of Canada and north-west China. In the Indian subcontinent, genetic improvement of seed yield is the prime breeding objective while in Western world, breeding for quality receives greater attention (Jonsson 1973).

2 History

Early history suggests that rapeseed has been cultivated for several thousand years with its origins in Asia. Sanskrit writings of 2000–1500 BC directly refer to oleiferous *B. napus* forms (sarson types) and mustard. Seeds of *B. juncea* have been found in the archaeological sites in India dating back to ca 2300 BC (Prakash 1980; Weiss 1983). Two species, *B. napus* and *B. campestris*, having a range of morphotypes, are the crops of antiquity in India where much before the Christian era, they were used for many purposes including oil for cooking and frying, spice for seasoning food articles, vegetables, and for religious ceremonies (Mehra 1966). Since time immemorial, the Brassica crops have been a part and parcel of human agriculture system, and at present also they occupy a predominant place in the world's agrarian economy. The Chinese word for rapeseed was first recorded ca 2500 years ago and the

oldest archaeological discoveries may date back as far as to ca 5000 BC (Yan 1990). The Greek, Roman, and Chinese writings of 500–200 BC refer to rapiferous forms of *B. rapa* and also describe their medicinal values (Downey and Robellen 1989). Seeds of *B. juncea* have been excavated from Chanhudaro, a site of Indus Valley civilization that existed in the plains of Punjab along the river of Indus ca 2300–1750 (Piggot 1950). Species from the genus Brassica were cultivated in ancient Rome and also in Gallia (Fussel 1955), and seeds of these species had also been found in the old German graves and Swiss constructions from the Bronze Age (Neuweiller 1905; Schiemann 1932; Witmack 1904). In Dodoneus's "Herbalist" (1578), a mention has been made regarding the growing of *B. rapa* var. *rapifera* in 1470 as a winter crop. In his "Herball," Gerarde (1597) had very clearly differentiated between turnips (*B. rapa*) and nevews (*B. napus*). Rape has been recorded as an oilseed crop in Europe at least since the Middle Ages, but it is still uncertain which species was cultivated (Appelquist and Ohlson 1972).

Domestication of rapeseed in Europe appears to have started in the early Middle Ages, although the true turnip was probably introduced by Romans. Since many other oil-yielding plants, particularly olive tree, were available in southern Europe, *B. rapa* initially spread mainly as turnip rape crop within Europe. However, in more prosperous countries like the Netherlands, the farmers used almost all Brassica seeds to produce vegetable oils. Oil was extracted from "raepsaet, koolaet, and mostaert saet" according to a Dutch reference of fourteenth century, which means "the seeds of *B. rapa*, *B. oleracea*, and *B. nigra* (or *Sinapis alba*)" (Reiner et al. 1995). As *B. rapa* was most intensively grown at that time, it can be concluded that this crop was the major source of producing large quantities of vegetable oils. Seeds of *B. rapa* were first recorded in Europe in 1620 by the Swiss botanist Casper Banhin. However, Boswell (1949) was of the view that these existed much earlier than this. As per some anonymous authors, rapeseed was grown in Europe as early as in the thirteenth century.

In the Netherlands, the commercial plantings of rapeseed were recorded in the early sixteenth century. It had limited industrial use at that time until the development of steam power, when it was discovered that rape oil was an excellent lubricant for steam engines. *B. rapa* was the dominant species in the western Canada in the early 1970s. It is comparatively a recent introduction in Canada and the United States and is found as an occasional weed or volunteer in the cultivated fields (Muenscher 1980; Munz 1968). In late 1980s, large acreages *of B. rapa and B. napus* were grown in the Prairie Provinces and these crops gradually started getting established. However, the production area sown to *B. rapa* decreased to about 15–20% in 1990s (The Biology of Brassica rapa 1999). In Austria, the annual wild-type *B. rapa* is found as a weed in rye and potato crops situated in relatively cool and high areas with an altitude of about 1,000 m (Holzner 1981). In the 1970s, the information on its distribution had been very uncertain due to incross and possibility of its escape from culture (Reiner et al. 1995). Canola is a modern, high-quality form of rapeseed and it has originated in Canada through genetic modification and emerged in the 1970s as a viable oilseed, equipped with the appropriate genetics to

transform the oil and meal from unacceptable to highly desired products for both human as well as livestock consumption (Shahidi 1990). Today, the fatty acid profile of canola is considered as the most desirable of all vegetable oil profiles by nutritionists (Stringam et al. 2003). Although superior edible oils had been developed by 1971, the presence of high amount of glucosinolates in the meal still remained a major concern in the expansion of market of the vegetable oil derived from Brassicas. In 1974, Dr. Baldur Stefansson from the University of Manitoba successfully developed the first "double low" variety with reduced levels of both erucic acid and glucosinolates (www.canola-council.org). This led to the evolution of a greatly improved crop, which met specific quality requirements of an oilseed fit for human as well as livestock consumption. As a result of these improvements, the FDA gave GRAS (generally recognized as safe) status to rapeseed oil in 1985 for use in the U.S. food products. The word "canola" was coined and trademarked for such type of rapeseed products, low in both glucosinolates and erucic acid to distinguish them from traditional rapeseed. The name canola was initially registered by the Western Canadian Oilseed Crushers' Association for reference to oil, meal, protein extractions, seed, and seed hulls from or of varieties with 5% or less erucic acid in the oil and 3 mg/g of glucosinolates (www.canola-council.org). Later, the control of the term was transferred to the Rapeseed Association of Canada in 1980, which subsequently changed its name to Canola Council of Canada (2006). The new target of achieving the ideal glucosinolate level at 15 μmol is underway. Keeping the above facts in view, it may be conveniently inferred that all canola is rapeseed but all rapeseed is not canola.

3 Origin and Evolution

The *Brassica* genus is a very complex member of the Cruciferae family, and as such it contains many cultivated plants and wild species. It, therefore, possesses several taxonomic and classification problems. Also, there is a lack of consistency in the names of different oil-yielding Brassicas throughout the globe, which aggravates the problem further. The scientific nomenclature is highly confusing, which makes it difficult for many to decide as to what particular scientific name should be used for a particular plant. Bailey (1922) has listed many reasons responsible for the chaotic nomenclature of Brassicas.

