Chapter 3.

Brassica crops (*Brassica* spp.)

This chapter deals with the biology of Brassica *species which comprise oilseed rape, turnip rape, mustards, cabbages and other oilseed crops. The chapter contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy for a range of* Brassica *species, their centres of origin and distribution, reproductive biology, genetics, hybridisation and introgression, crop production, interactions with other organisms, pests and pathogens, breeding methods and biotechnological developments, and an annex on common pathogens and pests.*

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Introduction

The plants within the family Brassicaceae constitute one of the world's most economically important plant groups. They range from noxious weeds to leaf and root vegetables to oilseed and condiment crops. The cole vegetables are perhaps the best known group. Indeed, the *Brassica* vegetables are a dietary staple in every part of the world with the possible exception of the tropics. The Food and Agriculture Organization of the United Nations estimates that world commercial production of cabbages, cauliflowers, broccoli and other *Brassica* vegetables in 2013 was over 93 million tonnes from about 3.7 million hectares, with a 2013 farm gate value of some USD 31 billion (FAOSTAT, 2013). These figures do not include the root vegetables or the production from kitchen gardens.

Less well known are the *Brassica* oilseed crops that annually occupy over 34 million hectares of the world's agricultural lands (FAOSTAT, 2013). Because of their ability to survive and grow at relatively low temperatures, they are one of the few edible oil sources that can be successfully produced in cool temperate regions. This characteristic makes them well adapted to cultivation at high elevations and as winter crops in the subtropics. In temperate regions, oilseed rape (*Brassica napus*)[1] and turnip rape (*Brassica rapa*) predominate, while in the subtropics of Asia, Indian mustard or rai (*Brassica juncea*) is the major oil source. Among all the commodities moving in world trade, only petroleum has a greater value than vegetable oils (United States Census Bureau, n.d.; United Nations, n.d.). In total, *Brassica* oilseeds provide 15% of the world's edible vegetable oil and are the third most important source of edible oil after soybean and palm (Table 3.1).

Table 3.1. **World production of edible vegetable oils, averages 1996-2000 to 2011-15**

Millions of tonnes

Crop	1996-2000 (MMt)	2001-05 (MMt)	2006-10 (MMt)	2011-15[2] (MMt)
Soybean	22.4	29.8	36.8	46.2
Palm	18.3	28.2	40.8	58.5
Rape/mustard	*11.8*	*13.7*	*19.1*	*25.7*
Sunflower	8.9	8.4	11.0	14.7
Groundnut	4.3	4.9	4.8	5.5
Cottonseed	3.7	4.1	4.9	5.1
Others[1]	7.9	9.5	11.2	13.1
Total	**77.3**	**98.6**	**128.6**	**168.7**

Notes: 1. Others include olive, coconut and palm kernel. 2. This column was added in January 2016.

Source: After USDA Foreign Agricultural Service (2015).

Species or taxonomic group

Classification and nomenclature

The family Brassicaceae (= Cruciferae) contains over 338 genera and 3 709 species (Al-Shehbaz, Beilstein and Kellogg, 2006; Warwick, Francis and Al-Shehbaz, 2006). The species of greatest interest to those concerned with genetically modified crops are given in Table 3.2 with their chromosome number, genome identification and common English name(s).

Table 3.2. **Nomenclature and genome relationships of cultivated *Brassica* species and related genera**

Species name	Common synonym	Haploid chromosome number and genome	Common name
Brassica rapa L.	*B. campestris* L.	10 AA	
subsp. *campestris* (L.) A.R. Clapham			Summer turnip rape, canola
subsp. *oleifera* (DC.) Metzg.			Winter turnip rape
subsp. *campestris* (L.) A.R. Clapham	subsp.*eu-campestris* (L.) Olsson		Bird or wild turnip rape
subsp. *trilocularis* (Roxb.) Hanelt	subsp. *sarson* (Prain) Denford		Yellow and brown Sarson
subsp. *dichotoma* (Roxb.) Hanelt			Toria
subsp. *chinensis* (L.) Hanelt	*B. chinensis* L. *B. chiensis* var. *parachinsis* (L.H. Bailey)		Pak-choi or bok choy, Chinese mustard, Chinese broccoli, Gai Lan
subsp. *pekinensis* (Lour.) Hanelt	*B. pekinensis* (Lour) Rupr.		Pe-tsai, Chinese cabbage
subsp. *nipposinica* (L.H. Bailey) Hanelt			Curled mustard
subsp. *Rapa*	*B. rapa* L.		Turnip
Brassica tournefortii Gouan		10 TT	Wild turnip
Brassica nigra (L.) W.D.J. Koch		8 BB	Black mustard
Brassica oleracea L.		9 CC	
var. *viridis* L.	var. *acephala* DC.		Kale, collard
var. *botrytis* L.			Cauliflower and broccoli
var. *capitata* L.			Cabbage
var. *gongylodes* L.	var. *caulorapa* Pasq.		Kohlrabi
var. *gemmifera* (DC.) Zenker			Brussels sprouts
var. *italica* Plenck.			Broccoli
var. *oleracea*	subsp. *sylvestris* (L.) Miller		Wild cabbage
subsp. *alboglabra* L.H. Bailey	*B. alboglabra* L.H. Bailey		Chinese kale, kailan
Brassica juncea (L.) Czern.		18 AABB	Brown and oriental mustard, rai
Brassica napus L.		19 AACC	
var. *napus*	subsp. *oleifera* (Delile) Sinskaya		Summer oilseed rape, canola
var. *napus*	*B. napus* f. *biennis* (Schübl. & G. Martens) Thell.		Winter oilseed rape, winter canola
var. *pabularia* (DC.) Rchb.			Rape-kale
var. *napobrassica* (L.) Rchb.	subsp. *rapifera* (Metzg.) Sinskaya		Rutabaga, swede
Brassica carinata A. Braun.		17 BBCC	Abyssinian mustard
Hirschfeldia incana (L.) Lagr.-Foss.	*Brassica adpressa* Boiss.	7 HH	Hoary mustard
Sinapis arvensis L.	*B. kaber* (DC.) L.C. Wheeler	9 SarSar	Wild mustard, charlock
Sinapis alba L.	*B. hirta* Moench	12 SalSal	Yellow or white mustard
Raphanus sativus L.		9 RR	Radish
Raphanus raphanistrum L.		9 RR	Wild radish
Diplotaxis muralis (L.) DC.		21 DD	Annual wall-rocket
Erucastrum gallicum (Willd.) O.E. Schulz		15	Dog mustard
Eruca vesicaria (L.) Cav. subsp. *sativa* (Mill.) Thell.	*Eruca sativa* Mill.	11 EE	Rocket salad

Source: Modified from Yarnell (1956).

Early humans recognised the edible value of many of these species and through selection modified nearly every plant part to suit their needs. Such modifications include the compacting of the leaves to form a head, the root or stem to form a bulb, the inflorescence to form a curd or bunch and the seed to provide both oil and condiment. Species grown as oilseeds include *B. napus*, *B. juncea*, *B. rapa* and *B. carinata*. The vegetable Brassicaceae includes *B. napus* (rutabaga, Siberian kale), *B. rapa* (Chinese

cabbage, bok choy, pai-tsai, mizuna, Chinese mustard, broccoli raab and turnip), *B. oleracea* (cabbage, broccoli, cauliflower, Brussels sprouts, kohlrabi, collards and kale), *Raphanus sativus* (radish), *Lepidium sativum* (garden cress) and *Nasturtium officinale* (watercress). The condiment crops include *B. juncea* (brown and oriental mustard), *Sinapis alba* (yellow mustard), *B. nigra* (black mustard, but now little used), *Armoracia rusticana* (horseradish) and *Eutrena japonica* (wasabi). There are a number of other minor potherbs and salad vegetables. There are numerous weedy species, but those of greatest interest with regard to cross-pollination with *B. napus* are *Sinapis arvensis* (wild mustard or charlock), *Raphanus raphanistrum* (wild radish), *B. rapa* (wild or bird rape) and *Hirschfeldia incana* (hoary mustard).

The genus *Brassica* is classified as follows:

Order Brassicales (= Cruciales)

Family Brassicaceae (= Cruciferae)

Tribe Brassiceae

Subtribe Brassicinae

Genus *Brassica* L.

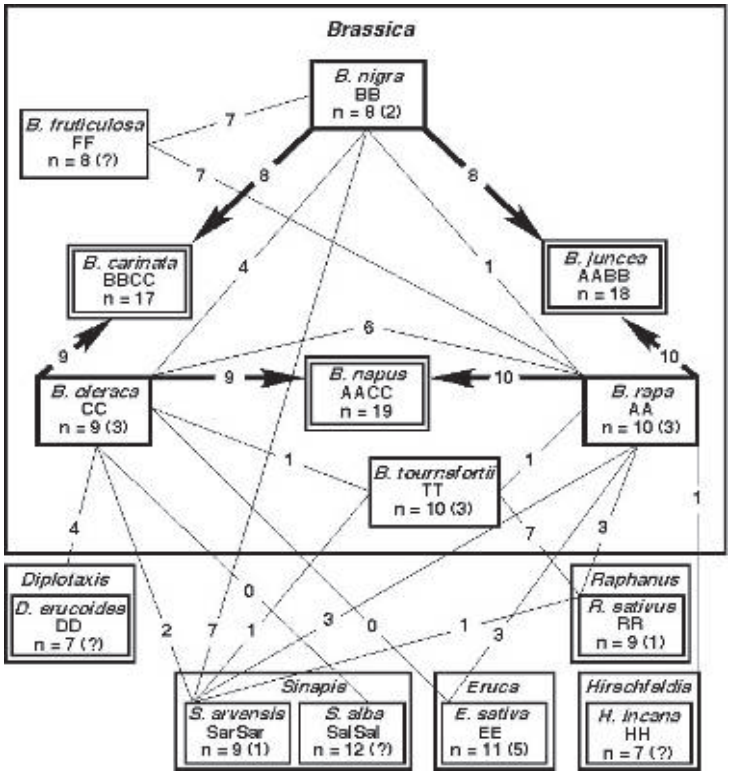
The Brassicaceae family is comprised of 25 tribes with about an additional 5 under study (Al-Shehbaz, Beilstein and Kellogg, 2006). The tribe Brassiceae, which contains the genus *Brassica* and its wild relatives, is made up of 48 genera and approximately 240 species (Warwick and Hall, 2009). Warwick, Francis and Al-Shehbaz (2006) have prepared a checklist and a current taxonomic database for the family on CD-ROM. Also on CD-ROM are chromosome numbers from the literature for 68.6% of the genera and 42.0% of the Brassicaceae species (Warwick and Al-Shebbaz, 2006; Warwick, Francis and Gugel, 2009). The morphological traits that characterise the tribe are conduplicate cotyledons (the radical enclosed by longitudinally folded cotyledons) and/or transversely segmented fruits, that have seeds or rudimentary ovules in both segments and, if present, only simple trichomes or hairs (Warwick and Hall, 2009). Modern molecular studies have reinforced the monophlyetic origin of the tribe.

Taxonomic research on the tribe conducted by Schulz (1936; 1919) established the basic classification that is followed today, although it has been modified and criticised (Al-Shehbaz, 1984). Within the tribe, Schulz (1936; 1919) recognised ten subtribes, with Gómez-Campo (1980) later recommending a reduction to nine. Of the nine subtribes, three are of greatest relevance to those concerned with *Brassica* crops, namely: Brassicinae, Moricandiinae and Raphaninae. Within these subtribes *Brassica*, *Sinapis*, *Diplotaxis*, *Erucastrum*, *Hirschfeldia*, *Eruca* and *Raphanus* are of primary interest.

The association and relationships among species within these subtribes have been studied cytogenetically, chemically and morphologically (reviewed by Prakash and Hinata, 1980; Takahata and Hinata, 1986, 1983) without providing a clear separation of the subtribes and their genera. Recent molecular, morphological and hybridisation data give strong support for a rearrangement of the three subtribes into two clades, namely, the Rapa/Oleracea and the Nigra lineages (see the section on "Centres of origin and ancestors", as well as Warwick and Hall [2009] and references therein). Such a division is also referred to in some publications as the *Brassica* and *Sinapis* lineages. It is expected that the realignment of the species from the three subtribes into the two clades will eventually require renaming of many of the species involved.

The difficulty in clearly separating the genera and species among the *Brassica* and their close relatives has arisen because similar plant forms and morphological traits occur in more than one genus or species. The difficulties encountered by early taxonomists in separating and classifying the various species and forms within the Brassicaceae family are well documented by Hedge (1976) and Prakash and Hinata (1980). As a result, there have been numerous changes and modifications to Schulz's (1919; 1936) original species names and arrangement. The cytological studies by Morinaga (1928; 1929; 1931; 1933; 1934a; 1934b) and his student, U (1935) clarified the broad relationships among the economically important *Brassica* species in which chromosome pairing clearly showed the three species with the higher chromosome number, *B. napus*, *B. juncea* and *B. carinata* are amphidiploids derived from the monogenomic or basic species, *B. nigra*, *B. rapa* and *B. oleracea* (Figure 3.1).

Figure 3.1. **Genome relationships of *Brassica* species and allied genera**



Notes: A, B, C… are the genome symbols. The number in brackets following the haploid chromosome number (n) indicates the maximum possible number of autosyndetic[2] chromosome pairs. The numbers within lines connecting two genomes give the maximum allosyndesis, i.e. the number of bivalents possible between the respective interspecific hybrids (Downey and Röbbelen, 1989).

Source: Modified from Mizushima (1980).

The genome relationships among the amphidiploids were confirmed by resynthesis of the three species from their diploid parents (Frandsen, 1947, 1943; Ramanujam and Srinivasachar, 1943). Further verification of these species' relationships were obtained from studies on phenolic compounds (Dass and Nybom, 1967), protein patterns (Vaughan, 1977), isozymes (Coulthart and Denford, 1982; Chen, Heneen and Simonsen, 1989) and nuclear DNA, restriction fragment length polymorphisms (RFLP; Song, Osborn and Williams, 1988a; 1988b). Additional verification has been achieved through

molecular analysis of nuclear and chloroplast DNA and fluorescence *in situ* hybridisation (Snowdon et al., 2003; Snowdon, 2007; Warwick and Sauder, 2005; Lysak et al., 2005).

To further establish the true relationships among the genus and species of the subtribe, Harberd (1976; 1972) proposed grouping them into "cytodemes" based on the crossability of related subspecies with the same chromosome number. Harberd (1976) defined cytodemes as follows: "If two populations have a common chromosome number and are easily crossed to form a hybrid, which is neither obviously weak in vigour nor of low fertility, then they belong in the same cytodeme. By contrast, different cytodemes (which sometimes have the same chromosome number) are (a) difficult to cross, or (b) give a weak hybrid, or (c) have a sterile hybrid, and frequently exhibit all three criteria." Harberd (1976; 1972) also defined the *Brassica* coenospecies as "the group of wild species sufficiently related to the six cultivated species of *Brassica* to be potentially capable of experimental hybridisation with them". On this basis and their chromosome number the coenospecies have been classified into 43 diploid and 13 tetraploid cytodemes (Warwick and Black, 1993: Table 3). This grouping, with the inclusion of *Raphanus* and *Enarthrocarpus* in the subtribe, is supported by both chloroplast and nuclear DNA analysis (Warwick and Black, 1993; Warwick and Hall, 2009).

Cytological analyses of chromosome pairing in interspecific crosses among some of the more important *Brassica* cytodemes by Mizushima (1980) provided information on the maximum possible number of autosyndetic[2] chromosome pairs (Figure 3.1). Harberd and McArthur (1980) extended the study of meiotic chromosome pairing to more than 50 species hybrids. These distant crosses were facilitated using embryo culture.

A chromosome analysis of the monogenomic *Brassica* species by Röbbelen (1960), established that only six chromosomes were distinctly different, the remaining being homologous with one or the other of the basic six. This evidence pointed to the presence, in the evolutionary pathway of the *Brassica* species, of a now-extinct, ancient progenitor with a basic chromosome number of × = 6. The long-standing hypothesis, that the cultivated diploid *Brassica* species are ancient polyploids, has been strongly supported by modern genomic investigations.

The genomes of *Brassica* species are extensively triploid (Lysak et al., 2007, 2005; Rana et al., 2004). In *B. nigra* Lagercrantz and Lydiate (1996) reported that every chromosome region appeared to be present in triplicate and the genomes of *B. oleracea* and *B. rapa* also exhibit tripling (Rana et al., 2004; Mun et al., 2009; Wang, 2010). High density comparative mapping of *Arabidopsis* and *B. napus* also supported the hypothesis of a hexaploid ancestor (Parkin et al., 2005). Indeed, chromosome tripling has been documented for the entire Brassiceae tribe (Lysak et al., 2005). Linkage maps and genome size data (Lysak et al., 2009) indicate that the *B. oleracea* genome, and probably the other monogenomic species which exhibit a range in chromosome number from 7 to 12, increased or reduced their chromosome number through duplication, translocations (Quiros, Ochoa and Douches, 1988; Hosaka et al., 1990; McGrath et al.,1990; Truco and Quiros, 1994), transposition of elements (Zhang and Wessler, 2004) as well as deletions (Hu and Quiros, 1991) and fusions (Lysak et al., 2006).

Table 3.3. **List of 43 diploid cytodemes and 6 amphidiplod taxa in the *Brassica* coenospecies**

N	CYTODEME
	Diploids
10	*Brassica barrelieri* (L.) Janka
7	*Brassica deflexa* Boiss.
11	*Brassica elongata* Ehrh.
8	[a] *Brassica fruticulosa* Cirillo (includes *B. maurorum* Dur., *B. spinescens* Pomel, *Erucastrum littoreum* (Pau & Font Quer) Maire subsp. *glabrum* (Maire) Gómez-Campo (=*Erucastrum laevigatum* subsp. *glabrum* Maire)
10	[a] *Brassica gravinae* Ten.
8	*Brassica nigra* (L.) W.D.J. Koch
9	*Brassica oleracea* L. (includes *B. alboglabra* L.H. Bailey, *B. bourgeaui* (Webb.) Kuntze, *B. cretica* Lam., *B. hilarionis* G.E. Post, *B. incana* Ten., *B. insularis* Moris, *B. macrocarpa* Guss., *B. montana* Pourr., *B. rupestris* Raf., *B. villosa* Biv.)
9	*Brassica oxyrrhina* (Coss.) Willk.
10	*Brassica rapa* L. (= *B. campestris* L.) (includes wild and cultivated varieties)
10	*Brassica repanda* (Willd.) DC. (includes *B. desnottesii* Emb. & Maire, *B. nudicaulis* (Lag.) O.E. Schulz, *B. saxatilis* DC.)
11	*Brassica souliei* (Batt.) Batt. (= *B. amplexicaulis* (Desf.) Pomel)
10	*Brassica tournefortii* Gouan
12	[a] *Coincya* spp. (=*Hutera* =*Rhynchosinapis*) (includes all species in the genus)
11	*Diplotaxis acris* (Forssk.) Boiss.
9	*Diplotaxis assurgens* (Delile) Gren.
9	*Diplotaxis berthautii* Braun-Blanq. & Maire
9	*Diplotaxis catholica* (L.) DC.
7	*Diplotaxis cossoniana* (Reut.) O.E. Schulz
7	*Diplotaxis erucoides* (L.) DC.
13	*Diplotaxis harra* (Forssk.) Boiss. (includes *D. crassifolia* (Raf.) DC., *D. lagascana* DC.)
8	*Diplotaxis siettiana* Maire (includes *D. ibicensis* (Font Quer) Gómez-Campo)
10	*Diplotaxis siifolia* Kunze
11	*Diplotaxis tenuifolia* (L.) DC. (includes *D. cretacea* Kotov., *D.* simplex (Viv.) Spreng., the latter species was incorrectly listed as *D. pitardiana* Maire)
9	*Diplotaxis tenuisiliqua* Delile
10	*Diplotaxis viminea* (L.) DC.
9	*Diplotaxis virgata* (Cav.) DC.
10	*Enarthrocarpus* spp. (includes *E. lyratus* (Forssk.) DC., *E. pterocarpus* (Pers.) DC., *E. strangulatus* Boiss.)
11	*Eruca* spp. (includes *E. vesicaria* (L.) Cav., *E. sativa* Mill., *E. pinnatifida* (Desf..) Pomel)
8	[a] *Erucastrum abyssinicum* R.E. Fr.
9	*Erucastrum canariense* Webb & Berthel. (includes *E. cardaminoides* (Webb) O.E. Schulz)
8	[a] *Erucastrum nasturtiifolium* (Poir.) O.E. Schulz (includes *E. leucanthum* Coss. & Dur.)
8	*Erucastrum strigosum* (Thunb.) O.E. Schulz
7	*Erucastrum varium* (Durieu) Durieu
7	[a] *Erucastrum virgatum* C. Presl
7	*Hirschfeldia incana* (L.) Lagr.-Foss.
9	*Raphanus* spp. (includes *R. raphanistrum* L., *R. sativus* L., *R. caudatus* L., *R. maritimus* Smith and *R. landra* DC.)
10	*Sinapidendron* spp. (includes *S. angustifolium* (DC.) Löwe, *S. frutescens* (Ait.) Löwe, *S. rupestre* Löwe)
12	*Sinapis alba* L. (includes *S. dissecta* Lag.)
9	*Sinapis arvensis* L. (includes *S. allionii* Jacq., *S. turgida* (Pers.) Delile)
7	*Sinapis aucheri* (Boiss.) O.E. Schulz (=*Brassica aucheri* Boiss.)
12	*Sinapis flexuosa* Poir.
9	[a] *Sinapis pubescens* L.
8	*Trachystoma* spp. (includes *T. aphanoneurum* Maire & Weiller, *T. ballii* O.E. Schulz and provisionally *T. labasii* Maire)
	Amphidiploids (with proposed parentage in parentheses)
17	*Brassica carinata* A. Braun (*B. nigra* × *B. oleracea*)
18	*Brassica juncea* (L.) Czern. (*B. rapa* × *B. nigra*)
19	*Brassica napus* L. (*B. rapa* × *B. oleracea*)
21	*Diplotaxis muralis* (L.) DC. (*D. tenuifolia* × *D. viminea*)
15	*Erucastrum gallicum* (Willd.) O.E. Schulz (*E. leucanthum* × ? unknown *n* = 7 taxon)
15	*Erucastrum elatum* (Ball.) O.E. Schulz (*E. littoreum* × ? unknown *n* = 7)

Note: N = haploid chromosome number. Information was obtained from the following sources: Gómez-Campo (1983), Harberd (1976, 1972), Harberd and McArthur (1980, 1972), Leadlay and Heywood (1990), Snogerup, Gustafsson and Von Bothmer (1990), Sobrino-Vesperinas (1988), Takahata and Hinata (1983) and Warwick, Black and Aguinagalde (1992). Nomenclature is based on that in USDA-ARS (The Germplasm Resources Information Network) (2011). a) Allotetraploids (4x) were also indicated for these cytodemes.

Source: Warwick and Black (1993) © Canadian Science Publishing or its licensors.

Based on cotyledonary studies of various taxa in the tribe Brassiceae, Gómez-Campo and Tortosa (1974) proposed that *Brassica* evolved from the Macaronesian plant taxon *Sinapidendron*. This Miocene relic survived several paleo-climatic changes that destroyed most of the Mediterranean Tertiary flora and is put forward as the archetype from which *Brassica* evolved through the *Diplotaxis* and *Erucastrum* complexes. However, the use of such morphometric data to establish evolutionary relationships withinBrassiceae has not always provided results that agreed with those from cytological and molecular studies.

Description

Prakash and Hinata (1980) have summarised the early taxonomic difficulties when only morphological characteristics were used to categorise the numerous and varied forms of the commercially important *Brassica* species. The early proliferation of species' names and misclassifications resulted from the wide array of plant forms that occur among plants within the same genome, plus the mimicking of the same morphological features in plants with a different genetic makeup. Although the application of advanced genetic techniques and chemical investigations has clarified relationships, there is still some disagreement among authorities as to whether a particular form should be considered a species, or a subspecies or variety within a species.

Brassica nigra

Figure 3.2. **Illustration of a *Brassica nigra* plant and its parts**

Source: Koehler's Medical-Plants (1887) provided by Wikipedia, the free encyclopedia.

Sinskaia (1928) identified two major geographic forms of *B. nigra*, a western form grown in Europe, Africa, Asia Minor and Afghanistan and an eastern form grown in India and as far west as Palestine and the Syrian Arab Republic. The early forms were of short season, spreading, with semi-erect growth up to a metre tall but taller, more erect material was selected for commercial production (Hemingway, 1995). The prevalent annual weedy form of today varies in height from 0.6-2.4 metres, depending on the competing vegetation and growing conditions. The plant is lightly covered with soft hairs; the lower

leaves are large with upper leaves reduced in size. *B. nigra* can be easily distinguished from the commercial *Brassica* crops in that *B. nigra* does not produce a rosette of basal leaves. A typical plant image, including the tap root, is shown in Figure 3.2. The siliques are short (2-5 cm), hirsute and appressed to the stem of the flowering raceme, with a beak about 0.6 cm long. The small, brown to black seeds exhibit primary dormancy and tend to germinate throughout the growing season.

Brassica rapa

Plants of *B. rapa* species are widely cultivated as leaf and root vegetables, fodder and oilseed crops. In addition, they can be a weed of cultivated land and disturbed sites. The widest array of vegetable forms evolved in the People's Republic of China (hereafter "China") with many of the selected forms corresponding to or mimicking those found in the *B. oleracea* complex. Because the selected forms exhibited significantly different morphological traits, early botanists classified them as separate species. Today they are more correctly classified as subspecies or varieties of *B. rapa*.

Brassica rapa vegetables

The plants in the *B. rapa* subsp. *pekinensis* group of vegetables are biennials that have been classified into three variant forms. The var. *cylindrica* has broad but thin, crinkled and conspicuously veined green leaves with white petioles (Figure 3.3). The leaves are usually tightly wrapped in a cylindrical formation to form a head with a length of 30-60 cm and a diameter about 10-17 cm. The var. *cephalata* forms a flat head similar to a drum-head cabbage (Figure 3.3) while the var. *laxa* forms a loose heart. In the second year of growth bolting occurs and the flowering stem is quickly thrust upwards reaching a height of 1.5 metres and bearing the characteristic raceme with typical *Brassica* yellow flowers. Common names for this group include pe-tsai, celery cabbage or Chinese cabbage.

Figure 3.3. ***B. rapa* subsp. *pekinensis***

A. var. cylindrica

B. var. cephalata

Source: Courtesy Evergreen Seeds.

The *B. rapa* subsp. *chinensis* group includes both annual and biennial forms. Bailey (1930) described the subspecies as "a very smooth biennial with large ladle-shaped upstanding radial leaves with thick ivory-white but not wing-margined or toothed petioles." The clasping, entire leaves have prominent veins and resemble leaves of Swiss chard (Figure 3.4). The common name for this plant group is pak choi or bok choy. If the plants are harvested in the early stages of growth they may be called "baby bok choy" or "Shanghai bok choy" (Figure 3.5). The subsp. *parachinensis* is usually included within

this group (Figure 3.6). It is grown for its thick stemmed flowering shoots that are cut for market as the first flowers open, allowing for several harvests. The common names for this variant include Gai Lan and Chinese broccoli. Tsen and Lee (1942) include the subsp. *rosularis* and subsp. *narinosa* in this *chinensis* group. The USDA *Germplasm Resources Information Network* (GRIN) database includes *rosularis* in the *chinensis* group, but keeps *narinosa* as a separate subspecies (USDA-ARS, 2011). The plants of the subsp. *narinosa* are stout, low growing, glabrous biennials. The lower leaves are small, puckered and orbicular-ovate with broad white petioles, arranged in short clusters. The upper stem leaves are very broad, entire and clasping. Siliques are about 2 cm long or less with a very short, stout beak about one-half or one-third as long as the pod. Tsen and Lee (1942) also place subsp. *japonica* and subsp. *nipposinica* within the *chinensis* group; however, the USDA keeps both of these subspecies separate (USDA-ARS, 2011). These two subspecies are considered synonyms for this form, exhibiting pencil-thin leaf stems supporting deeply indented feathery leaves (Figure 3.7). The flowering stalks produce siliques about 6 cm long.

Figure 3.4. ***B. rapa* subsp. *Chinensis*, Bok choy**

Source: Courtesy Tainong Seeds.

Figure 3.5. ***B. rapa* subsp. *Chinensis*, Baby or Shanghai bok choy**

Source: Courtesy Tainong Seeds.

Figure 3.6. ***B. rapa* subsp. *Parachinensis*, Gai Lan or Chinese broccoli**

Source: Courtesy Evergreen Seeds.

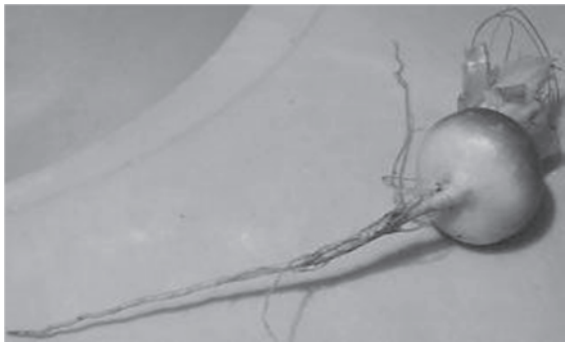
Figure 3.7. ***B. rapa* subsp. *Nipposinica***

Source: Courtesy North Carolina State University.

B. rapa subsp. *rapa*, the common turnip, develops a bulbous storage organ in the first year of growth. The top 1-6 cm above ground is an expansion of the hypocotyl that is fused with the expanded root below ground. A narrow tap root extends below the storage

organ (Figure 3.8). Most cultivars are white fleshed except for the exposed above-ground portion which, when exposed to sunlight, may turn purple, red or green. Yellow, orange and red fleshed cultivars are also grown. Leaves grow directly from the above-ground shoulder of the expanded hypocotyl and not from a visible crown or neck as occurs in rutabagas (*Brassica napus* var. *napobrassica*). The leaves may be harvested and eaten as "turnip greens". Turnip roots for edible purposes will each weigh about 1 kg but weight will vary with the variety and growing conditions. Cultivars grown for cattle and sheep feed produce much larger roots. The flowering stalk bolts from the overwintered root the following spring, producing a terminal raceme with siliques about 6 cm long.

Figure 3.8. ***B. rapa* subsp. *rapa* the common turnip**



Source: Courtesy Wikipedia, the Free Encyclopedia.

Brassica rapa oilseed and weedy forms

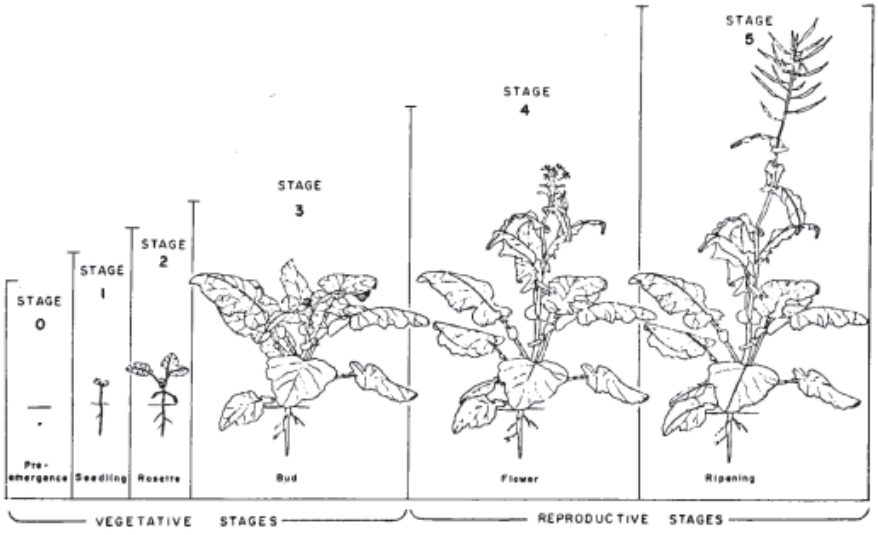
The oilseed form of *B. rapa* subsp. *oleifera* includes both annual and biennial varieties. Both the spring and winter forms of *B. rapa* mature earlier and withstand cold temperatures better than their *B. napus* counterparts. However, the seed and oil yield is normally lower than *B. napus* so production of the winter form is limited to the more rigorous climates of central Sweden, Finland, north-west China and the foothills of the Himalayan mountains. The plant and growth stages of spring *B. rapa* are illustrated in Figure 3.9. Following the emergence of the cotyledons, the plant quickly produces a tap root and a rosette of leaves that shades the surrounding area reducing weed competition. The lower leaves are stalked, lyrate-pinnatifid with a large end lobe exhibiting sparse hairs on the under side. The upper leaves are much smaller and slightly stalked. In the winter form, the plant remains in the rosette stage until exposed to a long vernalization period (40 days) at near freezing temperatures. Day length, and where required vernalization, determine when bolting of the flower stem will occur. Figure 3.9 shows only a single raceme but under field conditions the plant produces many flowering branches and with *B. rapa*, as opposed to *B. napus*, it can be difficult to identify the primary raceme. The plant grows to a height of a meter or less. The position of the flower buds on a raceme, relative to the just opened, self-incompatible flowers, can be used to distinguish plants of *B. rapa* from *B. napus*. In *B. rapa* the flowers over top the buds while the reverse is true for *B. napus*. Siliques, some 6 cm long, contain up to 30 brown to yellow seeds in 2 locules (Figure 3.10).

B. rapa subsp. *campestris* (formerly subsp. *sylvestris*), the weedy form of subsp. *oleifera*, is morphologically indistinguishable from the cultivated spring oilseed *B. rapa*, except that the seed of subsp. *campestris* exhibits primary dormancy, a recessively inherited characteristic.

B. rapa subsp. *dichotoma*, commonly referred to as toria, is an oilseed form grown on the Indian sub-continent. Morphologically it is indistinguishable from the spring form of *B. rapa* subsp. *oleifera.* Other forms grown on the sub-continent are termed yellow and brown sarson (*B. rapa* subsp. *trilocularis*). These forms have broad siliques containing larger seeds than toria. However, yellow sarson is distinguished by its introse anthers, self-compatibility and pure yellow seeds.

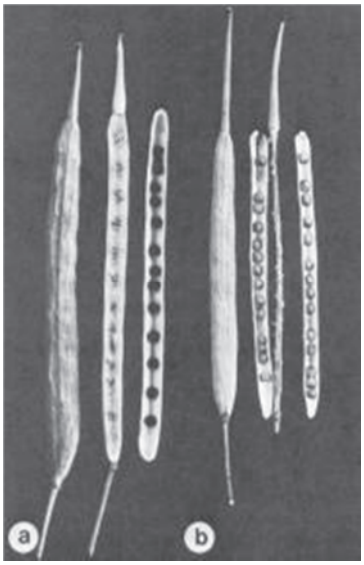
Figure 3.9. **Growth stages in turnip rape (*B. rapa*)**

Stages: 0: pre-emergence; 1: seedling; 2: rosette; 3: bud; 4: flower; 5: ripening



Source: Harper and Berkenkamp (1975). © Canadian Science Publishing or its licensors.

Figure 3.10. **Typical intact and opened siliques of *B. napus* and *B. rapa***



Notes: a) *B. napus* showing intact and opened siliques with seeds of the upper locule exposed, while those of the lower locule are partially obscured by the lamella. b) Intact and open silique of *B. rapa*.

Source: Downey (1983). Courtesy AAFC Reseach Station, Saskatoon (Photographer R.E. Underhood).

Autotetraploid *B. rapa* varieties have been developed for use as leafy vegetables, fodder (turnips) and green manure. Tetraploid plants have larger leaves, thicker stems, greater height and larger seeds than their corresponding diploids (Abel and Becker, 2007). However, tetraploids are not used as oilseed crops as their seed and oil yields are significantly lower than their diploid progenitors (Downey and Armstrong, 1962). The much larger tetraploid pollen also takes significantly longer to germinate than *B. rapa* diploid pollen. Thus, pollen from *B. rapa* diploid plants has a selective advantage resulting in triploid embryos, which abort (Downey and Armstrong, 1962; Håkansson, 1956), providing strong selection pressure against *B. rapa* tetraploid plants growing in *B. rapa* diploid populations. The slower pollen germination of tetraploid plants could predispose them to out-crossing with related species. On the other hand, since tetraploid *B. rapa* crops are normally consumed or ploughed down before flowering they are unlikely to be a significant factor in gene flow.

Brassica oleracea

The *B. oleracea* vegetables are often referred to as the "cole crops" and comprise cabbage, cauliflowers (including broccoli), kales and kohlrabi, but not the *B. rapa* vegetables.

Wild *B. oleracea*

Wild *B. oleracea* var. *oleracea* or wild cabbage is native to the western and southern seaboard of Europe where its tolerance of salt and lime, but its intolerance to competing vegetation, tends to restrict its presence to limestone sea cliffs (Heywood, 1964; Rich, 1991). The plants of this subspecies are biennial or perennial and in the first year produce a rosette of thick, fleshy leaves (Figure 3.11). Following vernalization a flowering stalk 1-2 metres tall arises from the centre of the rosette bearing a raceme of self-compatible, yellow flowers.

Figure 3.11. **Wild *B. oleracea* plants in their first year of growth**

Source: Wikipedia, the Free Encyclopedia.

B. oleracea var. *capitata*, cabbage

The cabbage is a biennial plant that in the first year of growth produces a dense, terminal head of tightly wrapped leaves on a short stout stem. The head is surrounded by a rosette of large fleshy leaves (Figure 3.12A). Three main types of heads – smooth green, red and Savoy – are commercially produced (Figure 3.12B). In the second year,

the head splits open and the flowering stalk bolts to 1.5-2.0 metres tall with branches bearing flowering racemes of self-incompatible flowers.

Figure 3.12. **Heads of *B. oleracea* var. *capitata* and Savoy cabbages**

A. Head of cabbage, *B. oleracea* var. *capitata* with its rosette leaves intact

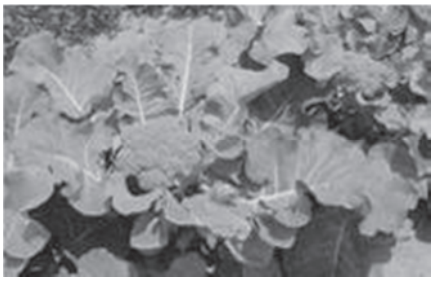
B. Heads of red, smooth green and Savoy cabbage with lower leaves removed

Source: Courtesy Floridata.

B. oleracea var. *botrytis*, broccoli and cauliflower

Cauliflower is derived from broccoli, being selected for short stout stems with a dense, terminal head or curd, made up of arrested inflorescence meristems, over topped by leaves (Figure 3.13). About 10% of the meristem mass will eventually develop into normal flowers and set seed (Sadik, 1962). Specific alleles of the *BoCAL*-a gene have been shown to be associated with discrete inflorescence morphologies (Smith and King, 2000; Purugganan, Boyles and Suddith, 2000). Smith and King (2000) present evidence suggesting that the cauliflower curd arose in southern Italy from a heading Calabrese broccoli via an intermediate Sicilian crop type.

Figure 3.13. **Head of cauliflower (left) and broccoli (right) *B. oleracea* var. *botrytis***



Source: Courtesy Cavaganaro, David/Sunset/Invision.

Broccoli differs from cauliflower in that broccoli flower heads tend to be smaller with more slender floret-stalks and are made up of arrested green (or purple) flower buds whereas the heads of cauliflower are formed by a condensed and thickened, malformed white (also purple or lime green) flower cluster. Both crops are biennial and, provided the plants have been vernalized, produce viable flowers and pods in the second year from the stump or parts of the head that remain. Vernalization requires a prolonged cold period of at least ten days with temperatures between 2°C and 10ºC. The larger the plant when exposed to the cold treatment the greater the incidence of bolting. Plants of both crops are

more susceptible to frost and less tolerant of heat and drought than cabbage. The cultural requirements of broccoli and cauliflower are similar but broccoli generally grows more rapidly. Most varieties are now F_1 hybrids.

The broccoli referred to above is more correctly known as "calabrese" broccoli. It produces a single head and is the form that is of greatest commercial importance. The "sprouting" broccoli, var. *italica*, produces a succession of small flowering heads over an extended period (Figure 3.14) while the "Romanesco" broccoli produces a head characterised by multiple cone shaped spirals consisting of masses of small flower buds (Figure 3.15).

Figure 3.14. **Sprouting purple broccoli**

Figure 3.15. **Romanesco broccoli**

Source: Courtesy Mr. Fothergill's Seeds Ltd. UK.

B. oleracea var. *viridis*, collards and kale

The kales and collards are biennials but are usually harvested in the first year for their edible leaves. They closely resemble their wild cabbage progenitors. Collards have large, smooth fleshy leaves with smooth margins (Figure 3.16). The leaves of kale are smaller and thinner than those of collards and many cultivars produce fringed, wavy-edged or feathery leaves (Figure 3.16). A thick flowering stem up to 1.5 metres tall emerges in the second year. One form called "Walking Stick" kale produces a tall straight stem which, when dried and polished, makes a fine walking stick.