The cytogenetic relationships between the rapeseed species as well as their closest allies were first explained systematically by U (1935) about 70 years ago. These relationships show that *B. campestris* (2n = 20, AA), *B. nigra* (2n = 16, BB) and *B. oleracea* (2n = 18, CC) are the primary species and *B. napus* (2n = 38, AACC), *B. carinata* (2n = 34, BBCC) and *B. juncea* (2n = 36, AABB) are the amphidiploids resulting from paired crossings between the primary species. Morinaga (1928, 1929a, b, 1934a, b) discussed that crop Brassicas include six cotydeme three elementary ones with 16, 18, and 20 chromosomes as diploid and three with higher

Table 3.1 Genus Brassica and their ecotypes

Species	Subspecies	2n Chromosome number	Common name	Use
B. nigra Koch	–	16	Black mustard	Condiment
B. oleracea	*aephala*	18	Kale	Vegetable/fodder
	aboglabra	18	Chinese kale	Vegetable
	botrytis	18	Cauliflower	Vegetable
	capitata	18	Cabbage	Vegetable/fodder
	gemmifera	18	Brussels sprouts	Vegetable
	gongylodes	18	Khol rabi	Vegetable
	italica	18	Broccoli	Vegetable
B. campestris				
	chinensis	20	Pak-choi	Vegetable
	japonica	20		Vegetable
	narinosa	20		Vegetable
	oleifera	20	Turnip rape	Oilseed
	pekinensis	20	Chinese cabbage	Vegetable
	rapa	20	Turnip	Vegetable/fodder
B. napus				
	oleifera	38	Rape	Oilseed
	rapifera	38	Rutabaga	Vegetable
B. juncea				
	rugosa	36	Chinese mustard	Vegetable
	oleifera	36	Indian mustard	Oilseed
B. carinata				
		34	Ethiopian mustard	Vegetable/ oilseed

Source: Kalia and Gupta (1997)

chromosome numbers of 34, 36, and 38 as tetraploid, the latter having evolved through interspecific hybridization in nature between any two of the elementary taxa (Table 3.1). Morinaga and his associates carried extensive cytogenetic studies in oilseed Brassicas and clarified the relationships between them (Prakash and Hinata 1980). According to the hypothesis of Morinaga (1934a, b), the three species with the higher chromosome number, *B. napus* L., *B. juncea* L. Czern. and Coss., and *B. carinata* A. Braun, are amphidiploids combining in pairs the chromosome sets of the low chromosome number species *B. nigra*, *B. oleracea*, and *B. rapa*. U (1935) verified the hypothesis with successful resynthesis of *B. napus*. Resynthesis of *B. juncea* and *B. carinata* was accomplished by Frandsen (1943, 1947). Robellen (1960) suggested that the low chromosome number species might have developed from the ancestral species, which could have even lower chromosome numbers. Also the chromosome analysis of the monogenomic species revealed that only six chromosomes were distinctly different, the remaining being homologous with one or another of the basic set of six.

4 Breeding Objectives for Varietal Development

Oilseed Brassicas includes number of crop species which have an amalgam of breeding systems ranging from complete cross-pollination to a high level of self-pollination. Therefore, they are quite interesting material from the breeding point of view. The different crop species of this group of crop, *B campestris* var. toria, lotni brown sarson, Banarasi rai (*B. nigra*), taramira (*Eruca sativa*), and so on, are highly cross-pollinated (because of the presence of self-incompatibility, presence of bright yellow opened petals, entomophily, high sucrose content ranging from 40 to 60% in their nectaries to attract honeybees and the extrorse anther condition which turns away from the stigmatic surface at the time of dehiscence), whereas *B. juncea*, gobhi sarson (*B. napus*), karan rai (*B. carinata*), tora brown sarson (*B. campestris*), and so on, are predominantly self-pollinated (because of the absence of self-incompatibility), light pale yellow petal color, low sucrose content (5–11) in the nectaries and introrse anther condition. However, even in the self-pollinated group, due to stray pollen contamination and visit by honeybees, bumble bees, and so on, the extent of out-crossing varies from 14 to 30% (Rai and Singh 1976; Rakow and Woods 1987; Rambhajan Chauhan and Kumar 1991; Singh 1958). The self-incompatibility is of homomorphic sporophytic type (Bateman 1955) and is genetically controlled by a series of "S" alleles. The presence of same allele in the pollen and stigma will inhibit the germination of the pollen grains or will prevent the pollen tube from penetrating the stigmatic surface of the style and effecting fertilization. The evolution of mating system in genus *Brassica* is also very interesting. There is strong intergenomic interaction affecting the mode of pollination. The three primary, monogenomic species are highly cross-pollinated, whereas their amphidiploid products are predominantly self-pollinated. The commercially cultivated species *B. campestris*, however, contains both self-compatible (yellow sarson, torabrown sarson) and self-incompatible forms (toria, lotni brown sarson). In this crop species, Iotni brown sarson appears to be the logical progenitor of its different cultivated forms. The evolution in this crop species has followed two independent pathways. On one hand, toria type has evolved as an escape from the onslaught of the biotic and abiotic stresses but retained its self-incompatibility gene complex. The early maturity (75–100 days) makes it a better material to survive the stresses imposed by frost injury, aphid infestation, and the threat from *Alternaria* leaf blight disease in comparison to its parental form lotni brown sarson, which usually takes 125–140 days for crop maturity and suffers heavily on account of these stresses. There is very good morphological similarity, chromosomal homology, and cross-compatibility between toria and Iotni brown sarson. The only visible difference between them is their relative number of days taken to crop maturity. On the other hand, tora brown sarson has evolved from lotni types, this is primarily because of the cultivator's preferences for the bold seeds, uniform types, and tall growing plants, which are considered very suitable for the mixed or intercropping systems being followed by the farmers. However, in the long process of human selection for uniformity, the self-incompatibility gene complex has been lost. Later, as a result of

macromutation(s) in tora brown sarson, the yellow sarson types have evolved and have been retained by the farmers for better seed and quality values. However, in India these types are being replaced by the *B. juncea* types because of their better yield performance, stability of production, and comparatively better tolerance to various biotic and abiotic stresses. Under European and Canadian conditions, both winter and summer (spring planted) forms of *B. campestris* and *B. napus* are being grown. But in *B. juncea*, only the spring form has evolved. Winter types of Gobhi sarson (*B. napus*) are largely grown under north European, Chinese, and Canadian conditions. But because of the short crop growing period and comparatively better winter hardiness, spring types of *B. campestris* are usually preferred and are largely grown in Sweden, Finland, and some parts of Canada and northwest China. In Indian subcontinent, the spring types of *B. juncea* and *B. campestris* cultivars are largely grown. Serious attempts are now being made to introduce *B. napus* for cultivation in northwest India but on the whole, *B. napus* is the dominant commercial species and covers nearly 75% of the total cropped area under oilseed Brassicas. It is, thus, clear that toria, Iotni brown sarson, taramira, *B. nigra*, and *B. tournefortii* are highly cross-pollinated crops, and maintain very high degree of heterozygosity. Panmixis generation after generation in nature eventually frustrates the efforts of enforced inbreeding or the fixation of genotypes. Hence, in such outbreeding population, breeding superior performing cultivars with high yield would obviously require adoption of a breeding procedure which maintains the balanced heterozygosity for the optimum plant productivity. This could be accomplished through selection (mass selection, recurrent selection, disruptive selection, and so on), breeding of synthetic and composite varieties, and ultimately by developing superior performing hybrids. On the other hand, for breeding purpose, the predominantly self-pollinated crops, such as yellow sarson, mustard (*B. juncea*), gobhi sarson (*B. napus*), *B. carinata*, and so on, should be treated as often cross-pollinated crops. The breeding objectives and appropriate breeding procedures for this group of crops are discussed in this chapter.