Figure 3.16. ***B. oleracea* var. *viridis*, collard plant (left) and row of kale (right)**

Source: Courtesy Floridata.

B. oleracea var. *gemmifera*, Brussels sprouts

B. oleracea var. *gemmifera* plants are cool season biennials with simple erect stems up to 1 metre tall, bearing round to heart-shaped simple leaves with lengthy petioles. The leaves are glabrous with the colour varying from light to deep greyish-green. In the first year, auxiliary buds or sprouts are borne beneath the leaves on an elongated stem (Figure 3.17). The buds are modified leaves that form small heads up to 30 mm in diameter. Following vernalization, a seed head is produced from which a flower stalk emerges bearing perfect, self-incompatible flowers on terminal racemes. The seeds, weighing about 2.8 g/1 000, are borne in typical, two locule siliques.

Figure 3.17. ***B. oleracea* var. *gemmifera*, Brussels sprouts**

Source: Courtesy Limagrain.

B. oleracea var. *alboglabra*, Chinese kale

The var. *alboglabra* is widely grown throughout south-east Asia as a leaf and stem vegetable. The perennial plants are grown as annuals, producing dull or glossy thick green, glaucous, elliptic leaves about 25 cm long. The plants commonly called Chinese kale and kailan attain a height of up to 40 cm in the vegetative stage and 1-2 metres at the end of flowering. Upper stem leaves are oblong, petioled or non-clasping. The white flowered inflorescences develop siliques 5-8 cm long (Herklots, 1972).

Brassica napus

B. napus var. *napobrassica*, rutabaga or Swede

B. napus var. *napobrassica*, the common rutabaga or Swede, is a biennial with similar characteristics to the turnip. The bulbous root develops from the hypocotyl in the first year of growth (Figure 3.18). The surface of the root may be purple, white or yellow with the inner content solid yellow or white fleshed. The thick, smooth, dark green leaves emerge from the crown or neck of the root to form a ground covering rosette that shades out competing weeds. The presence of a root crown or neck distinguishes rutabagas from turnips. Early in the second year the flower stalk bolts from the root crown and the self-compatible flowers produce short beaked siliques on short pedicels containing two rows of round black seeds. Rutabagas are used for human consumption and for late fall cattle grazing.

Figure 3.18. ***B. napus* var. *napobrassica*, rutabaga or Swede**

Source: Courtesy Floridata.

B. napus var. *pabularia*, Siberian or rape kale

This sub-species has both annual and biennial forms with much branched erect stems up to 1.5 metres tall. Lower leaves are glaucous and lobed. Upper stem leaves are lanceolate, sessile and clasping (Figure 3.19). The much branched inflorescence is an elongated raceme producing siliques 5-11 cm long and 2.5-4 mm wide with a slender 0.5-3 mm long beak. The tap root produces many side branches. The crop is grown as a leafy vegetable and for fodder.

Figure 3.19. ***B. napus* var. *pabularia*, Siberian or rape kale**

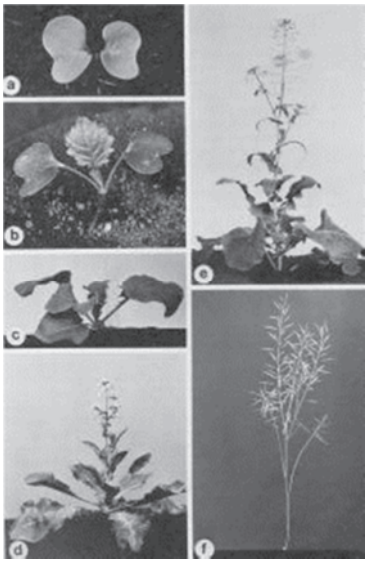
Source: Courtesy Floridata.

Oilseed rape, *B. napus* var. *napus* f. *annua* and f. *biennis*

Oilseed *B. napus* has both an annual (spring) and a biennial (winter) form. The biennial form is less winter hardy than winter wheat which restricts its production to areas with mild winter conditions such as northern Europe and central China. The annual form is grown as a spring crop in western Canada and northern China but also as a winter crop in Australia and other countries with very mild winters.

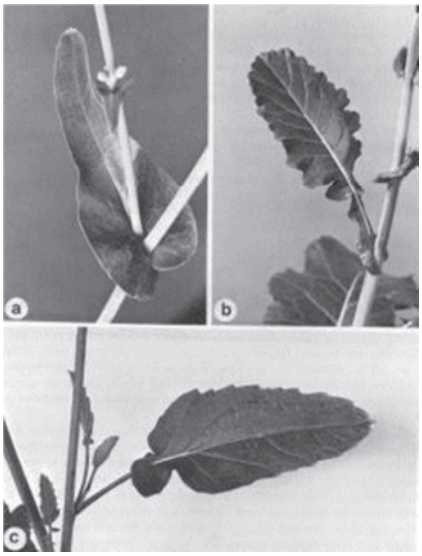
Growth stages of annual *B. napus* plant development are illustrated in Figure 3.20. The glaucous lower leaves form a rosette from which the flowering stalk emerges bearing a dominant, indeterminate main raceme. The upper stem leaves are small, lanceolate, sessile and clasping. Plants of the species *B. napus*, *B. rapa* and *B. juncea* can be distinguished by their upper leaf attachment to the stem as illustrated in Figure 3.21. Flowering begins with the lowest bud on the main raceme and continues upward with three to five or more flowers opening per day. The buds, unlike those of *B. rapa*, are held above the uppermost open flowers. Flowers on the secondary branches begin to open about three days after the opening of the first flowers on the main raceme. The siliques are ascending on slender pedicles and about 7-10 cm long with a beak about 1.3 cm long. Seeds are dark brown to black, and weigh 2.5-5.5 g per 1 000 seeds.

Figure 3.20. **Growth stages of *B. napus* var. *napus* f. *annua***



Notes: a) Seedling cotyledons; b) cotyledons and first true leaf; c) rosette; d) flowering; e) pod set; f) mature plant.

Figure 3.21. **Upper leaves of *B. rapa*, *B. napus* and *B. juncea***



Notes: a) *B. rapa*, fully clasping stem; b) *B. napus* partially clasping; c) *B. juncea*, non-clasping.

Source: Downey (1983). Courtesy AAFC Reseach Station, Saskatoon (Photographer R.E. Underhood).

Brassica juncea

B. juncea vegetables

In China and south-east Asia many vegetable forms of *B. juncea* have been developed and classified as species or subspecies under numerous names, depending on the morphological features given the greatest importance. Kumazawa and Able (1955) examined some 200 East Asian cultivars of *B. juncea* vegetables grown in China, Japan, Nepal and Chinese Taipei on the basis of their plant size, root form, tillering and leaf characteristics. All accessions of *B. juncea* and its subspecies were described as annuals and placed in 25 different groups within 8 classes. These classes were further condensed into four subspecies. The authors state that the subspecies evolved from the leafy and oilseed forms of brown mustard, *B. juncea* (L.) Cross. From the collection, the authors

illustrated a normal or "ordinal" root form, a turnip-like rooted form (also described by Dixon, 2007) and a little known form with a tuberous basal stem (Figure 3.22). The four subspecies were grouped and characterised as follows.

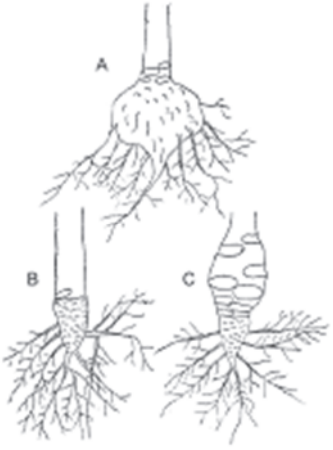
1. subsp. *napiformis* (Pailleux & Bois) Gladis, grown for its tuberous turnip-like root. This subspecies bolts late and has a high tolerance to cold.
2. var. *japonica* (Thunb.) L.H. Bailey, characterised by curled, narrow or dissected leaves.
3. subsp. *integrifolia* (H. West) Thell., characterised by entire or little lobed basal leaves. Herkots (1972) notes that some cultivars may form a tight head (Figure 3.23).
4. var. *rugosa* (Roxb.) N. Tsen & S.H. Lee, includes cabbage leafed forms with large entire or serrated radical leaves. The tuberous basal stem form (Figure 3.22) is included in this subspecies.

Herklots (1972) places var. *rugosa* within subsp. *integrifolia* but also puts forward the var. *sareptana* as characterised by lyrately-lobed basal leaves and var. *crispifolia* as having dissected, crisped lower leaves. More recently, a *B. juncea Biology Document* (Canadian Food Inspection Agency, 2007) quoted the grouping by Spect and Diederichsen (2001) into the following four sub-species:

1. subsp. *integrifolia*, used as a leaf vegetable in Asia.
2. subsp. *juncea*, cultivated mainly for its seeds, occasionally as fodder.
3. subsp. *napiformis*, used as a root-tuber vegetable. Dixon (2007) describes this subspecies as having a high tolerance to cold and an enlarged conical root.
4. subsp. *tsatsai* from which stalks and leaves are used as vegetables in China.

Figure 3.22. **Three forms of *B. juncea***

Bulbous root (a), normal or "ordinal" root (b) and tuberous basal stem (c)



Source: Kumazawa and Able (1955).

Figure 3.23. ***B. juncea* subsp. *integrifolia*, heading mustard, BauSin**

Source: Courtesy AgroHaitai Ltd.

B. juncea, oilseed and condiment mustards

Plants of this species, grown for their seed oil or condiment production, are normally referred to simply as *B. juncea* without the attachment of a subspecies name. However, Spect and Diederichsen (2001) classify this plant group as *B. juncea* subsp. *juncea*. Plant for both oil and condiment are similar in their morphology but differ in seed oil percentage and the type and amount of glucosinolates present in the seed. These forms are annuals that grow to about 1.2 metres as spring-sown crops in western Canada and Europe. On the Indian sub-continent they are grown as a winter crop where, under short days, plants grow up to 2.1 metres tall. The plants are green and sometimes slightly glaucous. The lower leaves of the rosette are rather thin, elliptic to obovate and lyrately-lobed or divided. The upper stem leaves are small, narrow and not clasping (Figure 3.21). Depending on the day length and temperature the flowering stalk bolts and produces a raceme with no terminal flower. As with *B. napus*, the buds are borne above the open flowers. Apical dominance is present with the secondary racemes initiated about three days after flowers open on the main raceme. The silique is about 7 cm long containing seed weighing 2.5-3.0 g/1 000 seeds.

Geographic distribution, ecosystems and habitats, cultivation and management practices, centres of origin and diversity

Introduction

From an ecological and agronomic point of view, both the spring and winter forms of oilseed rape exhibit two undesirable characteristics. First, mature pods tend to shatter, leaving large but variable amounts of seed on the ground at harvest (see below on the contribution of *B. napus* harvest losses to persistence). Pod shatter not only results in lost yield but also sets the stage for large numbers of volunteer plants in subsequent crops. Fortunately *B. napus* seeds have no primary dormancy so if moisture and temperature are adequate, the vast majority of these seeds germinate and are killed by frost, herbicides, cultivation or predators (see below). The opportunity for *B. napus* to acquire primary dormancy is limited due to the vast majority of fields being sown each year with high germination certified seed.

The second undesirable characteristic is the tendency for a proportion of the shattered seed to acquire secondary dormancy. Such dormancy is induced by abiotic stresses (see section on persistence below). Although most of the shattered seed will quickly be reduced by fatal germination, predation, disease and abiotic stress, a small percentage can remain dormant and viable for ten years or more (Schlink, 1998; Lutman, Freeman and Pekrun, 2003). Thus, *B. napus* is able to establish seed banks within cultivated fields (see Lutman et al., 2005 and below). As a result, traits or genes that have been genetically silenced or augmented within improved varieties may be reintroduced. Examples would be the genetic blocking of the biosynthesis of erucic acid in rapeseed oil, the reduction in linolenic acid content and the augmentation of oleic fatty acids in the oil, or reduction of glucosinolates in the oilseed meal.

It should be noted, however, that there is considerable genetic variability within the species and its close relatives in both the degree of pod shatter and the percentage of induced dormancy. Until recently these characteristics have not been a priority for oilseed rape breeders but progress is possible. Wang, Ripley and Rakow (2007) have clearly demonstrated that selection for reduced pod shatter in *B. napus* can be achieved. In addition, Østergaard et al. (2006) have shown that expressing the *Arabidopsis*

FRUITFULL gene in *B. juncea*, using a CaMV 35S promoter, produces shatter-resistant plants. Although the shatter-resistant pods held their seed too tightly for combine harvesting, a weakened form of the FRUITFULL gene could result in an economically and environmentally valuable advance. It is unlikely that conventional breeding will lead to complete elimination of the shattering characteristic, but there appears to be considerable room for improvement. Further, Pekrun, Potter and Lutman (1997); Gruber, Pekrun and Claupein (2004); Gruber, Emrich and Claupein (2009); and Gulden, Thomas and Shirtliffe (2004) have all shown that among *B. napus* varieties, of both spring and winter forms, there is a wide range in the percentage of seed susceptible to induced dormancy. Thus, the application of conventional breeding techniques to select varieties producing seed resistant to secondary dormancy should greatly reduce the presence of volunteers in subsequent crops.

Ecologically, *B. napus* is described as a cultivated crop where escaped plants become colonisers of waste places. However, they are not invasive of natural habitats. Colonisers are defined as species that occupy disturbed sites or habitats but with populations that keep moving, founding new populations while losing old ones (Williamson, 1996). Feral populations of *B. napus* are most frequently found along road and rail verges, field margins and in disturbed soils. The reports on the abundance and persistence of such feral populations vary considerably from country to country and between the spring and winter forms. Williamson (1996) noted that colonising species are not the same as invaders, even if they have high intrinsic rates of increase, as exhibited by *B. napus*. He classifies *B. napus* in Britain as intermediate between naturalised and casual. On the other hand, recent intensive surveys of feral sites in mainland Europe have identified feral populations in higher frequencies than anticipated, with some sites able to sustain themselves in a semi-permanent state (Pivard et al., 2008). Such reports have given rise to concerns by some that a proportion of feral populations could become permanent and in time result in the invasion of natural habitats.

Although the species does have the weedy characteristics noted above, producing many propagules (seeds), plus the ability to cross with some weedy relatives, it is not competitive with perennial grasses that dominate the natural habitat. It should also be noted that oilseed rape has been part of the European landscape for a very long time as have the truly weedy, related species, *Sinapis arvensis* and *B. rapa*. However, none have become invasive of natural habitats. In recent years, the area of oilseed rape cultivation and intensity of production has increased worldwide. For example, since 1970 oilseed rape production in France and Germany has increased 4.5- and 8-fold, respectively. In the same period, the Canadian oilseed rape acreage has quadrupled, thus a wider and more frequent occurrence of feral populations is to be expected. The spring form of oilseed rape is much less likely to form feral populations or to be self-sustaining since fall germination is normally fatal while frosts will kill many seedlings that germinate in the spring. Although Knispel et al. (2008) has reported some transient feral population in the province of Manitoba, Canada, such roadside populations are rare over most of the Prairie Provinces, except near collection points and to a limited extent along railroad verges. This is because in western Canada most road verges are mowed in late August before feral populations set viable seed. Such roadside mowing is essential to prevent snowdrifts across roads that tall vegetation can cause. In contrast, in Europe, the winter form can avoid being killed by the fall road maintenance since mowing does not usually affect the established first year rosettes, leaving some plants to flower and set seed before the next fall mowing.

Different agronomic practices also influence the size and persistence of volunteer populations. In Europe, the large amount of straw remaining after harvest plus the short time between the July harvest and August sowing dates encourages ploughing down of residue, resulting in seed burial. In Canada on the other hand, ploughing is not practiced and most fields are spring- sown into undisturbed stubble (minimum or zero tillage) from the previous year's September harvest (Hall et al., 2005). Thus, seed burial is minimised and harvest seed losses are exposed to environmental hazards. The result is that in Europe, old or discontinued cultivars or genotypes will persist in the seed bank for a much longer time than in Canada. This is clearly illustrated in the changeover from high to low erucic acid *B. napus* varieties. In the German oilseed rape growing province of Schleswig-Holstein, it required ten years to reduce the commercial crop from the traditional high erucic varieties (50% erucic) to the desired level of 2% (Sauermann, 1987). In Canada, the same results were obtained in three years (Daun, 1983).

Geographic distribution

The genus *Brassica* and its wild relatives are part of the tribe Brassiceae that has its origin in the Mediterranean basin and in south-western Asia. However, the geographic centre is thought to be in the south-western Mediterranean region (Algeria, Morocco and Spain) where some 40 genera have been shown to be endemic or exhibit maximum diversity (Hedge, 1976; Gómez-Campo, 1999, 1980; Al-Shehbaz, 1984; Al-Shehbaz, Beilstein and Kellogg, 2006; Warwick and Hall, 2009). For the subtribe Brassicinae, Hedge (1976) leaves little doubt that it originated in the Mediterranean basin. The species distribution of the Brassicaceae family is concentrated in the northern temperate zone and south-western and central Asia (Holm et al., 1997). Few species are found in hot, humid tropics.

B. nigra

B. nigra or black mustard was widely grown for the sharp pungency of its seeds and as a leaf vegetable. Prakash and Hinata (1980) placed the species origin in central and south Europe. It is one of the oldest recorded spice crops, which undoubtedly resulted in its early and widespread distribution across Europe, Africa, Asia and the Indian sub-continent, and its dehiscing siliques with primary dormancy of the seed ensured its persistence. The GRIN describes the species distribution as widely naturalised in the following regions and countries. In Africa: countries along the south shore of the Mediterranean as well as Eritrea and Ethiopia. In temperate Asia: Afghanistan, Armenia, Islamic Republic of Iran, Iraq, Israel, Kazakhstan, Lebanon, Syrian Arab Republic, Turkey and northwest China. For the Indian sub-continent: India, Nepal, Pakistan. In Europe: all countries in western and eastern Europe as well as the Balkans and Greece. The crop was introduced to the Americas and Australia as a spice. However, in the 1950s it was displaced by the higher yielding, pungent *B. juncea* that was better suited to mechanical harvesting. Although in many regions black mustard is now a weed of waste places, it has never become established on the Canadian prairies, although it is present throughout much of the United States.

B. rapa

B. rapa is thought to have originated in the mountainous areas near the Mediterranean sea (Tsunoda, 1980). The time of domestication is unknown. Sinskaia (1928) proposed two main centres of origin, one being the Mediterranean and the other the Afghanistan-Pakistan region. The species appears to have attained a wide distribution

throughout Europe, parts of Africa, Asia and the Indian sub-continent before recorded history. Excavations in China reported the presence of *B. rapa* seed at a 6 000-7 000-year-old archaeological site (Liu, 1985). Indian Sanskrit literature mentions the plant about 1599 B.C. (Prakash, 1961), and Renfrew (1973) indicated that *B. rapa* seed was consumed in Scandinavia as early as 350 B.C. *B. rapa* is grown as an oilseed crop in northern Europe, north-west China, the foothills of the Himalayas and northern India, while the vegetable forms were selected and modified in Asia, primarily in China. The oilseed form was introduced to Canada by a Polish immigrant about 1936 (Boulter, 1983) and Australia began its first investigations on the *B. rapa* crop in the early 1960s (Salisbury, 2002) but it has now been superseded by *B. napus* varieties. *B. rapa* also has a weedy form that differs from the cultivated plant in exhibiting primary dormancy and has a worldwide distribution (Figure 3.24).

Figure 3.24. **World distribution of *B. rapa* as a reported weed**

Source: Modified from Holm et al. (1997).

B. oleracea

The centre of origin for the *B. oleracea* species is along the European Atlantic coast while the wild related forms still grow on the islands and along the northern coast of the Mediterranean. The various forms of this species were developed in Europe and did not reach Asia until about the 16th century (Liu, 1985). The many cultivated forms of this species have been introduced and grown worldwide, with the exception of some tropical areas.

B. napus

B. napus is of relatively recent origin (<10 000 years; see the section on genetics at the end of this chpter and Figure 3.39) resulting from the interspecific cross between plants of *B. oleracea* and *B. rapa*. The cross must have occurred where the two species were growing in close proximity along the European Atlantic or Mediterranean coasts. Dispersal of the species is thought to have occurred throughout Europe in the 16th century with the introduction to the Americas in the 17th and 18th centuries and the Far East in the 19th century (Liu, 1985).