In the Indian subcontinent, genetic improvement of seed yield is the prime breeding objective, while in the western world, breeding for quality receives greater attention. In the Asian countries, centuries of rapeseed and mustard cultivation have led to the development of local land races of *B. juncea* and *B. campestris*, and these now form the basic raw material for the breeders.

In these crops, high number of siliquae/plant and more number of seeds/siliquae have been observed to be important yield attributes associated with its higher yield expression and could form suitable criteria to breed for high seed yield (Anand et al. 1975; Nagalakshmi 1992; Ramanujam and Rai 1963; Shabana et al. 1990). Dry matter accumulation at rosette stage and leaf area index (LAI) has also been observed to be positively associated with seed yield (Olsson 1990). Early maturing varieties (80–90 days) are usually required in the Indian subcontinent for fitting in the relay, multiple, and intercropping systems. These are suitable for escaping frost injury and for growing in the drought-prone or dryland areas with scanty rainfall.

Development of high yielding, early maturing varieties is also a major breeding objective in central China and in western Canada where frost-free days in growing

season are usually less than 100 days. The early maturing varieties complete their life cycle during this period and escape the frost injury. All over the world, breeding for resistance to diseases and insect pests has become as important breeding objective. In the Indian subcontinent, *Alternaria* leaf blight, white rust, downy, and powdery mildews are the major diseases, while in the western countries, blackleg (*Leptosphaeria maculans* Desm.) is important in Canada and Australia. Some other diseases which could cause considerable economic losses to these crops are clubroot (*Plasmodiophora brassicae*), root rot (*Rhizoctonia solani* Kuhn), stem root, and so on. In some areas, *Sclerotinia sclerotiorum* (Lib.) could pose an equal or even greater threat to cultivation of Brassicas than the blackleg disease. Races of white rust (*Albugo candida*) that could attack *B. campestris* (Race 7) and *B. juncea* (Race 2) have been identified (Pidskalny and Rimmer 1985).

European and Canadian *B. napus* cultivars are resistant to all known races of white rust, but many Chinese varieties are susceptible to Race 7 (Fan et al. 1983a, b). *B. juncea* varieties possess comparatively better field tolerance to leaf blight caused by *Alternaria brassicae* than that of the *B. campestris* selection (Rai et al. 1976). *B. carinata* selections have also been observed to show comparatively better tolerance to leaf blight than other *B. campestris* or *B. juncea* selections (Bansal et al. 1990). In the Indian subcontinent, mustard aphid (*Lipaphis erysimi* Kalt.), mustard sawfly (*Athalia proxima*), and leaf miner (*Bagrada cruciferarum*) are the important insect pests that cause considerable economic losses. *B. juncea* selections are reported to possess better tolerance to mustard aphid than *B. campestris* selection (Rai and Sehgal 1975; Rai et al. 1987). In *B. campestris*, two potential sources of dwarfing genes have been reported, and it has been suggested that they could be utilized meaningfully in developing semidwarf cultivars of toria and sarson for cultivation under high population densities for obtaining high seed yield (Rai and Kumar 1978; Rai and Singh 1993; Tyagi et al. 1983). The comparatively better salt tolerance of *B. juncea* than of *B. campestris* has made it a better choice for its cultivation under the salt-affected soils of north-western Indian states. The relative ability of the spring rapeseed cultivars to withstand the onslaught of frost at flowering time is considered important in northwest India and Sweden. There is a variable reaction of the Indian and Swedish cultivars for the frost tolerance (Aberg 1984). Several biotechnology groups are now working to transfer genes for tolerance to glyphosate, chlorosulfuron, and other herbicides into the agronomic background of the various oilseed *Brassica* varieties.

In Europe and Canada, breeding for oil and cake better suited to human nutrition, and livestock feeding has received higher research priority than anywhere in the Asian countries. While a high erucic acid rape oil is liked by industry, zero or low glucosinolate (00) oil is usually required for the human consumption. The rapeseed oil with zero erucic acid content is more or less parallel to groundnut or sesamum oil in its fatty acid composition. Consumers in east Indian states usually prefer mustard oil with pungency for frying fish or preparing pickles, while those in the west Indian states prefer oil with low pungency (Rai 1976). Now the development of “00” or canola quality varieties has been developed in different parts of the world.

4.1 Genetic Resources

The success of any crop breeding program normally depends on the extent of favorable genes available in the genetic stocks handled by the breeders. At international level, IBPGR collects, maintains, and handles the genetic diversity of a number of agrihorticultural crops. In India, National Bureau of Plant Genetic Resources (NBPGR) conserves about 19,600 accessions of different oilseed crops including 4,584 of the oilseed Brassicas and its wild allies. These are now being conserved under long-term storagein gene banks for its possible use in future (Singh and Rana 1994).

All these genetic stocks are being maintained by either sibmating or selfing. Under field conditions, an isolation distance of 400 m is required to be maintained. In the insect proof cages, glass house chambers, sibmating of the culture is affected by introducing honeybee. Pure stocks of the self-incompatible inbred lines have to be maintained by bud pollination and by selfing under muslin cloth bags in self-compatible lines. Over years, a number of genetic stocks have been identified for desirable agronomical attributes like earliness, tolerance or resistance to diseases and insect pests, shattering, frost tolerance, and so on. Some of these genetic stocks are now being utilized in crossing programs in India in intervarietal and interspecific crosses to create new genetic variability, and some are being utilized as base population for selection work. The exotic cultivars have so far not been used for direct commercial cultivation in India because of their late maturity and low yield.

4.2 Sources of Creating New Genetic Variability

In rapeseed, hybridization is accomplished by emasculating the flower buds that are due to open the following day. Next day, stigma of emasculated buds are dusted with the freshly dehisced pollen from the stamen of selected plant. Under storage conditions, pollen viability has been observed to last up to 35 days (Chiang 1974). In oilseed Brassicas, because of the cross-pollinating nature of the primary species, enough variability is available, but for searching new desired genes or gene complexes for resistance to diseases, insect pests, male sterility, fertility restoration, and so on, it requires to resort to purposeful intervarietal or distant hybridizations. Intraspecific crosses (i.e., in case of *B. campestris*; crosses between tora, Iotni brown sarson, tora brown sarson, and yellow sarson cultivars) are much successful and the success rate for such crosses, if carefully made, is greater than 90% and a single emasculated and pollinated bud may yield 10–20 crossed seeds per siliquae. However, the success rate of interspecific hybridization depends much on the genetic relationship, genomic constitution of parental species used, and also on the direction of cross. In general, the interspecific hybridization is more successful, if an amphidiploids species (*B. juncea*, *B. napus*, or *B. carinata*) is used as the female parent, which has one genome in common with the pollen parent. Hybrid between monogenomic primary species are rather more difficult to be obtained with success rate of 0.002 and 0.03 hybrids per pollinated flower (Downey et al. 1980; Mahapatra

and Bajaj 1987; Quazi 1988). The basic understanding of crossability relationship among the oilseed *Brassica* species is important to the breeders of these crops because there are good possibilities of transferring agronomically important attributes like diseases and insect pest resistance, cytoplasmic male sterility (CMS), fertility restoration, desirable quality attributes, and so on. Rao (1990) observed that out of six possible combinations between *B. juncea*, *B. napus*, and *B. carinata* including reciprocals, the *B. juncea* × *B. napus* cross was easier to be made. *B. juncea* × *B. napus* hybrid plants were observed to be more vigorous than their reciprocal. Good success was also obtained in *B. napus* × *B. carinata*, but the hybridization between *B. carinata* × *B. juncea* in either direction was rather difficult, primarily because of their nonsynchrony of flowering rather than any of its crossability barriers. In elaborate fraction I protein analysis and restriction pattern of chloroplast DNA studies, *B. nigra* and *B. campestris* have been identified as the female parental genomes in *B. carinata* and *B. juncea*, respectively (Uchimiya and Wildman 1978), and *B. oleracea* as the female parent of *B. napus* (Ichikawa and Hirai 1983; Prakash and Chopra 1991; Raut and Prakash 1985). It has now been possible to transfer blackleg-resistant genes from *B. juncea* to *B. napus* because of possible recombination between A and C genomes in *B. juncea* crosses and A and B genomes in *B. carinata* crosses (Sacristan and Gerdemann 1986). A line completely resistant to blackleg disease caused by *L. maculans* was selected from the F3 progenies of cross *B. juncea* × *B. napus* (Roy 1984). Resistance to *P. brassicae* has also been transferred from *B. napus* to *B. oleracea* (Chiang and Crete 1983). Same resistance has also been transferred to *B. napus* from *B. campestris* by subsequent backcrossing with *B. napus*. When a white rust-resistant line of *B. carinata* was crossed with that of *B. juncea*, the F1 was observed to be resistant to white rust with some additional resistance to *A. brassicae*. This has shown the possibility of transferring white rust resistance from *B. carinata* to *B. juncea* (Singh and Singh 1987, 1988). The Swedish rapeseed cultivar 821 has been developed from the cross *B. napus* × *B. chinensis* (He et al. 1987). The triazine resistance has been transferred from *B. napus* to *B. oleracea* (Ayotte et al. 1986, 1987). CMS has been transferred from *B. juncea* to *B. napus* through interspecific hybridization followed by four generations of backcrossing (Mathias 1985). The CMS was transferred from radish to *B. oleracea* (Bannerot et al. 1974; Mc Collum 1988). The fertility restorer genes for Polima-type CMS system of *B. napus* has been found in the *B. napus* var. Zem (Fan and Tai 1985). Genes for earliness have also been introgressed from *B. napus* and *B. carinata* to *B. napus* varieties. The genes for high linoleic acid have been transferred from *B. napus* to *B. napus* through selection in F2 generation (Roy and Tarr 1985; 1986). There are good possibilities of incorporating shattering resistance from *B. napus* and *B. carinata* to *B. napus* cultivars (Prakash and Chopra 1988; Rao 1990). Wide hybridization has been reported with some degree of success in the crosses of *B. spinescens* (2n = 16) × *B. campestris* (2n = 20) and for the production of *B. napus* × Raphanobrassica hybrids (Agnihotri et al. 1990a, b, c) by embryo rescue and ovary culture techniques. Protoplast fusion has helped in obtaining somatic hybrids of *B. oleracea* with Moricandia arvensis which possess intermediates C3–C4 photosynthesis carbon metabolism (Toriyama et al. 1987). From the

above-mentioned examples, it is clear that both interspecific and intergeneric hybridizations have much potential for creating new variability for rapeseed improvement. The fact is that the available natural variability of oilseed Brassica Landraces/germplasm has not yet been fully tapped and exploited with a few exceptions. If we search systematically, the needed characteristics could be found within the species of interest from close relatives. Few examples include spotting of the needed early maturing selections of *B. napus* in India and Canada, vary widely in seed size, oil content in *B. napus* in India, resistance to white rust, and blackleg in *B. napus* and *B. campestris* in France and Australia. But wherever usable variability is not available in the working germplasm, the induced mutagenesis could as well be explored and utilized.

4.3 Induced Mutagenesis for Creating New Variability

Induced mutagenesis is a useful tool for creating new variability hitherto not available, and a number of studies utilizing ionizing radiations (X-rays and cobalt 60) and chemicals such as ethylemethane sulfonate (EMS) have been used. Usually 60–80 Kr doses of rays are quite effective. Induced mutagenesis has been used to obtain mutant lines with 3% linolenic acid in *B. napus* (Rakow 1973; Robbelen and Nitsch 1974), for spotting seed color mutant in mustard (Verma and Rai 1980a), and for tolerance to leaf spot disease (Verma and Rai 1980b). It has also been very helpful in developing a number of rapeseed varieties in Sweden. Induced mutagenesis has also been used to create new variability for earliness, compact plant type, and yellow seed color in mustard at Bhaba Atomic Research Centre, Trombay, India. Two high-yielding lines of mustard (TM2 and T4) have emanated from this program.

5 Breeding Methods

Crucifers includes number of cultivated crops and wild species that have a breeding system ranging from complete cross-pollination to a high level of self-pollination. Therefore, these are quite interesting material from the breeding point of view. Selection procedure in cross-pollinated species vary from mass selection to recurrent selection and in predominantly self-pollinated ones, the desirable plants are usually selected from broad base population such as land races, segregated population, germplasm complexes, gene pools, etc., and are bulked. This bulked seed is repeatedly grown cycle after cycle. One cycle of mass selection in toria is reported to have given a yield improvement of 8.2% (Chaubey 1979).

Segregating populations or the progenies from the crosses could also make good base population for initiating recurrent selection program. In this method, the desirable individual open-pollinated plants (around 3,000) are harvested and threshed separately. A part of this seed is saved and other part of it is planted in a progeny rows,

evaluated visually and superior rows are selected, tagged and harvested separately. After harvesting and threshing, the seeds are analyzed for their 1,000-seed weight, oil content, glucosinolates and protein content, etc. Thereafter, equal quantities of the reserved seed from the selected plants are composited. This way, the first of recurrent selection cycle is completed and this composited seed is grown again in field in isolation, where intercrossing takes place among the plants within the composited populations. The second cycle of recurrent selection starts with the harvesting of the single plants (around 1,000) from this population. A bulk seed sample is harvested from the remaining plants of the population for use in replicated yield trials to determine response to selection in each recurrent cycle for character under improvement viz., oil content, seed yield or tolerance to a disease. Recurrent cycle selection is continued till reasonable level of improvement is achieved.

In self-pollinated crucifers, pure line selection is usually followed in India, which involves the isolation of superior performing lines from a genetically broad base population based upon their progeny performance. Various improved varieties like Varuna, Krishna, Kranti, Shekhar, Sita, RH-30 and Durgamani have all been developed from such simple breeding efforts (Rai 1983a, b).