B. juncea

B. juncea is believed to have arisen about 10 000 years ago as the result of an interspecific cross or crosses between plants of *B. rapa* by *B. nigra*. Evidence suggests that one primary centre of origin is China, where the greatest divergence of forms evolved (Prain, 1898; Sinskaia, 1928; Vavilov, 1949). A second centre of origin is thought to be Afghanistan and adjoining regions (Olsson, 1960; Mizushima and Tsunoda, 1967; Tsunoda and Nishi, 1968) from where it spread to a secondary centre on the Indian subcontinent and became a major oilseed crop (Hemingway, 1995; Prakash and Hinata, 1980). GRIN (USDA-ARS, 2011) lists *B. juncea* as native to temperate Asia including China, Kazakhstan, Kyrgyzstan, Mongolia, eastern and western Siberia, Tajikistan and Turkmenistan. It has been introduced as a condiment crop to Europe, the Americas, Australia and New Zealand. It has been designated a weed of southern European Russian Federation, the Caucasus, central Asia and southern Siberia, and a casual or feral plant in southern and southeast Asia, Africa and America (Canadian Food Inspection Agency, 2007).

B. carinata

B. carinata, like *B. juncea*, is believed to have arisen about 10 000 years ago as a result of an interspecific cross between plants of *B. nigra* and *B. oleracea*. The cross is thought to have occurred in the Mediterranean region where both species were present. As the climate in North Africa became dryer, *B. carinata*, along with the flora of the moist Mediterranean region, moved south to the highlands (1 300-1 500 metres) of Ethiopia. The species distribution from its Ethiopian centre of origin has been limited to neighbouring east African countries. Recently it has been introduced as an oil crop to India and as a species of commercial interest in Canada and Spain.

Ecosystems and habitats where the species occurs natively and has naturalised

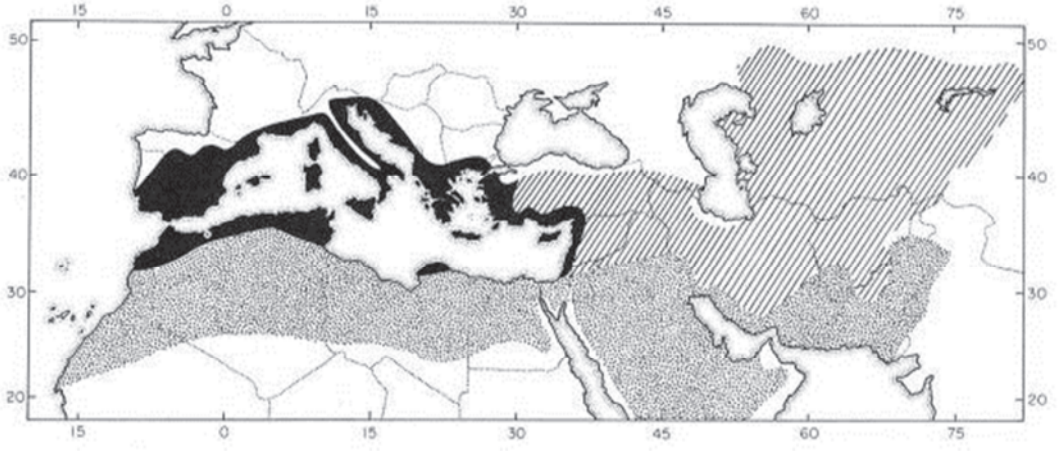
There are few areas of the world where members of the family Brassicaceae are totally absent. The exceptions are the Antarctic and some parts of the tropics. However, even in the tropics, the family is thinly represented by some introduced cosmopolitan weeds that have become established. The genera and species of the family occur in greatest number and diversity in the temperate zone of the northern hemisphere and in particular, the areas surrounding the Mediterranean basin and throughout the southwest and central regions of Asia (Figure 3.25) (Hedge, 1976). Although the generic and specific endemism in the family is highest in the Irano-Turanian region, the centre of the present-day subtribe, Brassicinae, lies in the Mediterranean basin (Hedge, 1976).

Feral populations in disturbed soils

Due to the large seed losses in commercial *B. napus* fields and the potential loss during transport and handling, the surviving seeds give rise to volunteers in subsequent crops and feral populations in non-cultivated areas (CETIOM, 2000; MacDonald and Kuntz, 2000; Orson, 2002; Pessel et al., 2001; Price et al., 1996). Volunteers are controlled by cultivation and herbicide application. In both Canadian and UK trials, the numbers of genetically modified (GM), herbicide resistant (HR) *B. napus* volunteers in the year following GM trials were comparable to, or less than, conventional *B. napus* (Crawley et al., 1993; Booth et al., 1996; Hails et al., 1997; Rasche and Gadsby, 1997; Sweet et al., 1999a, 1999b, 1997; Sweet and Shepperson, 1998; Norris et al., 1999). In their survey of Canadian commercial fields, MacDonald and Kuntz (2000) found the same trend, with similar numbers of volunteers in the year following cultivation of

GM HR canola compared to conventional varieties. Furthermore, prior to any field operations, they found an average over all fields of 200 volunteers/m^2. Initial soil disturbance was effective in controlling these emerged *B. napus* volunteers, but shallow cultivation resulted in the emergence of an even greater number of volunteers. A post-emergent weed control programme employed by the producer for the non-GM volunteers was also effective in controlling the GM volunteers (MacDonald and Kuntz, 2000). Downey and Buth (2003) reported that GM HR volunteers with single or stacked traits were readily controlled in western Canada by the same agronomic practices that are standard for controlling conventional canola volunteers. In Australia, post-harvest monitoring of GM HR (glufosinate or glyphosate) trial locations for six years indicated volunteer populations were adequately controlled by herbicide application or broadacre cultivation (either in-crop or by conservation tillage) (Salisbury, 2002).

Figure 3.25 **Approximate areas of the phytogeographic regions containing the world's greatest representation of Brassicaceae genera**



Note: They encompass the Mediterranean (black); the Irano-Turanian (striped) and the Saharo-Sindian (dotted) regions.

Source: After Hedge (1976).

Feral populations of *B. napus* can be found at various densities on road verges, along field margins and railway lines in all countries where it is grown (e.g. Crawley and Brown, 1995; Wilkinson et al., 1995; Squire et al., 1999; MacDonald and Kuntz, 2000; Agrisearch, 2001; Pessel et al., 2001; Orson, 2002; Salisbury, 2002). Populations may also become established in port areas where *B. napus* cargos are handled (Ramsay, Thompson and Squire, 2003; Saji et al., 2005; Aono et al., 2006). Annual recruitment to such sites is likely to be more from passing transport vehicles than from an established seed bank. *B. napus*, as with other *Brassica* species, is a coloniser of disturbed soils where it competes with other primary colonisers. However, *B. napus* is a poor competitor and is not regarded as an environmentally hazardous colonising species (European Commission, 2000, 1999, 1998a, 1998b; Beckie, Hall and Warwick, 2001; Dignam, 2001). Unless the habitats are disturbed on a regular basis, *B. napus* will be displaced (OECD, 1997).

In western Canada, roadside verges, field margins and railway lines were surveyed for canola plants (MacDonald and Kuntz, 2000). Only 13 and 27 volunteer *B. napus* plants were found in the mowed roadside over the respective 7 and 27 kilometres surveyed, and no plants were found in tall, unmowed grass. Surveys of rail beds leading

from local grain elevators, approximately 3 and 5 kilometres long, identified 287 and 29 plants, respectively, growing at the interface of the rail bed gravel and the tall grass of the right of way. No plants were located on the rail tracks or in the tall grass of the right of way. Similarly in Australia, a survey, making 400 observations in 5 × 20 m areas along 4 000 kilometres of roads in oilseed rape growing areas, found *B. napus* plants in only 31%, 20%, 13% and 9% of the observation points in southern New South Wales, Western Australia, Victoria and South Australia, respectively. Nearly all the plants were growing within five metres of the roadside, with the vast majority close to or alongside the road edge, suggesting they originated from seed dropped from passing vehicles (Agrisearch, 2001).

In the United Kingdom, Crawley and Brown (1995) found that along undisturbed roadways, the persistence of *B. napus* is about three to four years and that the density of such feral populations is correlated with human activities, such as vehicle transport. In a three-year assessment of feral populations in Scotland, Wilkinson et al. (1995) found that the turnover of populations was high, with only 19% of the 1993 population persisting into 1994 and 12% of the 1994 population persisting into 1995. Crawley and Brown (1995) obtained similar results in southern England. In a study conducted in Germany from 2001 to 2004, Dietz-Pfeilstetter, Metge and Schönfeld (2006) found persistence rates for feral populations of 29% between 2001 and 2002, of 12% between 2002 and 2003 and 80% between 2002 and 2004. However, molecular profiling using ISSR-PCR (inter-simple sequence repeats-polymerase chain reaction) revealed that plants appearing in successive years largely belonged to different genotypes, suggesting new seed input and an even higher turnover of populations.

Reuter et al. (2008) investigated a 500 km² area in the region of Bremen, Germany and reported average densities of 1.19/km² and 1.68/km^2 of feral and volunteer oilseed rape populations in rural and urban areas, respectively. The investigation showed that population density varies between years and feral plants tend to be smaller in stature (by at least 40%) than plants growing on cultivated land.

Surveys by Agrisearch (2001) and MacDonald and Kuntz (2000) suggest that to survive spring, *B. napus* roadside populations need to be regularly replenished. However, in France, Pessel et al. (2001) found roadside feral populations contained plants of old varieties that had not been grown for eight to nine years, indicating that the seed source was not entirely from recent vehicle spillage. These results are in keeping with previous reports that seed of old rapeseed varieties can persist for at least five to ten years after they were last reported grown (Squire et al., 1999; Orson, 2002). Pessel et al. (2001) suggested that the analysed roadside feral populations arose from multiple spillages from different fields or germination of seed from a mixed seed bank or most likely, both.

In Austria, Pascher et al. (2006) genetically analysed plants from 9 selected feral populations consisting of 50-150 individuals. They found the feral populations were genetically more diverse than could be explained by the dominant varieties grown in the area in the previous five years. They concluded that even though the feral populations largely reflected the genetic makeup of the dominate varieties being grown, a significant portion of plants had originated from seed banks older than five years. They also found that feral populations disappeared more quickly under dense grass cover than at sites with little vegetation, but genetic diversity remained unchanged. Their results indicated that genetic migration from commercial varieties to feral populations was five times greater than the inverse.

Feral populations in natural habitats

In natural (undisturbed) ecosystems, *B. napus* is not considered to be invasive or even a significant component of any natural plant community (AAFC, 1994; Warwick, Beckie and Small, 1999; Beckie, Hall and Warwick, 2001; Dignam, 2001).

Production and agronomy of Brassica *oilseed crops*

The world demand for edible oils and more recently for biodiesel has led to a rapid growth in the production of most oilseeds, with total seed oil produced increasing by about 4% each year. The percentage growth in the world *Brassica* seed oil production increased some 60% between 1996-09 and 2006-10 (Table 3.1). The locations of the major rapeseed/mustard producing regions over the two decades 1995-2014 are shown in Table 3.4. The expanded *Brassica* oilseed production has resulted from both an increase in the area sown globally, as well as the yield per unit area that has increased in most regions (Table 3.4).

Table 3.4. **Area harvested, production and yield by major *Brassica* oilseed producing countries, averages 1995-99 to 2010-14**

Producing country	Area harvested ('000 ha)				Production ('000 tonnes)				Seed yield (kg/ha)			
	1995-99	*2000-04*	*2005-09*	*2010-14*[3]	*1995-99*	*2000-04*	*2005-09*	*2010-14*[3]	*1995-99*	*2000-04*	*2005-09*	*2010-14*[3]
E.U.[1]	3 944	4 255	5 407	6 693	11 038	12 221	18 108	20 865	2 786	2 862	3 082	3 120
Canada	4 917	4 366	5 322	7 757	6 866	6 237	10 510	15 171	1 398	1 415	1 790	1 954
China (People's Republic of)	6 708	7 244	6 740	7 244	9 391	11 573	12 070	13 315	1 399	1 597	1 842	1 836
India	6 541	5 110	7 280	6 303	5 756	5 045	7 239	7 417	884	982	1 079	1 178
Australia[2]	929	1 335	913	2 425	1 230	1 529	1 395	3 133	1 370	1 146	1 084	1 277
United States	292	497	452	568	443	762	665	1 009	1 505	1 555	1 635	1 780

Notes: 1. E.U. = Total production of the 27 (28 from 2013) member states of the European Union. 2. Extreme drought greatly reduced Australian production and seed yield in the 2005-09 period. 3. Columns added in January 2016.

Source: FAOSTAT.

Cultivation and management of oilseed crops

The small seeds of the *Brassica* oilseed crops require that the seed be sown at shallow depths, 2-3 cm below the soil surface, into a firm, moist seedbed. Under favourable growing conditions the seedlings emerge within four to five days of sowing. Cotyledon expansion is quickly followed by the formation of a rosette of seven to eight true leaves from which the flowering stalk bolts. The length of time the crop remains in the rosette stage can vary from less than 30 days to more than 210 days depending on climatic conditions and the species and form grown. The complete growth cycle may be as short as 70 days (*B. rapa*) or as long as 380 days for winter *B. napus* varieties in China (Sun et al., 1991).

Although the *Brassica* oilseed crops prefer a deep loam soil, it does well when sown in a wide range of soil types and conditions and can tolerate a pH range from 5.5 to 8. Compared to most other grain crops, *Brassica* oilseed crops require greater nutrient inputs to achieve high yields. Generally speaking, they need about 25% more nitrogen, phosphorus and potassium and up to 5 times more sulphur than a wheat crop. Harvested seed should be stored at no more than 9% moisture when cooled to 10ºC to prevent deterioration due to fungal and/or insect activity. The usual rotation is as a break crop

with cereals. Wheat yields following a *B. napus* crop invariably improve in Europe and Australia due the reduced level of cereal pathogens present and the control of grassy weeds (Almond, Dawkins and Askew, 1986).

North and South America

The oilseed rape/canola grown in North America is concentrated in the northern part of the Western Great Plains (Figure 3.26). The species and form grown is almost exclusively the spring or annual *B. napus*. In western Canada, less than 1% of the 5 million ha is sown to spring *B. rapa*. Production of the winter or biennial form of *B. napus* in North America is confined to a few thousand hectares in the Province of Ontario, Canada and a few west and central states in the United States. In South America, both spring and winter *B. napus* is produced on some 17 000 ha in central Chile.

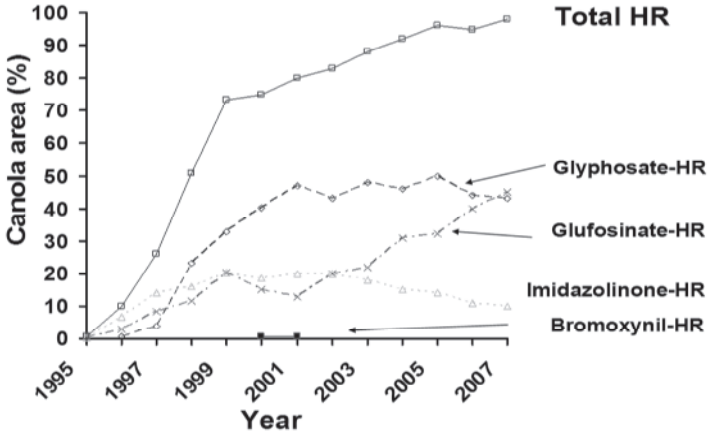
Figure 3.26. **Areas of oilseed rape/canola production in North America**

Notes: Light grey indicates heavier production concentration.

Source: Courtesy Canola Council of Canada.

Cultural practices in the main oilseed rape production regions of western Canada and the United States have changed in recent years. Traditionally the crop was sown into summer fallow, land laid fallow the previous year. With the shift to continuous cropping and minimum tillage, *B. napus* is now sown into the undisturbed stubble of the previous year's cereal crop. Weed control, which would normally be a problem with this direct seeding system, can now be easily achieved with the new broad spectrum, post-emergence herbicides such as glyphosate, glufosinate and the imidazolinones. The adoption of these herbicides and their associated herbicide resistant varieties has been extremely rapid (Figure 3.27).

Figure 3.27. **Percentage of the total Canadian *B. napus* production area sown to herbicide resistant varieties, 1995-2008**



Note: HR = herbicide resistant.

Source: Adapted from Beckie (2011).

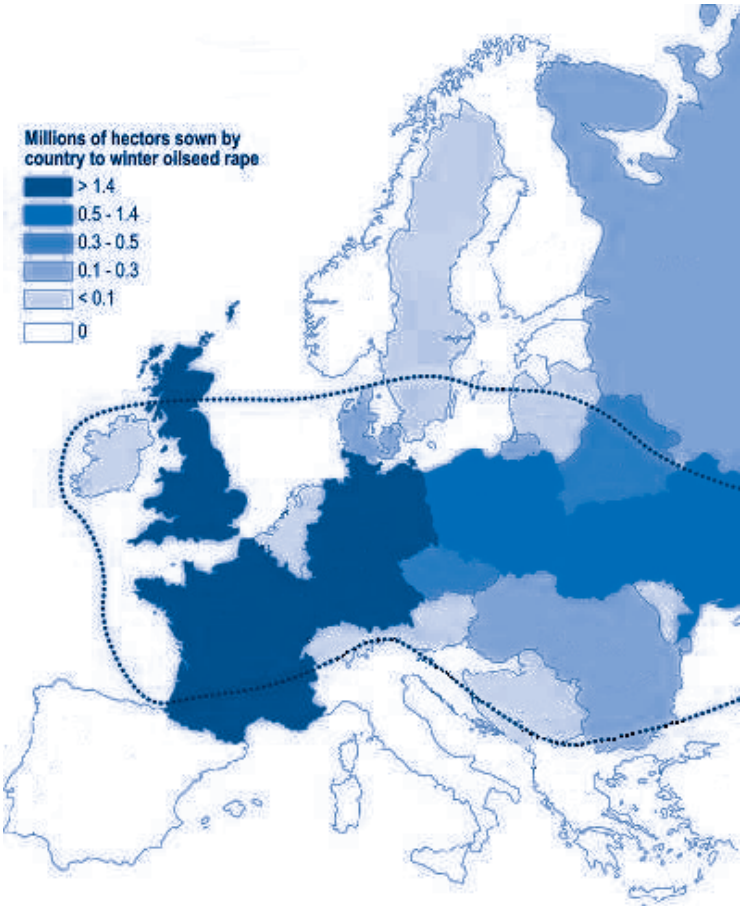
The double disc grain drill has now been largely replaced by large air seeders that place the seed and fertilizer some 2-3 cm below the soil surface, at a seeding rate of 5-8 kg per hectare. Seed is treated with an insecticide-fungicide coating. The herbicide glyphosate is usually spring applied prior to seeding to control early germinated or biennial weeds. In North America, seeding generally occurs in early May. The herbicide of choice is applied at the recommended rate when the weeds are small and the leaves of the *B. napus* plants have not fully covered the ground. *B. rapa* fields begin flowering in mid-June while *B. napus* fields begin to flower about two weeks later in late June or early July. Recommended fungicides and/or insecticides may be applied as a spray if the pest incidence warrants. At harvest, in late August through September, the crop is normally swathed into windrows to allow more uniform ripening and to protect against seed losses due to pod shatter. Combining the swaths is done when the seed is mature and dry. However, some straight combining of the standing crop is also practiced. Usually the seed is farm stored at less than 9% moisture until marketed.

Chile is the only country in South America that produces a significant quantity of oilseed rape with planting of winter and spring *B. napus* on some 17 000 hectares in the southern provinces of the central part of the country. The crop is predominantly winter *B. napus*. The winter crop is sown in March and April, flowers in October and November and is harvested by straight combining in January. Winter kill may occur in May or June due to the wet soil freezing and heaving, causing broken roots. The spring crop is sown in August-September, flowers in October and is harvested in late December or early January. The crop is normally sown on land broken out of grass pasture using a disk or mould board plough, disked twice with a double disk cultivator and packed. Seed is sown with a double disk seeder or the less satisfactory one-way disk at 7-8 kg per hectare. Fertilizer requirements vary widely due to the sharply different soil types encountered in rapeseed growing areas. Levels of macronutrients nitrogen, phosphorous and, in some soils, sulphur are very low. Also lacking in some soils are the micronutrients manganese, copper and boron. At harvest, a desiccant is applied and after the appropriate interval, the crop is straight combined. The seed is normally artificially dried to less than 9% moisture prior to storage or marketing.