6 Pedigree Method

This method may be effectively utilized for concentrating favorable genes for various economic traits and has been used to produce many cultivars in *B. napus* and *B. juncea.* In India, various high yielding varieties were developed following the pedigree selection. In this method, 5–10 F_1 plants are grown to obtain F_2 seed and 1,000–3,000 F_2 plants are grown and harvested individually from which F_3 progenies are secured. In F_4 generation, the selection is practiced. The variation among F_4 families is a good indication for the effectiveness of further selection. This method has been utilized to develop a low erucic acid high yielding and winter hardy *B. napus* variety from a cross between high erucic winter *B. napus* variety "Rapol" and the low erucic acid spring *B. napus* variety "Oro."

7 Backcross Breeding

When the desirable gene is available from unadapted or wild population, backcrossing would be the right choice, but if the favorable gene is available in an adapted or cultivated material's background, then pedigree method of selection would be the most appropriate procedure. The spring *B. napus* variety "Wester" had been developed by a combination of backcross and pedigree breeding. Backcross breeding has been used to transfer the low glucosinolate content of *B. napus* variety Bronowski, into a number of commercial cultivars of Gobhi Sarson (*B. napus*) in

various parts of the world. This method is also used to transfer new traits such as fatty acid composition, seed color, herbicide, and insect-pest resistance.

8 Development of Synthetics and Composites

In Indian subcontinent, development of composite varieties is being viewed as a possible way out for increasing the average yield production of Brassicas as these are largely grown under the rainfed conditions. These are subjected to all sorts of biotic and abiotic stresses (Rai 1979). Although synthetic in *B. napus* cultivars were also marketed in Europe, they were often not uniform and therefore this method of breeding is no longer used in *B. napus*. In Canada, efforts to develop synthetic in spring *B. napus* were not very encouraging for successful commercial cultivation. The composite breeding program in *B. rapa* and other Brassicas usually involves the production of number of intervarietal hybrid or by making their blends. This is followed by evaluation of inbreeding depression in seed yield from F_2 and later generation and the evaluation of the performance of the experimental checks against the ruling checks (Rai 1982).

Development of synthetic varieties requires the development of inbred lines, their testing for general combining ability (GCA) by making all possible cross-combinations, predicting F_2 performance constituting a number of experimental synthetic, testing the yield in trials over location and finally releasing those which excel the standard check.

9 Development of Hybrids

The basic requirement for developing commercial hybrids in crops like rapeseed is the availability of proven experimental hybrids (preferably with more than 20% standard yield heterosis), stable performing male sterile (A), maintainer (B), and fertility restoring (R) lines, good synchrony of flowering in seed and pollen parent, and adequate seed setting on male sterile seed parent through natural cross-pollination. High level of heterosis for seed yield in both spring and winter forms of *B. napus*, that is, quantitatively, 40% heterosis for yield has been reported in summer rape and 60–70% for winter form (Grant and Beversdorf 1985; Lefort-Buson and Datte 1982; Sernyk and Stefansson 1983).

A number of initial studies have demonstrated that there is considerable heterosis for yield in brassicas (Schuster and Michael 1976; Lefort-Buson and Dattee 1982), *B. rapa* (Sernyk and Stefansson 1983; Schuler et al. 1992) and *B. juncea* (Singh 1973; Larik and Hussain 1990; Pradhan et al. 1993). In India, 11–82% check parent yield heterosis has been reported in mustard (*B. juncea*), 10–72% in Gobhi Sarson, and 20–107% in *B. campestris* (Das and Rai 1972; Labana et al. 1975;

Yadava et al. 1974; Doloi 1977; Srivastava and Rai 1993), which is sufficiently high for its exploitation in hybrid cultivars. A range of 14–30% natural outcrossing is usually observed in these crops. So, this is sufficient to justify the efforts to develop cytoplasmic male sterile (CMS) lines and search for usable fertility restorer lines for producing the hybrids.

In oilseed Brassicas, a number of CMS sources viz., *Brassica carinata* CMS, *B. juncea* CMS, *B. oryrhina* CMS, *B. tournefortii* CMS, *Raphanus*-based ogura CMS, *B. napus*-based Polima CMS, *Siettiana* CMS, *Siifolia* CMS, etc., are now well known and some of them are being worked with rather intensively. Out of these CMS sources, fertility restoration has been identified in *Raphanus*-based Ogura CMS, 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. Fortunately, Punjab Agricultural University, Ludhiana in India has recommended to release first CMS-based Gobhi Sarson hybrid PGSH-51 for cultivation in Punjab state in India.

9.1 Commercial Hybrids in B. campestris

High level of exploitable yield heterosis has been reported in *B. campestris* hybrids (Das and Rai 1972; Hutchenson et al. 1981). In this species, CMS system has been developed by backcrossing *B. campestris* cultivar "Yukina" into the *Diplotaxis muralis* cytoplasm (Hinata and Konno 1979). The *B. campestris* "yukina" CMS was stable and restorer genes have been identified for this CMS source but the genes for maintenance of CMS will have to be transferred into the background of the adapted commercial cultivars of *B. campestris* before the hybrids could be put to test in *B. campestris*. In China, where adequate labor is available, gobhi sarson hybrids have been produced utilizing this type of male sterility (Lee and Zhang 1983).

9.2 Self-Incompatibility and Hybrid Seed Production

On the basis of sporophytic type of self-incompatibility, a theoretical model of triple cross $[(A \times B) \times C] \times [(D \times E) \times F]$ technique has been suggested by Thompson (1964) to exploit heterosis and produce commercial hybrid in these crops. However, practically it has not been put to commercial use. The difficulties of producing commercial quantities of selfed seeds of self-incompatible parental lines of the hybrid and also difficulties in detecting the breakdown of self-incompatibility in the production plots during flowering duration make it rather economically unviable and unprofitable. Self-incompatibility is a good outbreeding mechanism in nature, but unfortunately, due to very high self-incompatibility, heterozygosity, and as a result of high inbreeding depression, it frustrates the efforts to produce and maintain the homozygous lines which could produce the hybrid cultivars. It is a difficult task to maintain the inbred lines through continued selfing, primarily because of big loss of vigor of the inbred population to grow and produce seeds.

10 Artificial Synthesis of Amphidiploids for Commercial Cultivation

The commonest choice for direct interspecific hybridization is to double the chromosome number in the sterile hybrids and to establish fertile amphidiploids. This provides stability and could help in the preservation of gene complexes of both the componential species by enhancing the preferential pairing of the homologous chromosomes. Artificial synthesis of some of the commercially cultivated amphidiploid species, viz., *B. napus*, *B. napus*, and *B. carinata* has been reported long back (Frandsen 1943; Ramanujam and Srinivasachar 1943; UN 1935). Artificial synthesis of *B. napus* and *B. carinata* is comparatively easier than *B. napus*. Synthesis of *B. napus* has added to the usable variability for use in India. Undoubtedly, the derivatives of synthetic *B. napus*, *B. napus*, and *B. carinata* could provide additional material for widening the range of genetic variability. It is now possible to use some of these resynthesized digenomic strains in interspecific hybridization via backcross to obtain useful genotypes for commercial production programs. At IARI, New Delhi, Raut and Kaul (1982), Raut and Prakash (1985), and Prakash and Raut (1983) have synthesized early maturing and productive amphidiploids of *B. napus* by crossing early indigenous strain of *B. campestris* var. Iotni brown sarson with that of *B. oleracea* var. *botrytis*. The selections obtained are now being field tested for their comparative yield performance with commercial check varieties. The efforts to produce and improve tetroploids of toria variety T22 (Rajan 1955; Sikka and Rajan 1957) and various yellow sarson strains for genetic improvement of seed and oil yield has not met with much of success, though the induced tetraploidy has been of some use in developing high yielding fodder varieties of Brassicas in west European countries.

11 Development of Herbicide Tolerant Cultivars

Herbicides provide an inexpensive and effective means of control of weeds in crop Brassica. Development of herbicide-resistant cultivar in Brassica was started in 1960. The tolerance was cytoplasmically controlled and effective against triazine family of herbicide. Identification of triazine-tolerant biotype of bird's rape mustard led to the development of triazine-tolerant *B. napus* oilseed cultivars through introgression of the tolerant weed biotype cytoplasm in oilseed rape. Through an interspecific cross and backcross program, the tolerant cytoplasm of *B. campestris* was combined with the nucleus of *B. napus* to produce the first triazine-tolerant cultivar, OAC Trinton (Beversdorf et al. 1980). Triazine-tolerant *B. napus* cultivars are very useful and indeed essential in fields, where highly competitive weeds such as wild mustard (*S. arvensis* L.),stickweed (*Thlaspi arvense* L.), and quack grass (*Agropyron repens* L.) are found, suggesting that the growth rate and yield of triazine-tolerant cultivars will always be significant than that of recurrent parent (Downey and Rimmer 1993).

12 Development of *Alternaria* Blight and Aphid-Resistant Cultivars

Breeding for resistance to diseases and insect pests has now become an important breeding objective. In the Indian subcontinent, Alternaria leaf blight, white rust, and downy and powdery mildews are the major diseases in rapeseed, while in the Western countries, blackleg (*L. maculans* dasm.) is important in Canada and Australia. Yield losses may range up to 70% varying from location to location and year to year. No resistance has been reported against this disease in oilseed Brassicas. However, some of the interspecific crosses have been attempted between *B. napus* and *B. alba* with an objective to transfer Alternaria resistance from *B. alba* to *B. napus* (Brim et al. 1987; Chevre et al. 1991; Dueck and Degenhardt 1975; Rai 1976). Tewari (1991) have shown that more distantly related Crucifereae species may be very resistant to black spot. Some other diseases, which could cause considerable economic losses to these crops, are clubroot (*P. brassicae*), root rot (R. I Kuhn), stem rot, and so on. In some areas, *S. sclerotiorum* (Lib.) could pose an equal or even greater threat to the cultivation of Brassicas than blackleg disease. Races of white rust (*A. candida*) that could attack *B. campestris* (Race 7) and *B. napus* (Race 2) have been identified (Pidskalny and Rimmer 1985). European and Canadian *B. napus* cultivars are resistant to all known races of white rust but many Chinese varieties are susceptible to Race 7 (Fan et al. 1983). *B. napus* varieties possess comparatively better field tolerance to A. brassiceae-caused leaf blight than that of the *B. campestris* selections (Rai 1976). *B. carinata* selections have also been observed to show comparatively better tolerance than other *B. campestris* or *B. napus* selections (Bansal et al. 1990). In the Indian subcontinent, mustard aphid (*L. erysimi* Kalt.), mustard sawfly (*Sathalia proxima*), and leaf minor (*B. cruciferarum*) are the important insect pests that affect and cause economic losses. *B. napus* selections possess better tolerance to mustard aphid than *B. campestris* selections (Rai and Sehgal 1975; Rai et al. 1987).

13 DH Breeding and In Vitro Mutagenesis

Doubled haploidy (DH) breeding through microspore culture is very well developed in Brassicas (Maluszynski et al. 2003; Xu et al. 2007). The DH technology in Brassicas aims at developing fully homozygous plants in a single generation, which could be further used in mutation breeding, genetic engineering, in vitro screening for complex traits like drought, cold and salinity tolerance, and for developing mapping populations for linkage maps using molecular markers (Pratap et al. 2007). Several methods are available for DH production in Brassicas such as microspore culture, anther culture, and ovary/ovule culture. The possibility to produce haploids in *B. napus* from anther culture (Keller and Armstrong 1978) and microspore culture (Lichter 1982) has provided the breeders with a new tool for breeding improved cultivars of rapeseed and mustard (Zhou et al. 2002a, b).

The initiation of microspore culture experiments was followed by extensive investigations on various aspects of embryogenesis in anther and microspore culture and as a result, DH technology has been developed to its present form in Brassicas (Charne and Beversdorf 1988; Yu and Liu 1995; Wang et al. 1999, 2002; Shi et al. 2002). Microspore culture technique has widespread applications in *Brassica* breeding due to its relative simplicity, efficiency in haploid and doubled haploid production, mutation and germplasm regeneration, and gene transformation (Xu et al. 2007). Also microspore cultures provide the best material for mutation induction in haploid cells (Szarejko and Forster 2007). Microspore embryogenesis is affected by a number of factors such as donor plant genotype and conditions, pretreatment, growth stage of the anther/microspore to be cultured, culture media and environment, and diploidization process, etc. (Dunwell 1996; Gu et al. 2003, 2004; Zhang et al. 2006; Pratap et al. 2007).

Mutagenic treatments may have significant effects on the efficiency of DH breeding. McDonald et al. (1991) reported that UV light had harmful effects on embryo formation in rapeseed though regeneration remained unaffected and at the same time, gamma irradiation decreased the frequency of embryos and plants. The induction of mutation in haploid cells involves isolating the developing microspores at the late uninucleate stage followed by pretreatment and their culturing on specialized media, which lead to direct embryogenesis rather than formation of pollen (Szarejko and Forster 2007). Mutagenic treatment is given shortly after the isolation of microspores or after pretreatment, before the first nuclear division. Due to direct embryogenesis, uninucleate microspore is the ideal target for in vitro mutagenesis. Also, microspores are far more sensitive to mutagenic treatments than other explants and therefore yielded better results.

DHs also provide an efficient screening material for the desired mutants and other material for complex traits. Since through microspore-derived DHs we can obtain a very large number of synchronously developing embryos, we can modify the system to screen them in vitro for various desirable traits. For example, for development of herbicide-resistant Brassicas, the active chemical is introduced in the culture medium after mutation treatment (Beversdorf and Kott 1987) and the surviving plants after chromosome doubling could be raised under controlled conditions and later screened for this trait. Similarly, effective selection could also be done for drought, cold, and salinity tolerance. By using this technique, several herbicide-resistant mutants have been developed in rapeseed (Kott 1995, 1998; Swanson et al. 1988, 1989).