European crop cultivation and management

Winter oilseed rape (*B. napus*) is the dominant species grown in both Western and Eastern Europe (Poland, western Russian Federation and Ukraine); however, the area sown to spring *B. napus* is rapidly expanding (Figure 3.28). Some spring and winter *B. rapa* is grown in Finland and Sweden. In Germany over the past 13 years, the area sown to spring oilseed rape decreased from 10% to 1% of the oilseed rape growing area. Spring *B. napus* is used primarily as a replacement crop on winter oilseed rape fields that have been winter-killed. The optimum date for sowing the winter form varies with the latitude and the onset of winter. In northern European countries, the optimum sowing date is the last half of August while more southerly regions in France and Germany can delay seeding until early September. The objective is to produce plants that are large enough and have stored sufficient food reserves to withstand the rigours of winter. It is recommended that plants entering the winter show a vigorous growth, a well-developed root system (taproot about 8-10 mm in diameter) and have at least 6-8 true leaves. Seed is sown into well-worked soil at 5.0-5.5 kg per hectare when drilled and 8-9 kg per hectare if broadcast, to obtain fall stands of 50-85 plants/m^2 to allow for some winter kill.

Figure 3.28. **Oilseed rape (*B. napus*) production regions in Europe showing millions of hectares of winter rape per country**



Notes: The dotted line encircles the primary growing region for winter oilseed rape. Spring rape production is concentrated in Eastern Europe, primarily the Russian Federation (>0.5 M ha) and Ukraine (0.1-0.5 M ha).

Source: Adapted from information supplied by Norddeutsche Pflanzenzucht.

The seeding rates recommended for precision drilled hybrid varieties with a high branching density and a 1 000 seed weight of 4 g/1 000 is 1.2-1.6 kg per hectare and for seed of 7 g/1 000, 2.1-2.8 kg per hectare. The seeding rates for drilled hybrids are lower than for open pollinated varieties since the hybrid seed is likely to produce a more vigorous plant that better withstands the winter. The optimum spring plant population is reported to be 80-100 plants/m². Winter varieties are heavy users of nitrogen so frequently some nitrogen is incorporated prior to planting, with the balance top-dressed in the spring. Excessive nitrogen promotes vigorous fall growth but tends to make the crop more susceptible to winter kill. Phosphorus and potassium are applied before planting at the recommended levels. Sulfur is used in early spring in combination with N-fertilization. Boron is often applied in late spring in combination with fungicides. Nearly all seed is treated with a fungicide-insecticide combination (often with more than two active ingredients) to control seedling pests.

Disease, insect and weed control in the emerged crop is achieved by spraying the recommended products when needed. Flowering in northern Europe begins the last days in April, and harvest starts with some swathing at the end of July with the vast majority of the crop straight combined a week or so later. Harvest can continue through to the end of August. In southern regions, harvest commences about one to four weeks earlier.

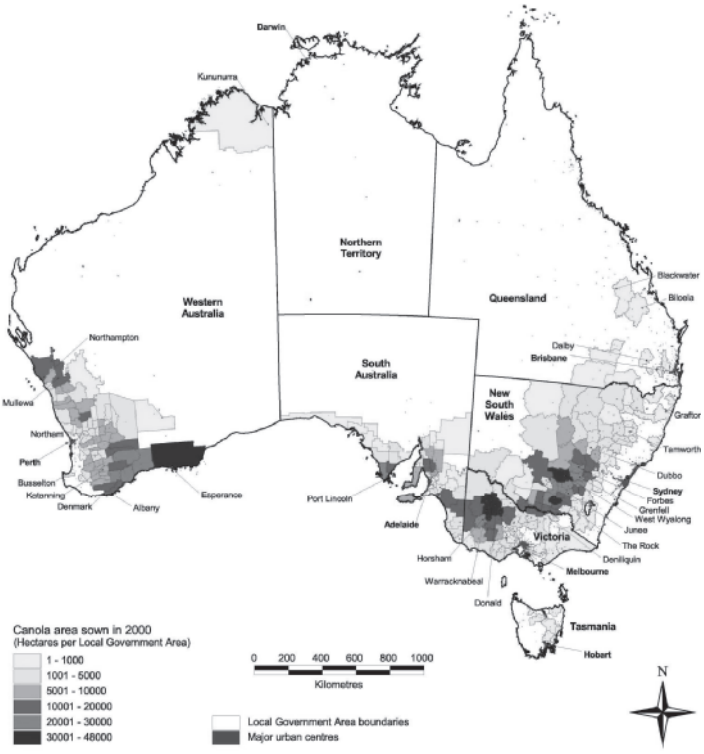
Australian crop cultivation and management

Oilseed rape production in Australia is relatively recent with the first commercial production undertaken in 1969. In the early years, both *B. rapa* and *B. napus* spring varieties from Canada were imported and grown in the winter season. Today production is almost exclusively from Australian-bred *B. napus* varieties. Canola is grown in most cropping areas of Southern Australia, including Western Australia (Figure 3.29). Most of the *B. napus* crop is sown in late autumn or early winter (April to June) during the rainy period. The seed is primarily sown with air seeders at seeding rates of 4-6 kg per hectare with hybrid varieties being sown at about 3 kg per hectare. All seed is treated to control blackleg disease (*Leptosphaeria maculans* [Desm.] Ces. et de Not.) and some seed is treated for control of the red-legged earth mite (*Halotydeus destructor* Tucker). Flowering occurs in August and September with harvest in late spring or early summer (November and December). The growing season ranges from about 150-210 days, depending on latitude, rainfall, temperature and sowing date. Growth and yield of the crop is almost always limited by the amount of water available to the crop, particularly during maturation.

Due to the age of Australian soils, macronutrients (particularly nitrogen, phosphorous and sulphur) and micronutrients are deficient. Deficiencies in boron, manganese, molybdenum and zinc have been reported for *B. napus* crops, as has toxicity on the more acid soils due to high levels of aluminium and manganese. Most soils are strongly acidic and liming is necessary to achieve high yields. Initially oilseed rape was sown into well-worked soil, but with the availability of glyphosate as a pre-planting herbicide and varieties resistant to triazine and imidazolinone herbicides, direct seeding has become standard practice.

Oilseed rape is most frequently preceded by a pulse crop or pasture while fallow and wheat are other alternatives. When the canola crop precedes wheat in the rotation, substantial wheat yield benefits occur.

Figure 3.29. **Areas and concentration of *B. napus* production by Australian government districts**



Data source: Australian Bureau of Statistics (2002).

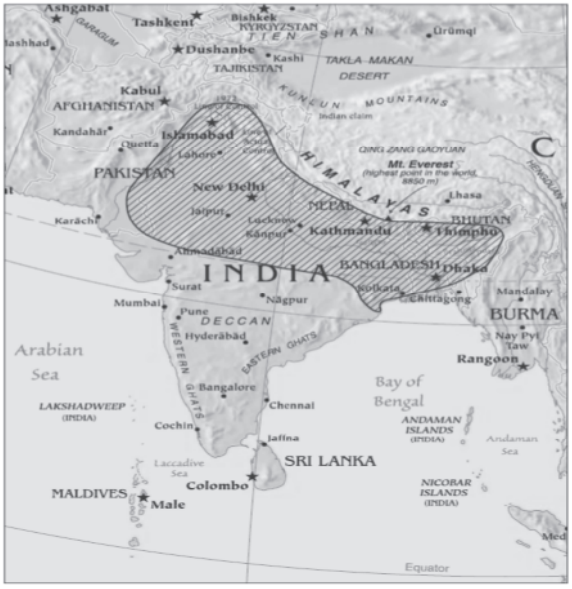
Indian sub-continent cultivation and management

The dominant *Brassica* oilseed crop on the Indian sub-continent is *B. juncea*, although a limited hectarage is sown to the *B. rapa* form, toria, which is grown from September through December in northern areas. *B. napus* and *B. carinata* are grown to a limited extent in some irrigated and dry land areas of northern and central Indian states, respectively. The major crop of *B. juncea* as well as small pockets of yellow and brown sarson (forms of *B. rapa*) are sown in October or early November and harvested in late March or early April. Flowering occurs in early January. Production is centred in the northern half of the sub-continent, in what is called the mustard belt (Figure 3.30). The untreated seed is normally broadcast on the ploughed and levelled fields and the seed buried by drawing a heavy plank over the field. The traditional practice of sowing the *Brassica* species mixed with a cereal grain is no longer employed to any degree and the sowing of pure stands of each crop is now normal practice.

However, mixed cropping is still practiced in several areas by few farmers. Double cropping in the mustard belt is the standard practice with mustard sown on the same land each year following the summer crop, which may be pulses (mung and urd bean) or green manure. Other alternatives are rice, cotton or millets (such as sorghum or pearl millet).

The recommended seeding rate for *B. juncea* is 4-5 kg per hectare. Fertilization with nitrogen-phosphorous-potassium, in the ratio of 80-40-40 kg per hectare, together with 40 kg of zinc and 25 kg of sulphur, is recommended.

Figure 3.30. **Major production region (striped area) of oilseed mustard (*B. juncea*) and toria (*B. rapa*) on the Indian sub-continent**



Source: Courtesy R.K. Downey

Chinese cultivation and management[3]

China is the world's largest producer of *Brassica* oilseed crops, annually producing some 11.5 million tonnes. Species contributing to this output include winter and spring *B. napus*, *B. juncea* and both winter and spring forms of *B. rapa*. Production is primarily from *B. napus* (representing 95% of the total), but both *B. juncea* (4%) and *B. rapa* (1%) oilseed crops are also grown at various concentration in the different provinces.

B. napus is grown throughout the country with the winter form dominating in the southern provinces and the spring form in the north.

The provinces along the Yangtze River provide the bulk of China's production. The level of winter hardiness required is not great. Indeed, Canadian and European varieties of the spring form have successfully survived the winters in the Chinese winter-growing region.

The spring-sown crops (*B. napus*, *B. juncea* and *B. rapa*) are sown in May, flower in June or early July and are harvested in September. The growing cycle for *B. napus* takes about 120 days. In the southern portion of the spring-growing area, half a season may be used to grow a forage or vegetable in conjunction with *B. rapa*. Because of the small field sizes, most are sown by hand or walking plough, although some large fields are mechanically sown. In the winter rape areas, the seed is sown into small seedling beds in September and the seedlings later transplanted into the production fields in mid- to late September. Flowering takes place in late March and harvest is in May. The total production cycle is about 220 days. The rotation in the triple cropping winter rape area is either rape-rice-rice or rape-maize-potato and in the double cropping regions rape-cotton or rape-rice.

Soil fertility is a limiting factor in production, with the area devoted to winter rape being particularly deficient in phosphorous. While all soils require nitorgen, phosphorous

and potassium, significant areas are deficient in the micronutrients zinc and boron, while shortages of manganese, copper and iron also occur.

Herbicide resistant *B. napus*

B. napus is not considered a significant weed in managed ecosystems (AAFC, 1994). However, due to the high level of seed lost during harvest it can be an abundant weed in subsequent crops. Légère et al. (2001) ranked *B. napus* as 18th in relative abundance among Canadian weed species in western Canada, and Leeson et al. (2005) found *B. napus* plants in 10.5% of the fields surveyed. Studies in both Canada and Europe have shown that the incorporation of genes for resistance to specific herbicides imparts no altered weediness or invasive potential for glyphosate, including different events (AAFC, 1995b, 1996a; Norris et al., 1999: Crawley et al., 2001); glufosinate-ammonium, including its combination with the hybrid system (AFFC, 1995a, 1995d, 1996b; Rasche and Gadsby, 1997; Norris et al., 1999; MacDonald and Kuntz, 2000); bromoxynil (PBO, 1998) and non-GM imidazolinone (AAFC, 1995c). Experience in western Canada from 1995 through 2011, with all HR systems, have confirmed the validity of these earlier assessments (Beckie, 2011; Warwick, Beckie and Hall, 2009; Beckie et al., 2006).

However, GM-HR volunteers can occur in subsequent *B. napus* crops. The level will depend on the interval between oilseed rape crops in the rotation and how well the producer has controlled volunteer *B. napus* in the intervening years. The shorter the rotation and the less volunteer control, the greater the contamination level in the second planting. The presence of one GM-HR canola plant per square metre throughout a field of conventional oilseed rape calculates to a GM content of 2.5% in the harvested conventional crop (planted at 40 plants/m^2). This calculation assumes that the number of seeds produced by a volunteer plant is the same as that produced by the conventional plants (CETIOM, 2000). However, Gruber and Claupein (2007) report that volunteer winter *B. napus* plants, growing in a sown rapeseed crop only yield 45% of the seed produced by corresponding sown plants.

Off-type volunteer plants can come from multiple sources, including the seed bank from previous crops, movement of farm equipment and animals, pollen flow and contaminated seed stocks. In Australia, Stanton, Pratley and Hudson (2002) found sheep can excrete viable or germinable *B. napus* seed up to five days after ingestion. Similarly, Martens (2001) claimed that manure from oilseed rape-fed chickens resulted in volunteer plants when the manure was spread on a field 12 months later. In Canada, Downey and Beckie (2002) and Friesen, Nelson and Van Acker (2003) found certified pedigreed seed lots of conventional varieties contained unacceptable levels of GM seeds, apparently resulting from pollen flow in breeding nurseries. The seed industry quickly purified their breeding stocks but absolute exclusion cannot be guaranteed. Feral populations may disseminate genes to nearby oilseed rape crops but the incidence would be very small and far less than several of the sources noted above (CETIOM, 2000; Wilkinson et al., 1995).

In all oilseed rape growing regions, leaving the soil untilled for a period after harvest and using non-inversion tillage is an effective strategy for minimising the size of the seed bank (Gruber and Claupein, 2007; Gulden, Shirtliffe and Thomas, 2003a). Ploughing, as done in Europe, will bury the seeds below germination depth but when the field is again ploughed the dormant seeds will be brought to the surface. Pre-emergence and in-crop post-emergence herbicide applications are effective in controlling volunteers even if they contain one, two or three different herbicide-resistance genes (Table 3.5; Downey and Buth, 2003). In western Canada, where herbicide tolerant oilseed rape has been grown

extensively for 15 years, there is no evidence that volunteer *B. napus* has increased or is more prevalent because of the herbicide resistance traits (Hall et al., 2000; Beckie et al., 2006, 2004).

Oilseed certified seed production

The production of oilseed *Brassica* sowing seed is normally undertaken within the areas where the *Brassica* crop is commercially grown. The rules under which pedigreed seed is produced and identified in the market place are stringent and extensive. Regulations vary from country to country but the minimum requirements for certified seed moving in international trade are governed by two international certification organisations. Both the OECD Seed Schemes and the Association of Official Seed Certifying Agencies (AOSCA) were developed to facilitate seed trade through mutual recognition of the official certification labels of member agencies. Member countries must meet OECD and AOSCA standards, but countries can – and most of them do – have domestic certification standards that exceed those minimums.

Table 3.5. **Number of herbicide products available for control of volunteer *B. napus* with nil, single or multiple herbicide tolerances in western Canada**

Herbicide system	Number of products
Susceptible	27
Liberty Link (LL)[1]	26
Roundup Ready (RR)[2]	25
Clearfield (CF)[3]	19
RR × LL	24
RR × CF	17
LL × CF	18
RR × LL × CF	16

Notes: 1. LL Glufosinate; 2. RR Glyphosate; 3. CF Imidazolinone.

Source: Downey and Buth (2003). Courtesy AAFC Reseach Station, Saskatoon.

AOSCA has a focus on the United States but its members include also Argentina, Australia, Canada, Chile, New Zealand and South Africa. AOSCA standards cover not only varietal certification of seed but also germination, physical purity, disease and other quality traits. Their varietal certification requirements include a maximum variety impurity "seed" standard that is used for post-control verification testing.

The OECD Seed Schemes, which largely reflect the requirements of the European Union seed certification system, are increasingly implemented at the global level. They comprise 58 member countries including most of the countries discussed above. China and Pakistan are currently not members of the OECD Seed Schemes (situation November 2012). However, China is developing standards for *Brassica* crops and Pakistan has regulations that are similar to those of India. OECD seed standards do not deal with germination or physical purity but focus on varietal certification, based mainly on morphological characteristics during inspections of seed-production crops. In addition, minimum requirements and standards for verification, using post-control field testing, are mandatory.

Seed classes allowed are normally designated by the breeder or maintainer of the variety. For *Brassica* oilseed, the seed multiplication factor for each generation is typically large (>1 000:1). Thus, the seed classes designated for its species are normally

limited to three and identified under the OECD Seed Schemes as "basic", "certified 1st" and "certified 2nd" generations, with the equivalent generations designated under AOSCA as "breeder", "foundation" and "certified seed". Normally only one generation is allowed for the foundation and certified classes. The OECD seed regulations for Brassicaceae oilseeds require a five-year interval between crops of the same species. AOSCA standards for production of foundation seed of *B. napus*, *B. juncea* and *B. rapa* require four years between crops of these species and a two-year interval when producing certified seed. Under OECD regulations, basic and certified seed-production fields of *B. napus* must be isolated from any possible source of cross-pollinating pollen by a minimum of 200 m and 100 m, respectively. AOSCA regulations require foundation producing fields of *B. napus*, *B. juncea* and *B. rapa* to be isolated from any other crop of the same kind by 201 m, 402 m and 402 m respectively. For certified producing fields of these three species, the respective isolation distance required is 100 m, 402 m and 100 m. Both sets of regulations require all seed-production fields to be inspected by the designated authority at least three times for basic seed production and three times on each parental line for the production of certified seed of hybrid varieties, i.e. before the flowering stage, in the early flowering stage and before the end of the flowering stage. Fields must also meet stringent standards for varietal purity (visual characteristics) as well as freedom from cross-pollinating species and other crop kinds.

It must be emphasised that the above are minimum standards, with most countries having higher requirements as well as many seed companies exceeding the more stringent domestic regulations. Open-pollinated varieties of *B. napus* are rapidly being replaced by F_1 hybrid varieties, and a similar situation is likely to occur in *B. juncea* within the next few years. The requirement for nearly absolute purity of the female parent is mandatory if the hybrid is to produce the desired level of heterosis. The male restorer parent must also breed true for restoration of hybrid fertility. Thus, the hybrid regulations for isolation distances under AOSCA are much greater at 804 m while most seed companies use 1 000 m or more. Also, foundation and certified producing crops for hybrid seed production cannot be grown on land which has grown *B. napus*, *B. rapa*, *B. juncea* or oilseed *R. sativa* in the past five and three years, respectfully.

The studies by Downey and Beckie (2002) and Friesen, Nelson and Van Acker (2003) that identified some Canadian certified *B. napus* seed lots as containing undesirable levels of foreign herbicide resistance traits are often cited as sources of contamination. Regulators and the seed industry moved quickly to correct this situation. Today the Canadian Food Inspection Agency (CFIA) carries out seed testing of *Brassica* oilseed varieties for: 1) adventitious presence (AP) of approved events; and 2) herbicide trait purity of glyphosate and glufosinate ammonium resistant varieties. All official reference control samples for oilseed rape varieties submitted to the CFIA's Variety Registration Office at the time of registration of a new variety are subject to AP testing and if the variety is herbicide resistant, to herbicide purity trait testing. Furthermore, the CFIA also monitors AP and trait purity of foundation and certified seed. In instances where AP and/or trait purity issues are identified, the breeder of the variety is notified and appropriate action is taken (Canadian Food Inspection Agency, 2009).

The Canadian Seed Growers Association (CSGA) (2009) have also revised its "Regulations and Procedures for Breeder Seed Crop Production" so that seed certificates are only issued for breeder seed crops that are produced within a third-party audited quality management system (QMS) and verified to preserve varietal identity. Further, non-compliance with QMS requirements can lead to suspension or cancellation of the

professional recognition of a plant breeder, which is required in both CFIA variety registration and CSGA seed crop certification.

Brassica *vegetable seed-production locations and management*

The market for *Brassica* vegetables has, in recent years, experienced a steady increase in demand. This expansion has been aided by widespread refrigerated transportation systems that can provide a year-round supply of such vegetables to most markets. The *Brassica* vegetable crop with the greatest demand for seed is cabbage, followed by the *B. rapa* Asian vegetables and broccoli. The world requirement for cauliflower seed is less while the demand for turnip, rutabagas and kohlrabi is relatively small. Accompanying the increased commercialisation of *Brassica* vegetable production has been the need to provide large quantities of seed of high quality and varietal purity. This requirement has resulted in the majority of the seed being produced in specific locations where climate and isolation from other *Brassica* crops are favourable for consistent high yield and quality. To aid the growing international trade in vegetable seed, the OECD has established a Scheme for the Certification or Control of Vegetable Seed which requires field and seed inspection by an accredited authority, within the country of origin, to ensure the seed meets varietal purity standards, including freedom from cross-pollinating species. The OECD Vegetable Seed Scheme provides for the production of "certified seed", and the designation of "standard seed", corresponding to two different control requirements. Other organisations that facilitate the seed trade include the International Seed Federation (ISF), which has defined trading terms and rules dealing with sales, can arbitrate settlements and assists with import and export licenses: ISF regional seed industry organisations, such as the Asia and Pacific Seed Association (APSA), which seeks to improve vegetable seed production and trade in the region (George, 2009). Many companies also use a QSM as described above for oilseed seed production.

Locations of concentrated vegetable seed production

In developing countries, vegetable seed is primarily supplied from farm-saved seed, and more rarely from the formally organised seed sector. In countries with strong agricultural and horticultural industries, nearly all the seed is from commercial pedigreed sources. For large-scale seed production of the biennial *Brassica* vegetables, seed companies have concentrated production in areas with relatively mild winters and moderate summer temperatures. In Europe, such areas are found in Belgium, Brittany (France), northern Italy and the Netherlands.

In North America, among the *Brassica* vegetables, broccoli has the greatest seed demand followed by cabbage and cauliflower. The market for the seeds of collard, Brussels sprouts and the Asian vegetables is much smaller. Seed production of these crops is concentrated in valleys of Oregon and Washington states (e.g. Oregon's Willamette Valley). Selected areas in California and Arizona are also important producers of broccoli and cauliflower seed. Essentially all broccoli and cabbage varieties produced in the United States are F_1 hybrids. In contrast, most cauliflower varieties are highly inbred and uniform, self-pollinating populations, but in recent years more and more F_1 hybrids have entered the market (Farnham, 2007). F_1 and inbred varieties of collards, Brussels sprouts and kale provide seed to the commercial market. In South America, Chile is a significant supplier of vegetable *Brassica* seed.

In Australia, seed production of *Brassica* vegetable crops is centred in Tasmania in the regions of the Coal River Valley, Derwent Valley, central East Coast,

Hagley/Westbury and Devonport. Tasmania is climatically suited for "counter-season" seed production for the northern hemisphere markets of Asia and Europe. Major seed crops produced in 2001 were hybrid cabbage (150 ha) and cauliflower (97 ha) (Government of Tasmania, 2003). Cabbage and cauliflower are high-value autumn planted crops while the lower value mustard and Chinese vegetable types are spring sown. Locations for hybrid seed production of cabbage and cauliflower are determined by the need for an isolation zone of 1.5-3 km from other crops of the same botanical family. Grower awareness and consultation between companies ensures adequate isolation distances.

In New Zealand, the Canterbury Plains and other smaller areas of the South Island (43º south) have become a major vegetable seed-production location, particularly for the Asian *Brassica* vegetables. In this region, the seed merchants and growers have put in place an isolation mapping system to avoid cross-pollination among different species and varieties. The system is operated by a government-owned company called AgriQuality that displays an Internet map of every farm field involved in seed production. When a seed contract is arranged and a field is selected, the seed merchant logs the details into the system and can see if there are any conflicts within the isolation distance required. Normal minimum isolation distance for the *Brassica* crops is 1 000 m, but that can be extended, particularly with hybrid seed production.

Not all seed-production regions are maritime based. In China, cauliflower and broccoli seed production is concentrated in semi-desert regions around the cities of Jiuquan and Jiayuguan in Gansu and in Yunnan provinces. In these high-elevation areas precipitation is minimal, but irrigation is available and the temperatures remain within the required range. Cabbage and Chinese cabbage seed production is located further south in Hebei, Henan, Shandong and Shanxi provinces (X.-W. Wang, personal communication). In these regions the normal isolation distances between production fields is 1 000 m.

On the Indian sub-continent, no concentrated areas for seed production were identified. However, small individual fields occur scattered in the foothill valleys of the Himalayas. For the production of certified cauliflower seed, the minimum isolation distance is 1 000 m (Indian Minimum Certification Standards).

In Japan, no concentrated area exists for large-scale seed production. However, various *Brassica* vegetables (*B. rapa* and *B. juncea*) are cultivated locally (Inomata, 2007) and seed production is practiced on a small scale. The minimum isolation distance required for seed production is 600 m.

George (2009) notes that most authorities recommend having a greater distance (up to 1 500 m) between different types of *B. oleracea* (cabbage vs. kohlrabi) than between varieties of the same type (two cabbage varieties, up to 1 000 m).

Vegetable seed cultivation and management

The optimum pH for cole crops is reported to be 6.0 to 6.5 with the generally recommended ratio of N-P-K nutrients being 1:2:2 at soil preparation, but it varies depending on the production region (George, 2009). The lower ratio of nitrogen is to avoid "soft plants" that are less winter hardy. Extra nitrogen is normally topdressed in the spring. It is important to ensure that adequate levels of sulphur as well as the micronutrients boron, manganese and molybdenum are available. The development of hybrids in Brussels sprouts has become very important (George, 2009) with self-incompatible and cytoplasmic male sterility (CMS) hybrids becoming more frequent

in cabbage, cauliflower, kale and kohlrabi. The ratio of male to female in hybrid production fields is normally 1:1 or 1:2 (Takahashi, 1987).

Most cabbage and cole crops and some Asian vegetables are biennials and will not bolt until they have been exposed to temperatures of 4-7°C for 6-8 weeks. Day length has no affect on bolting or flower initiation (Nieuwhof, 1969). At the end of the first year, cabbage plants can withstand temperatures of -12°C to -14°C for extended periods, but lower temperatures can cause much damage, as can alternating periods of frost and thaw (Nieuwhof, 1969). The usual practice in producing cabbage seed is to sow in the summer with the plants over-wintering, bolting in the spring and to harvest the seed in summer. Cultivars differ in their winter hardiness with red cabbages the least hardy and savoy the hardiest. Summer temperatures are also important in determining seed yield. Temperatures above 25°C arrest growth and cause seed abortion. Because of these environmental constraints, commercial production tends to be concentrated in areas with mild winters, sufficiently cold to ensure vernalization without winter kill, combined with moderate summer temperatures. The availability of irrigation is also important to obtaining uniform high yields.

Seeding of the biennial crops in the northern hemisphere is normally done in mid-June to mid-August. If the seed is to be sown in beds for transplanting, rather than direct seeding into the field, seeding should be done about ten days earlier than the field sowing to allow for the plant setback brought on by transplanting (Nieuwhof, 1969). The recommended rate for field sowing is 3-5 kg per hectare, unless precision sowing is practiced, where only 1-2 kg per hectare is needed. Plants are thinned to 35-40 cm between plants within the row. To increase the over-wintering survival rate, plants may be earthed up covering the most sensitive plant portion just below the head. Weed control is critical, as in mild winters weeds may over grow the crop. Most of the cole crops are self-incompatible and depend on insects, primarily honey bees, to effect fertilisation. Harvesting is done once the pods have turned yellow and the seedsbrown. Depending on field size and seed value, harvesting may be done by various methods from hand cutting and threshing to straight combining. Kohlrabi, although a true biennial, can be vernalized by initiating germination through pre-soaking the seed for 8-9 hours at 20ºC followed by a cold treatment of -1ºC for 35-50 days. The treated seed can then be sown directly into the field in the spring and the seed crop harvested in the fall. Brussels sprouts and kale are grown for seed in the same manner as cabbages.

For cauliflower and broccoli crops, only a mild vernalization period is required so environmental limitations are less stringent. However, as a seed crop, these forms normally require an extended growing season. Selection of cauliflower varieties for a tighter curd has resulted in slow and incomplete bolting, thus further extending the required growing season. In Western Europe, cauliflower is sown in September and over-wintered under glass with transplanting to the field in early spring. Transplants are spaced on a 50 × 50 cm or smaller grid. Flowering occurs in July or August and the crop is harvested in September or early October. Seed production of tropical and subtropical cauliflower is discussed by Lal (1993).

Drying the harvested *Brassica* vegetable seed is frequently required. To maintain germination capacity, the maximum air drying temperature should not exceed 60ºC. If seed is to be stored for a year, maximum moisture content should not exceed 9% with a storage temperature of 5-10ºC.

The biennial turnips and Swedes (rutabaga) regenerate from growing points at or near ground level. This means they can benefit from a large underground source of nutrients

for seed production. Thus, these crops are more winter hardy than cole crops and can be grown for seed over a much wider environmental range. However, the market for their seed is relatively small, so seed companies tend to contract their production with growers in areas already producing seed of other *Brassica* vegetable crops.

The *B. rapa* vegetables prefer a soil pH between 6.0 and 7.5 with an N-P-K fertilisation ratio at planting of 2:1:1. Additional nitrogen fertiliser is normally applied at anthesis (George, 2009). The seed is produced by either the head-to-seed or the seed-to-seed method described by Opeña, Kuo and Yoon (1988). As with the cole crops, the ratio of male to female in hybrid production fields is 1:1 or 1:2 (Takahashi, 1987).

Centres of origin and ancestors

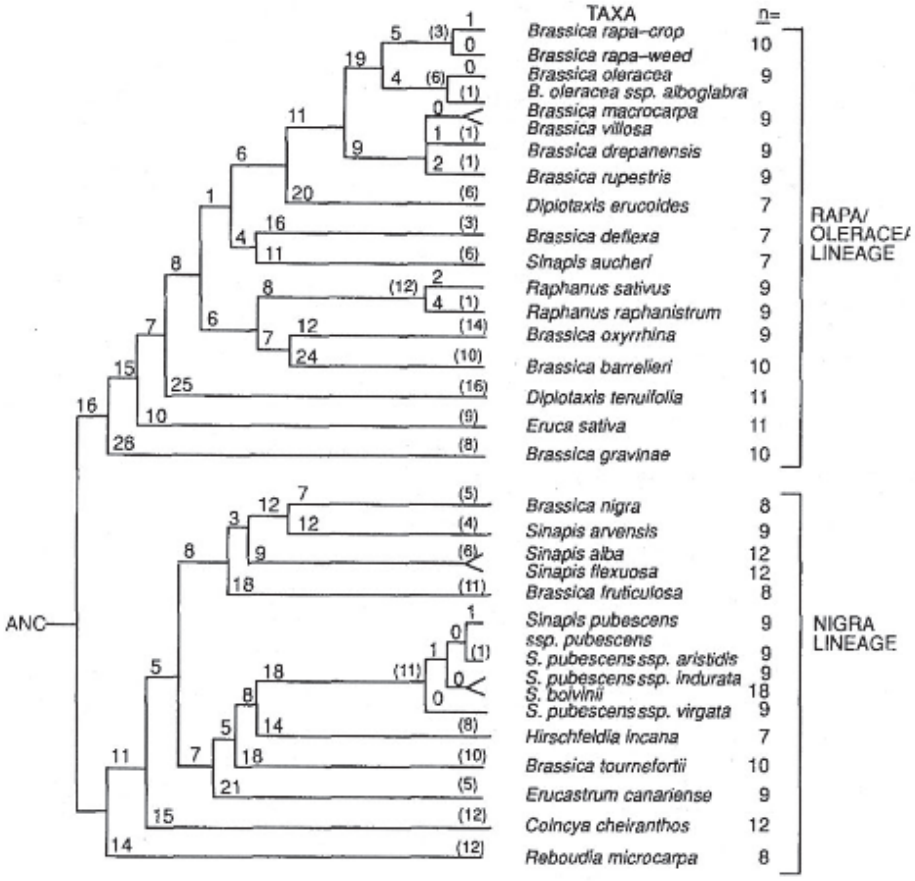
Introduction

There are few areas of the world where members of the family Brassicaceae are totally absent. The exceptions are parts of the tropics, were the family is thinly represented, but where some introduced cosmopolitan weeds have become established. The genera and species of the family occur in greatest number and diversity in the temperate zone of the northern hemisphere and in particular, the areas surrounding the Mediterranean basin and throughout the southwest and central regions of Asia (Figure 3.31; Hedge, 1976). Although the generic and specific endemism in the family is highest in the Irano-Turanian region, the centre of origin of the current subtribe Brassicinae, lies in the Mediterranean basin (Hedge, 1976).

Using chloroplast DNA restriction sites together with cpDNA probes, Warwick and Black (1991) surveyed 33 diploid taxa of the Brassicinae. The phylogenetic results indicated there were clearly two ancient and distinct evolutionary lineages within the subtribe. They found the "Nigra" lineage to include *B. nigra*, *B. fruticulosa*, *B. tournefortii*, *Sinapis pubescens*, *S. alba*, *S. flexuosa*, *S. arvensis*, *Coincya cheiranthos*, *Erucastrum canariense* and *Hirschfeldia incana.* The other lineage, termed "Rapa/Oleracea", was made up of *Brassica rapa*, *B. oleracea* and subsp. *alboglabra*, the *B. rupestris-villosa* complex (*B. rupestris*, *B. drepanensis*, *B. macrocarpa*, *B. villosa*), *B. barrelieri*, *B. deflexa*, *B. oxyrrhina*, *B. gravinae*, *Diplotaxis erucoides*, *D. tenuifolia*, *Eruca sativa*, *Raphanus raphanistrum*, *R. sativus* and *Sinapis aucheri.* In the "Nigra" lineage, *B. nigra* was most closely related to the annual *Sinapis* species *S. arvensis* and *S. alba* (Figure 3.31). Only a single mutation difference was found between the crop and weedy accessions of *B. rapa* and between crop accessions of *B. oleracea* and wild accessions of *B. oleracea* subsp. *oleracea* and subsp. *alboglabra* (Warwick and Black, 1991). The weedy species *R. raphanistrum* and the crop species *R. sativus* differed by only four mutations.

Although the economically important *Brassica* species arose from ancestors in the Mediterranean region, wars and trade ensured their wide dispersal, resulting in islands of isolated environmental and selection pressure. The earliest widely distributed species were those that exhibited seed dormancy combined with useful traits. Seed dormancy allowed the introduced seed to survive long after its introduction. The fast-growing, weedy type of *B. rapa*, providing lamp oil and animal feed, and *B. nigra* as an oil and spice source, would be prime candidates. The Mission Trail in Southern California is a case in point: priests scattered *B. nigra* seed to mark the trail between the early Missions. Parts of those trails can still be seen each year as the black mustard blooms on the California hillsides.

Figure 3.31. **Phylogenetic tree for the subtribe Brassicinae, based on PAUP analyses of the chloroplast DNA restriction site/length mutations shared by two or more taxa/accessions**



Notes: PAUP is a computational phylogenetics programme for Phylogenetic Analaysis Using Parsimony that infers evolutionary trees (phylogenies), Tree length in this tree is 489 steps, consistency index is 0.491. Tree topology indicates how accessions are related, and branch length (numbers above the branches) indicates the minimal number of mutational steps occurring during the evolution of a particular taxa. Mutations unique to a given species and to the genus *Raphanus* (number indicated in brackets at the end of a branch) should be added to determine terminal branch length. ANC indicates a common hypothetical common ancestor.

Source: Warwick and Black (1991). © Canadian Science Publishing or its licensors.

B. nigra

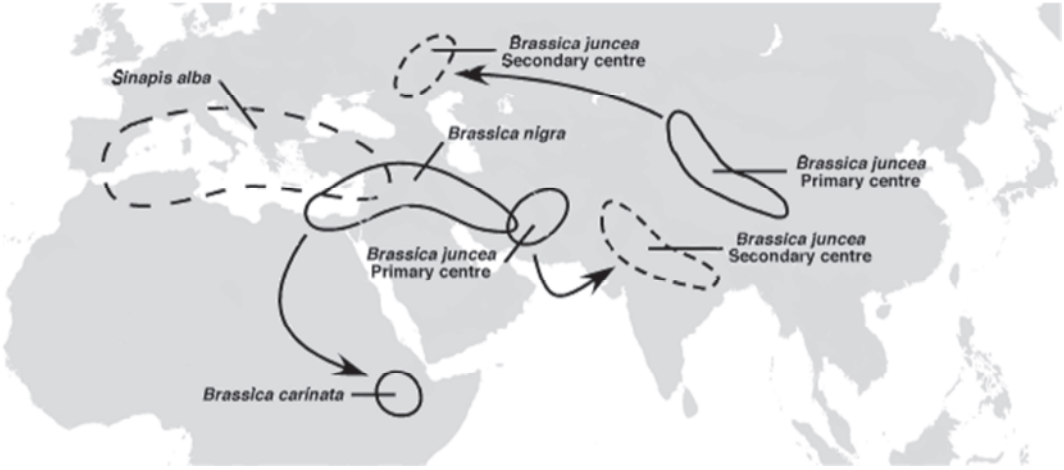
B. nigra is amongst the oldest recorded spices, being noted in the Sanskrit writings of about 3000 B.C. as "Sarshap" (Prakash, 1961). However, little is known about *B. nigra*'s true centre of origin. Hemingway (1995) placed it in Irano-Turanian, Saharo-Sindian region (Figure 3.32). However, Prakash and Hinata (1980) favoured an origin in Central and south Europe. Its use as a commercial spice ensured its very early, widespread distribution across Europe, Africa, Asia and India, and its dehiscing siliques ensured its persistence. The crop was grown for the sharply pungent chemical (allyl isothiocyanate) released when the crushed seed was mixed with a small amount of water, in the same way that *B. juncea* powdered mustard is used today. Until the 1950s *B. nigra* was the world's major source of pungent mustard, but because it shatters as soon as the pods are ripe it required hand harvesting. Thus, it was replaced in a single decade by highly pungent

B. juncea varieties well suited to mechanical harvesting. Today there is essentially no commercial production of *B. nigra* and it has become a weed of waste places in many regions. It is an introduced species to the Americas and Australia. It has never become established on the Canadian prairies although it is present throughout much of the United States.

B. rapa

B. rapa is generally believed to have originated in the mountainous areas near the Mediterranean sea rather than the coastal areas (Tsunoda, 1980). As with *B. nigra*, *B. rapa* had a wide distribution before recorded history. Indian Sanskrit literature first mentions the plant about 1599 B.C. as "Siddharth" (Prakash, 1961). Burkill (1930) proposed that the leafy vegetable forms were developed in China from the oilseed form about 2 000 years ago. Seeds of both *B. rapa* and *B. juncea* were found in excavations of the ancient village of Banpo, Xian, Shanxi Province, China that existed in Neolithic times 6 000-7 000 years ago (Liu, 1985). Turnip seeds were also found in pottery jars from the 5th century B.C. at the Yang-shao agrological site in Shensi Province (Chang, 1970). Cultivation of *B. rapa* is also mentioned in the oldest collection of Chinese poetry, Shi Jing (the book of Odes), written during the Chunqui period about 535 A.D. (Liu, 1985; Chapman and Wang, 2002). In Scandinavia, *B. rapa* seeds were being consumed as early as 350 B.C. as indicated by their presence in the stomach of the Tollund man (Renfrew, 1973).

Figure 3.32. **Evolutionary geography of *B. juncea*, *B. carinata* and *Sinapis alba***



Source: Greatly modified from Hemingway (1995).

Sinskaia (1928) proposed two main centres of origin, with the Mediterranean area as the primary centre for the European form, and Afghanistan with the adjoining portion of Pakistan as the other primary centre. Asia Minor, the Transcaucasus and Iran were considered secondary centres. Alam (1945) concluded that the Sarson and Toria types of *B. rapa*, now grown as oil crops in India and Pakistan, evolved in the Afghan-Persian area and migrated south to India and further east. McNaughton (1995a) concluded that multiple domestication of the wild forms for oilseed occurred from the Mediterranean to India about 2000 B.C. with later selection for short stature and leafiness in the Far East (China) resulting in the numerous *B. rapa* vegetable forms. Tsunoda and Nishi (1968) proposed that, with selection for increased leaf number, subsp. *chinensis*, and *japonica*

evolved and with increased leaf size and head forming, *pekinensis*, *narinosa* and *nipposinica* were selected. Cultivation of the oilseed form in Europe as a source of lamp oil is thought to have been under way by the 13th century, first as an annual form from which the biennial form was selected (Appelqvist and Ohlson, 1972). In northern Europe, turnip evolved from the biennial oilseed form through selection for bulbous roots (McNaughton, 1995a). Cartier in 1540 is credited with the first introduction of turnips into North America and more specifically to eastern Canada. They were also being grown in the Virginia colony by 1609 (Sauer, 1993). Canadian commercial production of the oilseed form began in 1943.