Though embryogenic microspores are the prime targets for mutagenic treatment, other haploid tissues and cells have also been treated with mutagens in Brassicas. In *B. napus*, isolated microspores have been treated with chemicals such as EMS (Beversdorf and Kott 1987), NaN_3 (Polsoni et al. 1988), MNU (Cegielska-Taras et al. (1999) and ENU (Swanson et al. 1988, 1989) and physical mutagens such as gamma rays (Beversdorf and Kott 1987; McDonald et al. 1991), X-rays (McDonald et al. 1991) and UV rays (Ahmad et al. 1991; McDonald et al. 1991). *B. napus* anthers have also been treated with gamma rays and fast neutrons by Jedrzejaszek et al. (1997). Similarly, microspores of *B. carinata* have been treated with EMS and UV rays (Barro et al. 2001, 2002) and *B. campestris* with UV rays (Zhang and

Takahata 1999; Ferrie and Keller 2002). In *B. juncea* also, isolated microspores as well as haploid embryos have been treated with chemical mutagens.

Despite great promise, the use of DH technology as a routine breeding tool for *Brassica* improvement is yet to be seen, mainly due to problems associated with anther/microspore culture (Pratap et al. 2007). These include low regeneration rate, highly genotype-specific response and high frequency of callogenesis but low recovery of DH plants. The focus of rapeseed breeders has lately shifted toward more specific and practical goals such as development of herbicide-tolerant varieties, development of male sterile lines for hybrid seed production, oil and meal quality improvement, and also drug production (Gupta and Pratap 2008). For this, DH breeding has to be adopted in conjugation with newer ideas such as directed in vitro mutagenesis, in vitro screening for desirable traits, and incorporation of molecular markers.

14 Genetic Transformation

Biotechnology has opened up new horizon for novel agronomic and quality traits in responsive crops such as Brassicas by providing access to novel molecules, ability to change the level and pattern of gene expression and development of transgenics with insecticidal genes. With the development of genetic transformation techniques, it has become possible to bring about quick and dramatic improvements in the tolerance to many Lepidopteran and other insect pests, and herbicides, improvement in oil quality for industrial and domestic use and development of pharmaceuticals and industrial products. Much emphasis is now placed on the transgenic technology toward the improvement of cultivated Brassicas. As a result, the global area of biotech canola has reached to an estimated 5.5 million ha in 2007 (James 2007), majority of it being under herbicide-resistant canola.

Successful genetic transformation systems have been developed in many economically important Brassicas such as *B. napus* (Moloney et al. 1989), *B. oleracea* (De Block et al. 1989), *B. juncea* (Barfield and Pua 1991), *B. carinata* (Narasimhulu et al. 1992), *B. rapa* (Radke et al. 1992) and *B. nigra* (Gupta et al. 1993). However, among all the systems, *Agrobacterium tumefaciens*-mediated gene transfer is most widely used in *Brassica* and it is also quite efficient and practical in most of the species in the genus (Cardoza and Stewart 2004).

Rapeseed cultivars tolerant to herbicides such as imidazoline, glyphosate, and glufosinate are available commercially in USA and Canada (Cardoza and Stewart 2004). For insect resistance, the gene from *Bacillus thuriengiensis* has been introduced in canola cultivars (Stewart et al. 1996; Halfhill et al. 2001), which leads to overproduction of δ-endotoxins in the insects feeding on transgenic canola. This crystalline prototoxin gets inserted into the midgut plasma membrane of the insect, leading to lesion formulation and production of pores that disturb the osmotic balance. These cause swelling and lysis of the cells and as a result, the larvae stop feeding and die (Hofte and Whiteley 1989; Schnepf et al. 1998; Shelton et al. 2002).

Canola varieties with increased linolenic acid (Liu et al. 2001), stearate (Hawkins and Kridl 1998), laurate (Knutzon et al. 1999), and increased enzyme activity (Facciotti et al. 1999) have been developed through genetic transformation. Further, Brassicas have been transformed to develop various industrial and pharmacological products also. For example, *B. carinata* has been transformed for the production of hirudin, a blood anticoagulant protein (Chaudhary et al. 1998) while *B. napus* has been used for the production of carotenoids (Shewmaker et al. 1999). Development of male sterile lines and fertility restoration systems has also been achieved through genetic transformation in *B. napus* (Jagannath et al. 2001, 2002), which could be of tremendous potential for development of commercial hybrid cultivars. Similarly, salt- and cold-tolerant tolerant lines have also been developed in *B. juncea* by engineering of the bacterial *codA* gene (Prasad et al. 2000). Transgenic lines of "Wester" having high palmitic and stearic acids have been developed by Hitz et al. (1995) High oleic acid containing *B. napus* and *B. juncea* lines with better shelf life have also been obtained through the transgenic technology (Stoutjesdijk et al. 2000).

15 Quality Improvement

Brassica oil is nutritionally superior to most of the other edible oils due to the lowest amounts of harmful saturated fatty acids and a good proportion of mono- and polyunsaturated fatty acids in it (Agnihotri et al. 2007). However, the value of its oil and meal gets restricted due to the presence of two major antinutritional substances, erucic acid – a long carbon chain unsaturated fatty acid, and glucosinolate – the sulfur-containing compounds.

Oil quality mainly relates to fatty acid composition of the seed. High contents of erucic and eicosenoic acids in *Brassica* oils decrease the profile of oleic, linoleic and linolenic acids, rendering them inferior in quality to those from other oilseeds (Gupta and Pratap 2007). Therefore, one of the most important breeding objectives in *Brassica* breeding has been the genetic modification of the seed quality by changing the proportion of fatty acids suitable for nutritional as well as industrial purposes. Modifications in the compositions in fatty acids have been achieved in past through various conventional breeding methods coupled with biotechnological techniques such as induced mutation, in vitro embryo rescue, DH technique and genetic engineering, especially posttranscriptional gene-silencing (Agnihotri et al. 2007).

Dietary recommendations in many countries focus attention on limiting total fat intake to 30% of energy and saturated fat intake to 10% of energy. Breeding approaches in reducing the saturates include interspecific crosses followed by selection, reconstitution of *B. napus* from *B. rapa* and *B. oleracea* strains with reduced saturate levels, and mutagenesis in both *B. rapa* and *B. napus*. For reduction in linolenic acid, both mutagenic source and genetic transformation can be used. Gas liquid chromatography (GLC) (Craig and Murphy 1959) and the technique of using only half of the cotyledon to test the erucic acid content together provide quick means to screen very large populations necessary to identify genetically changed

Brassica strains with low or zero erucic acids. With this technique, the desirable strains with half cotyledon intact have been grown and carried forward and with this, low erucic acid strains of *B. napus* (Stefansson et al. 1961; Downey and Harvey 1963) and *B. campestris* (Downey 1964) had been developed in early 1960s. Later, such strains were developed in *B. juncea* (Kirk and Oram 1981) and *B. carinata* (Alono et al. 1991). Gupta et al. (1994, 1998) identified low erucic acid genetic stocks among the Indian accessions of *B. juncea.* Several low erucic acid *B. juncea* genotypes have been developed in India through interspecific hybridization (Khalatkar et al. 1991; Malode et al. 1995), and transgressive segregation through interspecific/intergeneric hybridization, followed by pedigree method (Agnihotri et al. 1995; Agnihotri and Kaushik 1998, 1999a, b). Similarly, other fatty acids have also been modified in oleiferous Brassicas and high oleic and low linolenic acid *B. juncea* genotypes have been developed (Oram et al. 1999; Potts et al. 1999).