B. oleracea

B. oleracea has its centre of origin in the Mediterranean region (Snogerup, 1980). The wild forms of the *B. oleracea* complex still grow along the coast of the Mediterranean sea and Atlantic ocean from Greece to England (Figure 3.33). Snogerup, Gustafsson and Von Bothmer (1990) concluded from morphological and crossing studies among the wild *B. oleracea* forms, including *B. oleracea*, *B. cretica* Lam., *B. bilarionis* Post., *B. insularis* Moris., *B. villora* Biv., *B. incana* Ten., *B. macrocarpa* Guss. and *B. montana* Pourr., that these species should be considered subspecies of *B. oleracea* along with the cultivated forms. These conclusions were confirmed by Von Bothmer, Gustafsson and Snogerup (1995) through a crossing programme involving ten wild taxa and six major cultivated forms. Snogerup, Gustafsson and Von Bothmer (1990) reported that all wild forms of the *B. oleracea* complex were suffrutescent perennials, exhibiting no primary dormancy. They are also self-compatible and readily intercross within the group and with cultivated forms. They also identified some wild *B. oleracea* tetraploid plants and reported a higher fertility rate in F_1 hybrids between the wild *B. oleracea* and the cultivated forms than with the other wild subspecies.

Mutation, adaptation and selection within these populations yielded the present-day forms of cabbage, savoy, kales, collard, broccoli, Brussels sprouts, cauliflower and kohlrabi. The kales, several thousand years ago, were probably the first cultivated forms. They were grown as early as 600 B.C. by the Greeks while ancient Roman writers described heading cabbage and possibly kohlrabi (Thompson, 1976). De Candolle (1885) suggested cabbage was first domesticated somewhere in Western Europe by the Celts during the first millennium B.C. Support for this conclusion comes from the respective English, German and French common names “cabbage”, “kopf or kohl” and “cabus”, which are all probably derived from the Celtic word “cap” or “kap”, meaning head (Prakash and Hinata, 1980). A number of authors have theorised, but lacked the research to support their views, as to which species in the *B. oleracea* complex gave rise to the various cultivated forms (Helm, 1963; Neutrofal, 1927; Schiemann, 1932; Schulz, 1936; Lizgunova, 1959). After considerable investigation, Snogerup (1980) concluded that: 1) headed cabbages originated from west European *B. oleracea* and savoy cabbage may have resulted from introgression with other cole crops; 2) branched bush kales originated from *B. cretica* in Greece; 3) stem kales probably arose from the *rupestris-incana* complex; 4) the origin of the inflorescence kales such as cauliflower and broccoli is uncertain although Schulz (1936) provided some evidence that *B. cretica* could be the ancestor; and 5) *B. alboglabra* originated from *B. cretica* in Greece and was carried east by traders. Today, *B. oleracea* var. *alboglabra*, or Chinese kale, is among the ten most important market vegetables in Southeast Asia, including Thailand and China (Rakow, 2004). Little is known as to when forms of *B. oleracea* arrived in Asia but Schafer (1977) noted that kohlrabi was being cultivated in Tang’ times (600-900 A.D.).

Figure 3.33. **Distribution of wild "species" of *B. oleracea* in 1990**

Note: Introductions of *B. oleracea* outside its spontaneous area are not mapped.

Source: Modified from Snogerup, Gustafsson and Von Bothmer (1990).

B. napus

B. napus with its oilseed, forage and root forms is a relatively recent species. The Greeks and Romans knew of the Swede or rutabaga root crop, but reference to these forms does not appear in the ancient literature. Although Prakash and Hinata (1980) state that no wild *B. napus* populations have been found, Linné reported wild forms growing on the beaches of Gothland (Sweden), the Netherlands and Britain (cited by De Candole, 1885). Since the species is the result of an interspecific cross between a plant or plants of *B. rapa* and the *B. oleracea* complex, it could only have arisen in the Mediterranean or the European west coastal regions, where the two species were growing in close proximity (Figure 3.33). Olsson (1960) suggested that *B. napus* could have arisen several times by spontaneous hybridisation between different forms of *B. rapa* and *B. oleracea.* Evidence from chloroplast and mitochondrial DNA suggests that *B. montana* might be closely related to the maternal prototype that gave rise to *B. napus* (Song and Osborn, 1992). That *B. oleracea* was the maternal parent is supported by both Erickson, Straus and Beversdorf (1983) and Ohkawa (1986). However, Flannery et al. (2006), using SSR (simple sequence repeat) *Brassica* plastid markers, noted that *B. rapa* always grouped with *B. napus* and concluded that *B. rapa* is the more likely plastid genome donor. Further, Allender and King (2010), using chloroplast and nuclear markers, concluded that it is highly unlikely that *B. oleracea* or any of the C genome species are closely related to the maternal progenitor of most *B. napus* accessions. They suggest that a *B. rapa* strain from northern Italy called "spring broccoli raab" may be the closest extant relative of the *B. napus* maternal ancestor. However, the data also suggest that the interspecific cross may have occurred more than once, with *B. napus* having multiple origins. Thus, the

Swede or rutabaga could have originated in medieval gardens where turnips and kale grew side by side (McNaughton, 1995b). There is general agreement that the winter or biennial form of *B. napus* originated in northern Europe. On the other hand, forage rape almost certainly evolved from the oilseed form.

Cultivation of oilseed rape in Europe was under way by at least the Middle Ages (Appelqvist and Ohlson, 1972). It is only in relatively recent times that *B. napus* oilseed forms have been introduced to other parts of the world (Figure 3.34). *B. napus* did not arrive in China or Japan until about 1860-70, with the coming of European traders (Liu, 1985; Shiga, 1970). European immigrants introduced the forage and root crop forms into North and South America in the 17th and 18th centuries. In China, Japan and Korea *B. napus* proved to be more productive than the indigenous oilseed forms of *B. rapa*. Today most of the oilseed rape produced in China, Japan and Korea is harvested from *B. napus* cultivars that have been bred from interspecific crosses between introduced *B. napus* and the older indigenous *B. rapa* cultivars (Shiga, 1970). *B. napus* is less adapted to the Indian sub-continent due to the short days and warm growing conditions. Commercial production of the oilseed form did not occur until 1942 in Canada and 1969 in Australia.

Figure 3.34. **Dispersal of the *B. napus* species from a proposed centre of origin**

Notes: Distribution occurred throughout Europe in the 16th century, the Americas in the 17th and 18th centuries, and China and the Far East in the 19th century.

Source: Modified from Liu (1985).

B. juncea

B. juncea appears to have a much longer history than *B. napus*, even though it is also the result of an interspecific cross (*B. rapa* × *B. nigra*). Fraction 1 protein data (Uchimiya and Wildman, 1978) and chloroplast DNA analysis established that *B. rapa* functioned as the female parent in the formation of this species (Erickson, Straus and Beversdorf, 1983; Palmer, 1988; Palmer et al., 1983; Song, Osborn and Williams, 1988a, 1988b; Warwick and Black, 1991; Yang et al., 2002). However, Qi, Zhang and Yang (2007) reported that some Chinese phenotypes may have evolved with *B. nigra* as the maternal parent. They investigated the nuclear internal transcribed spacer (ITS) regions of ribosomal DNA from 15 different Chinese vegetable phenotypes and one oilseed form (pictures of the 16 phenotypes, including 2 root forms, are provided in the publication). They found that four of the accessions, including the oilseed form, apparently had *B. nigra* as the maternal

parent, a finding at odds with the RFLP and chloroplast DNA investigations noted above. However, the difference may be related to the limited Chinese genotypes that were available to other researchers.

There has been much speculation in the literature as to the centre(s) of origin for *B. juncea.* However, Prain (1898), Sinskaia (1928) and Vavilov (1949) all agree that China, where the greatest divergence of forms occurs, is one centre of origin. In addition, Vavilov (1949) also identified Afghanistan and adjoining regions as a second primary centre. This observation was supported by Olsson (1960) and Mizushima and Tsunoda (1967) as well as Tsunoda and Nishi (1968), who found wild forms growing on the plateaus in Asia Minor and southern Iran. India and the Caucasus have also been put forward as secondary centres (Hemingway, 1995; Figure 3.32). There is strong evidence for China as a primary site. As noted in the *B. rapa* section above, *B. juncea* has a long history in China. Leafy, vegetable forms of *B. juncea* mustard are also consumed in great quantities in China and other Asian countries (Herklots, 1972; Nishi, 1980). The greatest range in leaf types occur in Sichuan Province within the varieties of *rugosa*, *japonica*, *integrifolia* and *cernua*. A root-forming type has also been selected and cultivated in northern China with the variety names of *napiformis* and *tumida* (Nishi, 1980; Chen et al., 2005).

The *B. juncea* from Afghanistan and Asia Minor is believed to have migrated south to Pakistan and India where a secondary centre of origin was established (Figure 3.32). The earliest direct reference to *B. juncea* is in the Indian Sanskrit literature about 1500 B.C., where it is mentioned as "Rajika" (Prakash and Hinata, 1980). The existence of two primary centres in China and the Middle East-India is supported by the fact that the Indian sub-continent and Chinese oilseed forms not only differ in morphological traits (Sinskaia, 1928), but also chemically and in day-length requirements. The seed from Indian *B. juncea* material contains mainly 3-butenyl glucosinolate and the crop is day neutral, while the Chinese spring-sown oilseed forms contain only 2-propenyl (allyl) glucosinolate and are long day requiring. The Chinese material also contains pure yellow seeded strains which are absent in the Indian material. The Russian material displays most of the same characteristics as the Chinese material and although it may also have resulted from an independent interspecific cross, more likely it was carried into the Russian Federation from China or Mongolia via the Northern Silk Road. Wu et al. (2009) investigated the relationships among 95 *B. juncea* accessions originating from China, France, India, Japan and Pakistan, using sequenced related amplified polymorphisms (SRAPs). They found the Chinese vegetable phenotypes formed a highly diverse group with the spring- and winter-sown oilseed forms split into two separate groupings. The winter-sown accessions exhibited more genetic diversity than the spring-sown accessions but less than the vegetable group. The SRAP markers did not provide a clear-cut separation between the Indian/Pakistan and Chinese winter-sown mustards. Srivastava et al. (2001), using AFLP markers, investigated the relatedness of oilseed *B. juncea* cultivars from Australia (2 cultivars), Canada (2), China (2), Europe (6), India (7) and Tibet (1). Their data separated the cultivars into an Indian/Chinese group and a second cluster of the remaining ones. Their findings and that of Wu et al. (2009) suggest a close relationship between the Chinese northern spring-sown oilseed cultivars and the European mustards, while the winter-sown cultivars are closely associated with the Indian form. The data from both Wu et al. (2009) and Qi, Zhang and Yang (2007) support the contention of Song, Osborn and Williams (1988b) that the vegetable and oilseed mustards had a polyphyletic origin and evolved separately.

B. carinata

B. carinata, commonly called Abyssinian or Ethiopian mustard or simply "carinata", is an amphidiploid species derived from and containing the full genomic complement of the putative parental species, *B. nigra* (black mustard) as the female and *B. oleracea* as the male (Uchimiya and Wildman, 1978; Palmer et al., 1983; Song, Osborn and Williams, 1988b; Erickson, Straus and Beversdorf, 1983). The plant is cultivated on a small scale on the Ethiopian plateau. *B. carinata* may have originated from a hybrid between kale, which is grown on the plateau, and wild *B. nigra*, which is also present. However, this species, as with others in this group, almost certainly originated in the Mediterranean basin where the two putative parental species were growing in close proximity. It is believed that the cross occurred many eons ago when the climate on the African side of the Mediterranean was moist and lush. However, as the climate of this region became dryer and hotter, *B. carinata*, together with the plant community of the region that included castor oil plant and coffee, moved to the south and became isolated in the Ethiopian highlands. Thus, Ethiopia in effect preserved the environment of the centre of origin of *B. carinata* (Figure 3.32). Farmers of northeast Africa grow the plant both for its leaves, which are plucked, boiled and eaten, and for the edible oil in the seed. The local common name for the crop is *gomenzer*. The interspecific cross that created this species does not appear to have occurred elsewhere in nature or, if it did, the progeny did not survive. There is no commercial production of this species, other than in Ethiopia and neighbouring countries where the crop is grown on small holdings or in kitchen gardens. However, the species is being investigated and bred for potential commercial production in Australia, Canada, India and Spain.

Sinapis alba

Sinapis alba has its centre of origin in the eastern Mediterranean region (Figure 3.32) and wild forms are present around most of the Mediterranean littoral (Hemingway, 1995). In China, *S. alba* appears to have been cultivated by the middle of the first millennium A.D. (Hemingway, 1995).

Reproductive biology

Generation time and duration under natural and managed conditions

Generation and flowering times are discussed in the above sections dealing with cultivation and management.

Reproduction

Floral biology[4]

The basic floral characteristics of all the *Brassica* species included in this chapter are essentially the same, differing only in flower size. The floral arrangement in *Brassica* species is typically a corymbiform raceme. Flowering is indeterminate beginning at the lowest part of the main raceme and auxiliary branches, and continuing upward. The inflorescence may attain a length of 1-2 m. The buds begin opening under the pressure of the rapidly growing petals. The process of flower opening begins in the afternoon and is all but complete very early the following morning. The stigma is receptive from three days before to three days after the flower opens (Mohammad, 1935). Day length can play a critical role in initiating bolting of the flowing stem. Species such

as *S. alba* are very day-length sensitive while some cultivars of *B. napus* and *B. juncea* are day neutral.

Both the onset of flowering and duration of the flowering period are variable and quite dependent on weather, particularly temperature. Low temperatures decrease the rate of plant development and hence the onset and rate of flowering is delayed. Low plant density results in secondary branching, thus extending the flowering period. If plants are pruned back when still green, regrowth and a second flush of flowers can be obtained. Flowers produced on regrowth are typically smaller and less productive than the first formed flowers (Downey, Klaasen and Stringham, 1980).

The flowers of the *Brassica* species are regular, bisexual and hypogenous. The differentiation of the flower proceeds through the successive development of four free sepals in two whorls, medium and transverse, six stamens, two carpels and four free diagonally placed petals (Figures 3.2 and 3.35). The flowers have one pair of lateral stamens with shorter filaments and four median stamens with longer filaments. When the anthers are a few millimetres in length, the pollen mother cells, after meiosis, give rise to the tetrads. The pollen grains are 30-40 µm in diameter and have three germination pores. The sutures of the anthers are introse in the bud stage, but the four long anthers become extrose as the flower opens (except in the *B. rapa* Yellow Sarson form where they remain introse).

Figure 3.35. **Typical flower of *B. napus***

Note: This photo shows the typical four petals with the stigma in the centre surrounded by four median stamens and a pair of shorter lateral stamens.

Source: Downey, Klaasen and Stringham (1980).

Two functioning nectaries are located at the base of the short stamens and two non-functional nectaries at the base of the pairs of the long stamens. The anthers dehisce when the petals completely unfold. The pollen is shed through two longitudinal slits on the upper side of the anthers. If the weather is warm and dry, nearly all the pollen is shed the day the flower opens. In the evening the flowers tend to close, approaching a funnel shape but open again the following morning. On the third day the flower remains almost closed and the petals and sepals begin to wilt.

Studies on pollen-tube growth indicate that fertilisation is effected within about 24 hours of pollination (Khanna and Chowdhury, 1974). The two carpels (although flowers on some plants may produce three or four carpels) form a superior ovary with a "false" septum and two rows of campylotropous ovules. After fertilisation, the ovary develops into a bivalve silique with a longitudinal septum (Figure 3.2). When the buds

are about 5 mm long, the megaspore in each ovule divides twice, producing four cells, one of which becomes the embryo sac, while the others abort. The nuclear tissue is largely displaced by the remaining embryo sac and at flower opening, the ovules mainly consist of two integuments and the ripe embryo sac.

Pollination, pollen dispersal and viability

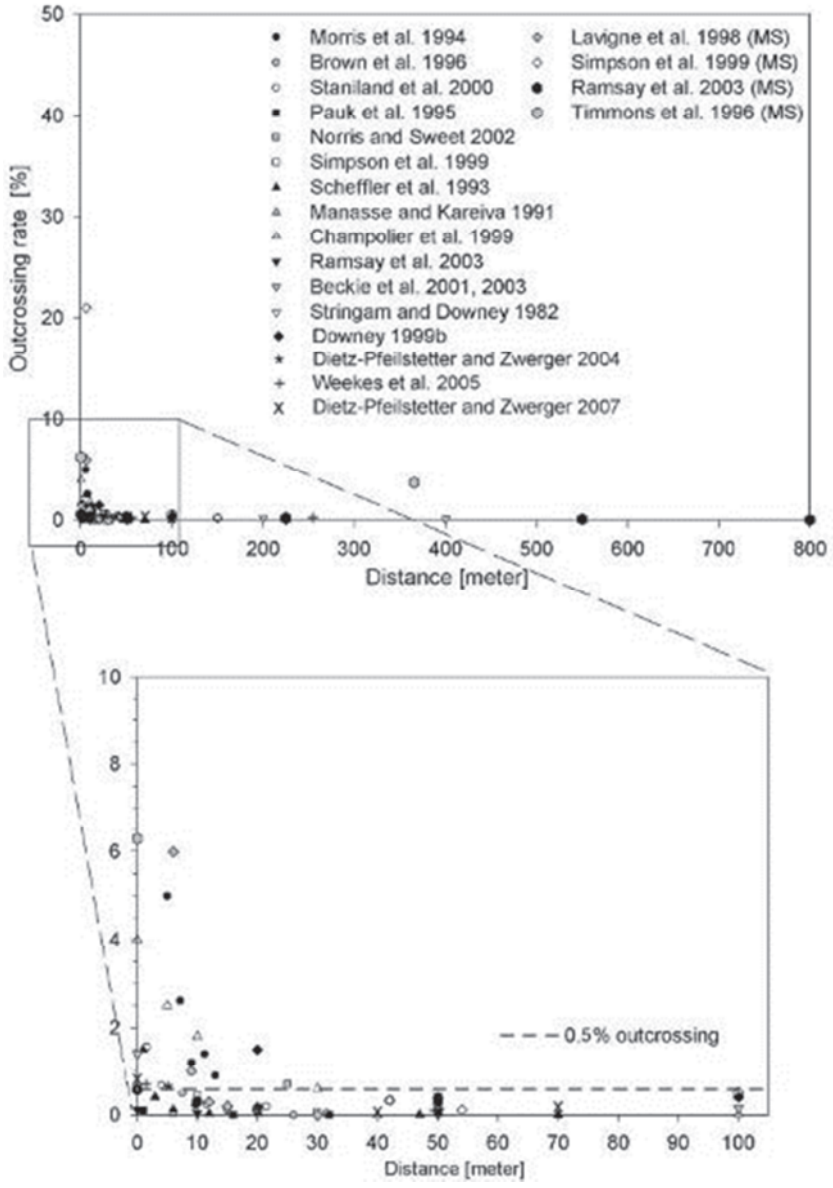
Brassica pollen, although heavy and slightly sticky, can still become airborne and float on the wind due to its minute size (30-40 µm). In addition to wind, pollen can be transferred by insects, primarily honey bees (Williams, Martin and White, 1987, 1986; Scheffler, Parkinson and Dale, 1993; Paul, Thompson and Dunwell, 1995; Timmons et al., 1995; Thompson et al., 1999). Physical contact between flowers of neighbouring plants also results in pollen dispersal while animals, including humans, passing through flowering *Brassica* fields can act as pollen vectors.

Pollen movement can be detected using pollen traps for airborne pollen or by using bait plants (either male sterile or emasculated) to detect outcrossing, usually through the use of marker genes such as herbicide resistance. An effective pollen trap, developed in Germany, combines a sampler that determines pollen deposition rate (Sigma-2 sampler) and a pollen mass filter apparatus that collects sufficient pollen for polymerase chain reaction (PCR) analysis (VDI Richtlinien, 2007). Pollen from the Sigma-2 sampler is analysed as to species and amount under a light microscope and/or by automated imaging analysis. Strategically located bee hives can also be used to monitor pollen flow whereby pollen in honey and bee bread samples is concentrated and analysed under a light microscope or subjected to PCR analysis (VDI Richtlinien, 2006).

Under natural conditions, Ranito-Lehtimäki (1995) reported a gradual decrease in pollen viability over four to five days. In the laboratory, Mesquida and Renard (1982) found pollen remained viable between 24 hours to 1 week. However, Chiang (1974) reported that *B. oleracea* pollen stored at 4ºC germinated above 20% for the first 10 days, and even after 6-7 weeks an average 4.5% of the test pollen remained viable.

The greatest pollen outflow from flowering *Brassica* fields is undoubtedly wind borne. Studies have shown that the vast majority of the pollen cloud travels less than 10 m and approximately half the pollen produced by an individual plant falls to the ground within 3 m (Lavigne et al., 1998). In a two-year study, Bilsborrow et al. (1998) reported that the pollen concentrations at 10 m was reduced by 48% and 67% compared to that recorded 2 m from the field border. McCartney and Lacey (1991) found that the amount of pollen detected at 20 m from the field border was 90% less than that recorded at the field edge. Over longer distances of 360 m and 400 m, relative to the field margin, Timmons et al. (1995) and Thompson et al. (1999) reported reductions of 90% and 95%, respectively. These findings, combined with outcrossing data, established that *Brassica* pollen follows a leptokurtic distribution i.e. the presence of pollen shows a steep decline with distance, but with a long tail containing long-distance events (Figure 3.36; Thompson et al., 1999; Staniland et al., 2000). These data indicate that at a distance of 50 m from the pollen source, the level of outcrossing is less than 0.5%, even when male sterile bait plants are used as pollen recipients (Figure 3.36).