Brassica seed meal is an important source of nutrition for animals. However, undesirable components in the meal such as glucosinolates render them unfit for animal and human consumption. In high concentrations, in nonruminants like swine and poultry, it hydrolyzes to form thiocynates, isothiocynates or nitriles and can adversely affect iodine uptake by the thyroid gland and can reduce their weight gains (Fenwick et al. 1983). The high-performance quantitative GLC technique (McGregor et al. 1983a, b, Spinks et al. 1984; Brazezinski et al. 1986) has made it possible to obtain the profiles of glucosinolates and also measure their absolute levels. Besides glucosinolates, other antinutritional factors such as sinapine, phenolic acid, tannins and phytic acid also interfere with the digestive enzymes, especially those affecting protein hydrolysis. To improve the quality of *Brassica* seed meal, the glucosinolate contents should either be decreased or altogether eliminated from the meal through appropriate breeding techniques. However, unfortunately, the genes controlling glucosinolate content in rapeseed are either pleiotropic or in linkage with the seed filling stage and have a positive correlation with 1,000-seed weight (Oliveri and Parrini 1986). This renders the strict selection difficult for quality traits in early segregating generation, lest genotypes for high seed yield could be lost. Therefore, it is advocated to keep the population heterozygous for quality characters and select the plants for these characters in advanced generation.

Till date, the "Bronowski" gene is the only known source for low glucosinolates content and no natural germplasm source for stable low glucosinolates genes has been reported (Agnihotri et al. 2007). "Bronowski" is a Polish *B. napus* cultivar, which has a glucosinolate content of about 12 μmol/g oil free meal and 7–10% of erucic acid in the oil. Considerable success has been achieved in Australia in the development of low glucosinolate genotypes using mutagenesis, interspecific hybridization, and tissue culture coupled with pedigrees election (Oram et al. 1999). In India, two transgressive segregants (TERI 5 and TERI 6) with low glucosinolate and a Canadian accession BJ-1058 have been used to develop low-glucosinolate genotypes in the background of *B. juncea* var. Pusa Bold (Agnihotri and Kaushik 2003a, b; Agnihotri et al. 2007).

Breeding of "canola" type of cultivars in Brassica with less than 2% erucic acid in the oil and less than 30 μmol/g of glucosinolate in defatted meal (commonly

known as "00") has been done and several such varieties viz., Cyclone (Denmark), Shiralee (Australia) and AC Excel (Canada) are now available (Rakow 1995). In Australia, several double low cultivars of *B. juncea* have shown promising yield potential (Burton et al. 2003b). In India, Agnihotri and Kaushik (2003a, b) have reported successful introgression of double low traits in *B. juncea* cultivar "Varuna" using low erucic acid donors TERI (OE) M21 and Zem-1, and low glucosinolate line BJ-1058. The double low *B. napus* varieties GSC-865 and TERI-Uttam-Jawahar have been released for commercial cultivation in the states of Punjab and Madhya Pradesh, respectively (Agnihotri et al. 2007).

Introduction of double low (low erucic acid–low glucosinolates) genotypes of *B. napus* has followed their extensive cultivation in many countries of the world and experimental work toward development and improvement of low erucic acid germplasm for other species is being pursued at global level (Rakow and Raney 2003). At present, the breeding efforts in the development of canola quality double low *B. napus* cultivars in improving the oil composition and enhancing vitamin levels are underway in many countries of the world including Germany (Luhs et al. 2003), Canada (Raney et al. 2003a,b), United States (Corbett and Sernyk 2003), Australia (Gororo et al. 2003), France (Carre et al. 2003) and Poland (Spasibionek et al. 2003). Yellow seed coat color also adds to high oil content and therefore this could also be another breeding objective for improved Brassicas.

16 Conclusion

The current trends in the rapeseed breeding research indicate that to maintain the tempo of progress in quality and yield improvement work in these crops, much expanded research efforts would be needed to solve the emerging and challenging problems ahead. Now, there would be far greater need for the collection, computerization, and creation of new usable genetic variability, greater application of cytoplasmic male-sterility techniques, chromosomal mapping studies, and assimilation of many new and novel ideas for tackling new problems. It would also be desirable to make broad-based genepools of different cross-pollinated species of oilseed Brassicas and maintain at least one gene pool having all the available collection of the group. Such a population is likely to have more natural recombination hitherto not available in nature and provide an opportunity to break some of the existing undesirable genetic linkage, and provide good base population for future recurrent selection programs. Exploitation of heterosis in oilseed Brassicas would require more intensive and concerted efforts for effective utilization of cytoplasmic male sterility. In years to come, meaningful basic work would also be needed on the stability of sterility in CMS lines, understanding of the mechanism of fertility restoration, extent of cytoplasmic penality that would normally be expected on using CMS lines from a very distant wild types/species of genera and on perfectization of hybrid seed production techniques. In the Indian subcontinent, some of recently introduced materials of the disease-resistant or canola quality lines from outside may not be

high yielders per se under the local condition, but they could possibly make good parental lines for production of hybrids and if that possibility exists, it should be explored. The production of doubled haploid lines, either by microspore or anther culture techniques, is now possible to rapidly produce homozygous inbred lines from the promising *B. napus* and *B. campestris* genotypes as well. Such inbreds could be produced and used to develop more productive hybrid cultivars. The inputs available from biotechnology may more purposefully be utilized in solving the pressing problems of male sterility, fertility restoration, crossability, and oil and seed meal quality. An exciting and challenging area of rapeseed breeding research would be to develop cultivars with built-in genetic resistance to devastating insect pests like mustard aphids and *Alternaria* leaf blight disease in the Indian subcontinent and for the important diseases like blackleg (*L. maculans* Desm.), clubroot (*P. brassicae*), *S. sclerotiorum*-caused damage, and white rust diseases in some of the western countries where these diseases threaten production. Development of fertilizer responsive, nonlodging, compact plant types with high population densities would be more rewarding breeding preposition in the years to come. Incorporation of dwarfing genes and development of semidwarf varieties of *B. campestris* could pay rich dividends. Presently, in the Indian subcontinent, much emphasis is being laid on the genetic improvement of yield but the future will see much expanded genetical and breeding investigations to improve quality characteristics of the commercial cultivars to meet the export needs. The search for genes governing thermo- and photoinsensitivity and also better photosynthetic activity would receive far greater attention than what is at present. The incorporation of such genes in rice and wheat has proved useful in expanding the areas of their production. So, why not in rapeseed? The cultivars with these genes could be grown over a wide range of crop growing conditions and this would help in increasing the overall production of these important oil crops.

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