Figure 3.36. **Outcrossing percentages as affected by distance from the pollen source**



Note: (MS) indicates male sterile bait plants used.

Source: Modified from Andersson and de Vicente (2010).

Where fields are large (>60 hectares) and/or production regions are extensive, as in Australia, Canada and India, wind is considered to be the primary pollen vector since bee populations cannot service the vast number of exposed flowers. However, in the United Kingdom and other parts of Europe where field size is small and bees and pollen beetles are abundant, insects play an important role in pollen dispersal, especially over long distances (Ramsay, Thompson and Squire, 2003; Ramsay et al., 1999; Thompson et al., 1999). Pollen distribution by insect can vary greatly depending on the production region, the environment and the experimental design (Barber, 1999; Thompson et al., 1999; Ramsay, Thompson and Squire, 2003). Honey bees visiting a new field are covered with pollen from that field after visiting about four flowers, thus

reducing the chances of cross fertilisation between plants of the new field and fields previously visited (Cresswell, 1994). Honey bees are also more efficient pollinators than wind-borne pollen over longer distances. This is to be expected since to effect fertilisation, wind-borne pollen must fall from the sky and land on an unfertilised stigma. Using published measurements of pollen dispersal, Hayter and Cresswell (2006) estimated that when bees are scarce, wind can contribute to pollination of fields 1 km distant at a level of up to 0.3%, but only up to 0.007% when bees are abundant. However, with a non-GM pollen source 500 m from a beehive and a GM field 800 m from the same hive, Ramsay et al. (1999) detected some pollen grains from the GM field in largely non-GM pollen loads. They concluded that there was either switching between fields or a long persistence of pollen grains on the bees, or there was pollen mixing within the hive. Ramsay et al. (1999) also found that honey bee colonies can forage up to 2 km from their hive, indicating a potential for pollen transfer around the hive covering an area 4 km in diameter. The maximum 4 km distance for pollen dispersal by bees corresponds closely with the 4 km maximum for the wind-borne pollen model reported by Timmons et al. (1996).

A number of models have been developed to predict the level of gene flow that might be expected among *B. napus* fields and feral populations as well as interspecific crosses with *B. rapa* (among others, Bateman, 1947a, 1947b; Lavigne et al., 1998; Colbach et al., 2005; Klein et al., 2006; Devaux et al., 2007; Graziano Ceddia, Bartlett and Perrings, 2007). However, as many biotic and abiotic factors affect gene flow, the models currently only provide an approximation. Further, the models have tended to focus on pollen dispersal and its arrival on the stigma, and have paid little attention to hybridisation and introgression.

Outcrossing in the field

Although *B. napus* is self-compatible (autogamous), pollen from neighbouring and distant *B. napus* plants compete with the plant's own pollen to effect fertilisation. There are no genetic or morphological barriers to cross-pollination among *B. napus* plants, so crossing between fields does occur (Becker, Damgaard and Karlsson, 1992; Becker et al., 1991; Rakow and Woods, 1987). The outcrossing rate within fields varies considerably, averaging between 20% and 40%, mainly depending on the environmental conditions during flowering (see Becker, Damgaard and Karlsson, 1992 and references therein). It is estimated that one hectare of spring oilseed rape produces 9.3 ± 0.5 kg of pollen each 24 hours during a 17-day flowering period with *B. rapa* fields producing 20.2 kg/ha/day, more than twice that of *B. napus* (Szabo, 1985). Most of the crossing occurs between neighbouring plants (Rakow and Woods, 1987), but long-distance pollen transfer can occur by both wind and insects (primarily bees). The measurement of pollen flow via wind or insects, or estimating the amount of outcrossing using male sterile or emasculated bait plants, provides information on the potential for outcrossing; however, it is not an accurate indicator of the actual outcrossing level that can occur between fully fertile oilseed rape crops. In reality, male sterile plants would normally be growing in association with fully fertile plants, so data from male sterile bait plants significantly overestimate the level of outcrossing that would normally be expected. Ramsay, Thompson and Squire (2003) concluded that bait plants over-estimate the outcrossing level by at least one order of magnitude.

Numerous experiments have been undertaken in recent years to determine the frequency of outcrossing that occurs between two populations of *B. napus*, with increasing distance between the pollen donor and recipient populations. The availability

of HR genes and other markers have facilitated the detection of such genes in non-HR *B. napus* plots and fields and multiple HR types in single HR crops. However, measurement of the rate of outcrossing is complex as it can vary with the experimental design, environmental conditions, cultivars grown, synchrony of flowering, insect pollinator activity, local topography, and the relative size and arrangement of the donor and recipient populations. Two types of designs have been used in these studies. In the continuous design, the recipient population surrounds the donor, while in the discontinuous designs the recipient populations are distributed in locations at increasing distances from the pollen source (Hüsken and Dietz-Pfeilstetter, 2007). Using continuous designs, over short isolation distances (0-30 m), researchers observed a rapid decline in outcrossing rates as they sampled from the field edge into the recipient population (Scheffler, Parkinson and Dale, 1993; Morris et al., 1994; Brown et al., 1996; Staniland et al., 2000; Reboud, 2003; Dietz-Pfeilstetter and Zwerger, 2009, 2004). Examples from such studies, conducted in Canada, the United Kingdom and the United States are given in Table 3.6. These results underline the importance of determining outcrossing data across the whole field and not just the level at a particular spot or distance into the field. However, using commercially sized fields in a discontinuous design, Rieger et al. (2002) found that fields situated within 100 m of the pollen source showed very little edge effect while fields far from donor sources displayed a low and variable edge effect.

Table 3.6. **Short distance pollen mediated gene flow from *B. napus* pollen donor to recipient field/plots in Canada, the United Kingdom and the United States**

Metres into recipient field	Outcrossing %	Reference, location and trial year
0.5	4.8	Scheffler, Parkinson and Dale (1993) United Kingdom, 1991
1.0	1.5	
3.0	0.4	
6.0	0.11	
12.0	0.016	
24.0	0.004	
36.0	0.001	
0.0	2.0/3.5	Morris et al. (1994) United States, 1992 east/west wind direction
0.3	1.0/1.5	
0.6	0.75/1.2	
3.0	0.65/0.6	
4.6	0.50/0.6	
0.0	0.70	Staniland et al. (2000) Canada 1994-95 Data averaged over wind directions and years
2.5	0.30	
5.0	0.10	
10.0	0.07	
15.0	0.08	
20.0	0.07	
25.0	0.04	
30.0	0.03	

The mean rate of outcrossing at various isolation distances is a valuable statistic, but the more important question might be "what is the maximum outcrossing that might be expected at various distances?" There is now considerable evidence that the highest rate of outcrossing that might be expected at 50-100 m is <0.5% and at 200 m the maximum would be <0.1% (Tables 3.7 and 3.8 and Figure 3.36).

In most of the small plot trials, arranged in a continuous design, the area occupied by the donor population is small in relation to the recipient populations, the ratio being about 1:4. This unequal availability of pollen tends to dilute the amount of donor pollen accessible to both wind and bee vectors. As a result, outcrossing rates reported for small

plot trials with isolation distances of over 30 m tend to be lower than those recorded in larger scale investigations where the area devoted to the pollen donor are substantially greater (Table 3.7).

Table 3.7. ***B. napus* to *B. napus* outcrossing rates, by isolation distances, reported from small plot trials and/or large fields**

Isolation distance	Small plot trials		Large field trials (0.05 ha or more)	
	% outcross	Reference	% outcross	Reference
30-60 m	0-0.0003	Scheffler, Parkinson and Dale (1993)	<0.01	Champolivier et al. (1999)
	0.022	Manasse and Kareiva (1991)	0.1	Simpson[2]
	0.11-0.16	Sweet et al. (1999a)	0.2	Beckie, Hall and Warwick (2001)
	0.02-0.24	Monsanto[1]	0-0.4	Downey (1999a, 1999b)
	0.05-0.33	Simpson et al. (1999) (MS)	0.1-0.65	Norris[2]
	2.1	Stringam and Downey (1982)	0.02	Ramsay, Thompson and Squire (2003)
	0.02	Staniland et al. (2000)	<1	CETIOM (2000)
	0.05	Von Ernst et al. (1998)	0.05	Wilkinson et al. (1995)
	0	Lavigne et al. (1998) (MS)	0.1-0.08	Dietz-Pfeilstetter and Zwerger (2004)
	0.02-0.05	Wilkinson et al. (1995) (MS)	0.2-0.4	Weekes et al. (2005)
	0.33	Ramsay, Thompson and Squire (2003)	0.00-0.09	Rieger et al. (2002)[3]
90-150 m	0.01-0.02	Manasse and Kareiva (1991)	0.05	Simpson[2]
	0.00-0.07	Kamler (2000)	0.1	Downey (1999a, 1999b)
	0.11-0.22	Simpson et al. (1999)	0.15	Beckie, Hall and Warwick (2001)
	0.01-0.13	Simpson[2] (FB)	0.25-0.5	Norris[2]
	0.01-0.21	Monsanto[1]	<0.5	CETIOM (2000)
	0.5	Timmons et al. (1996) (MS)	0.01-0.02	Weekes et al. (2005)
175-225 m	0.02-0.03	Simpson[2] (FB)	<0.1-0.2	Norris[2]
	0.017-0.6	Dietz-Pfeilstetter et al. (1998)	0.2	Beckie, Hall and Warwick (2001)
	0-0.9	Monsanto[1]	0.02	Ramsay, Thompson and Squire (2003)
	0.15	Scheffler, Parkinson and Dale (1995)	0.00-0.005	Rieger et al. (2002)[3]
	0.21	Ramsey, Thompson and Squire (2003) (MS)		
360-400 m	0.0038	Scheffler, Parkinson and Dale (1995)	0.1	CETIOM (2000)
	0.06	Simpson[2] (FB)	0.14	Beckie, Hall and Warwick (2001)
	0.6	Stringam and Downey (1982)	0.00-0.025	Rieger et al. (2002)[3]
	0.0	Monsanto[1]		
	3.7	Timmons et al. (1996) (MS)		
500-800 m	0.02-0.1	Ramsey, Thompson and Squire (2003) (MS)	0.00-0.053	Ramsay, Thompson and Squire (2003)
			0.001-0.03	Rieger et al. (2002)[3]

Notes: 1. Cited by Salisbury (2002). 2. (FB) indicates use of fertile bait plants, cited by Easthan and Sweet (2002). 3. Ranges estimated from published graph. (MS) indicates the use of male sterile bait plants.

Crawford, Squire and Burn (1999) estimated that a square donor plot of at least 400 m^2 would be needed if a sharp decline in the effectiveness of donor pollen is to be avoided. Positioning of the donor and recipient fields can also affect the outcrossing measurements. Ingram (2000) noted that the rate of outcrossing would be higher when the long sides of donor and recipient fields faced each other. Hüsken and Dietz-Pfeilstetter (2007) statistically analysed published outcrossing results for both continuous and discontinuous designed studies. Their data indicate that with the discontinuous design, the mean outcrossing rate between *B. napus* fields at 50 m and 100 m would be 0.11% and at 200 m, 0.05% with lower rates for the continuous design studies (Table 3.8).

Under short isolation distances, surrounding the pollen source with a synchronous flowering recipient border may be effective in reducing pollen outflow (Staniland et al., 2000; Reboud, 2003). Staniland et al. (2000) found that surrounding a spring *B. napus* pollen donor with a 15 m and 30 m wide *B. napus* border/pollen trap, separated from the pollen donor by a cultivated 1.5 m strip, reduced the outcrossing level to 0.02% at 30 m,

a level they equated to the outcrossing rates observed at 200 m by Scheffler, Parkinson and Dale (1995) (Table 3.7). They concluded that under western Canadian conditions, the current regulations, which require a 10 m wide continuous border surrounding the pollen donor, would effectively contain the majority of pollen-mediated gene flow, but would not completely eliminate gene escape.

Table 3.8. **Mean outcross percentages of pollen donor to *B. napus* recipient populations, for various isolation distances and two design classes**

Distance from pollen source (m)	Continuous design			Discontinuous design		
	Mean	Standard deviation	Number of data points	Mean	Standard deviation	Number of data points
0-10	1.78	2.48	26	0.94	0.51	10
10-20	0.33	0.45	7	0.40	0.47	8
20-50	0.05	0.05	10	0.14	0.11	11
50-100	0.04	0.04	3	0.11	0.11	11
>200	n.d.	n.d.	n.d.	0.05	0.05	6

Note: n.d. = insufficient data.

Source: Hüsken and Dietz-Pfeilstetter (2007).

Field size experiments by Reboud (2003), using 24 m borders, indicated that for short isolation distances gaps of bare ground between the donor and recipient plots/fields should be avoided. Outcrossing declined more rapidly when there were intervening plants, e.g. when the pollen donor was separated from the recipient field by a 3-4 m gap the level of outcrossing was similar to that found 1 m into the crop where the gap was zero. The same effect was noted by Dietz-Pfeilstetter and Zwerger (2004) when a bare gap between donor and recipient fields was increased from 0.5 m to 10 m.

In the large field studies, not all the factors contributing to gene flow have been controlled. Weekes et al. (2005) found the level of outcrossing to be considerably higher in winter than in spring oilseed rape (Table 3.9) while Ramsay, Thompson and Squire (2003) found the opposite to be true. They attributed the low value in the winter rape trial to poor pollinating weather in May.

However, Reboud (2003) and Dietz-Pfeilstetter and Zwerger (2009) observed that varieties used as pollen donors differed significantly in their outcrossing potential. The outcrossing values in some fields in the Rieger et al. (2002) study may have been overestimated since seed sown in the recipient fields was not tested as to the possible presence of imidazolinone-tolerant seeds (Salisbury, 2002). Such contaminant HR seed could have been present in seed sown in the recipient fields as a result of outcrossing or admixture during the breeding and multiplication of the donor and recipient varieties, as was observed in Canada by Downey and Beckie (2002) and Friesen, Nelson and Van Acker (2003).

Also, it has been suggested that outcrossing levels were underestimated due to the segregation of the two genes required to provide full tolerance to the selective herbicide. Hall et al. (2000) identified some herbicide-resistant seedlings from recipient plants situated some 650 m from an HR field. However, Downey (1999b) suggested the seed may have been transported by the farmer's swathing and harvesting equipment as observed in the Dietz-Pfeilstetter and Zwerger (2009) study.

The outcrossing percentages reported by Stringam and Downey (1982) are substantially higher than recorded for other studies listed in Table 3.7. However, it should be noted that in the Stringam-Downey trials the pollen donors were fields of >60 hectares

which resulted in the overloading of the small 42 m^2 recipient plots with donor pollen. Similar high outcrossing rates were recorded by Ramsey, Thompson and Squire (2003) where blocks of ten male-sterile plants were placed at increasing distances from a large commercial field. These results have implications for feral populations situated near commercial fields. Other observations suggest that field-to-field crossing is likely to be highest in fields just commencing or finishing flowering when a nearby field is in full bloom.

Table 3.9. **Predicted outcrossing rates for spring and winter oilseed rape at three isolation distances (with 95% confidence limits), based on 2000-03 multilocation UK field trials**

Oilseed rape type	Percent outcrossing		
	2 m	50 m	150 m
Spring	0.46 (9.97)[1]	0.02 (0.39)	0.01 (0.14)
Winter	0.76 (12.25)	0.04 (0.84)	0.02 (0.40)

Note: 1. 0.46 is the average percent outcrossing with a 5% chance that outcrossing could be as high as 9.97%.

Source: Weekes et al. (2005).

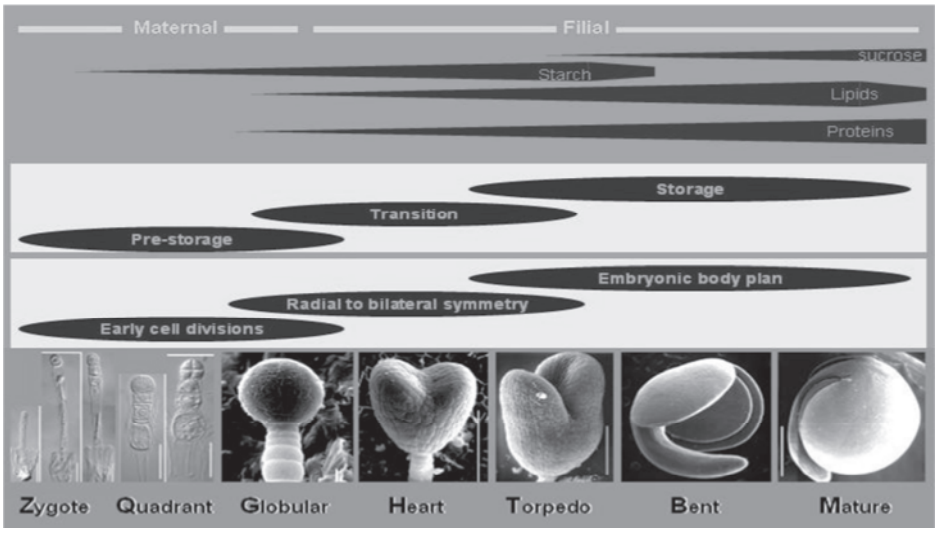
Downey and Beckie (2002) and Friesen, Nelson and Van Acker (2003) illustrated how easily pedigree seed can be contaminated in breeding nurseries. Admixture during seeding, harvesting or cleaning was also identified as a contaminant source (Downey and Beckie, 2002). These studies alerted seed companies to the problem of contamination in breeders seed stocks, leading to tighter controls (see the section "Oilseed certified seed production").

However, the present rapid development and acceptance of *B. napus* hybrid varieties dictates that certified seed-production fields will contain at least 66-75% male sterile plants. This increases the risk of outcrossing. In Canada, all hybrid producing seed fields are regulated and inspected to ensure that they are isolated from other rapeseed plants and fields by at least 800 m and free of certain *Brassica* weeds within the production field and the regulated isolation area. The isolation distance used by most seed companies for hybrid seed production of *B. napus* in Canada is at least 1.6 km (Wescott and Nelson, 2001). To further reduce the possibility of fertilisation by foreign pollen, the fields are heavily stocked with honey bees. Such fields are also saturated with leaf cutter bees (*Megachile rotundata* [Fabricius]), which have a short foraging range, to ensure the desired rapid and complete fertilisation of the male sterile female parent.

Seed development, production and natural dispersal

After fertilisation the endosperm develops rapidly, while embryo growth does not start for some days. The embryo is generally still small two weeks after pollination but by three to five weeks has almost completely absorbed the endosperm and filled most of the seed coat. Nutrient reserves for germination are stored in the cotyledons which are folded one over the other so that there is a smaller inner and a larger outer cotyledon (Figure 3.37).

Figure 3.37. **Development stages of the *Brassica napus* zygotic embryo**



Source: Courtesy Plant Biotechnology Institute, Saskatoon, Canada.

The size of seeds can be defined by both their physical dimensions and weight. The range in seed weight among the *Brassica* crop species is given in Table 3.10. Typical seeds of *Brassica* species and subspecies are illustrated in Figure 3.38. These drawings, produced by the USDA many years ago, are still valid and can be used as a starting point to distinguish many of the species and subspecies according to the reticulation patterns on the seed surface. The different patterns are the result of variation in the size of the palisade cells that form the outer cell layer of the seed coat.

Table 3.10. **Typical seed weight ranges (or averages) of *Brassica* crop plants by species and form**

Species	Form	g/1 000 seeds	Source
B. napus	Winter oilseed rape	4.5-5.5	Bengtsson et al. (1972)
	Spring oilseed rape	2.5-4.6	Elliott, Franke and Rakow (2008)
B. rapa	Winter turnip rape	3.0-4.0	Bengtsson et al. (1972)
	Spring turnip rape	2.0-3.0	Bengtsson et al. (1972)
B. juncea	Condiment and oilseed mustard*	2.5-3.0	Rakow and Rode (2009); Rakow et al. (2009)
B. oleracea	Cabbage	3.6	Ohio State University (2009)
	Broccoli	2.7-5.8	Heather and Sieczka (1991)
	Brussels sprouts	2.8	George (2009)
	Kohlrabi	3.2	George (2009)

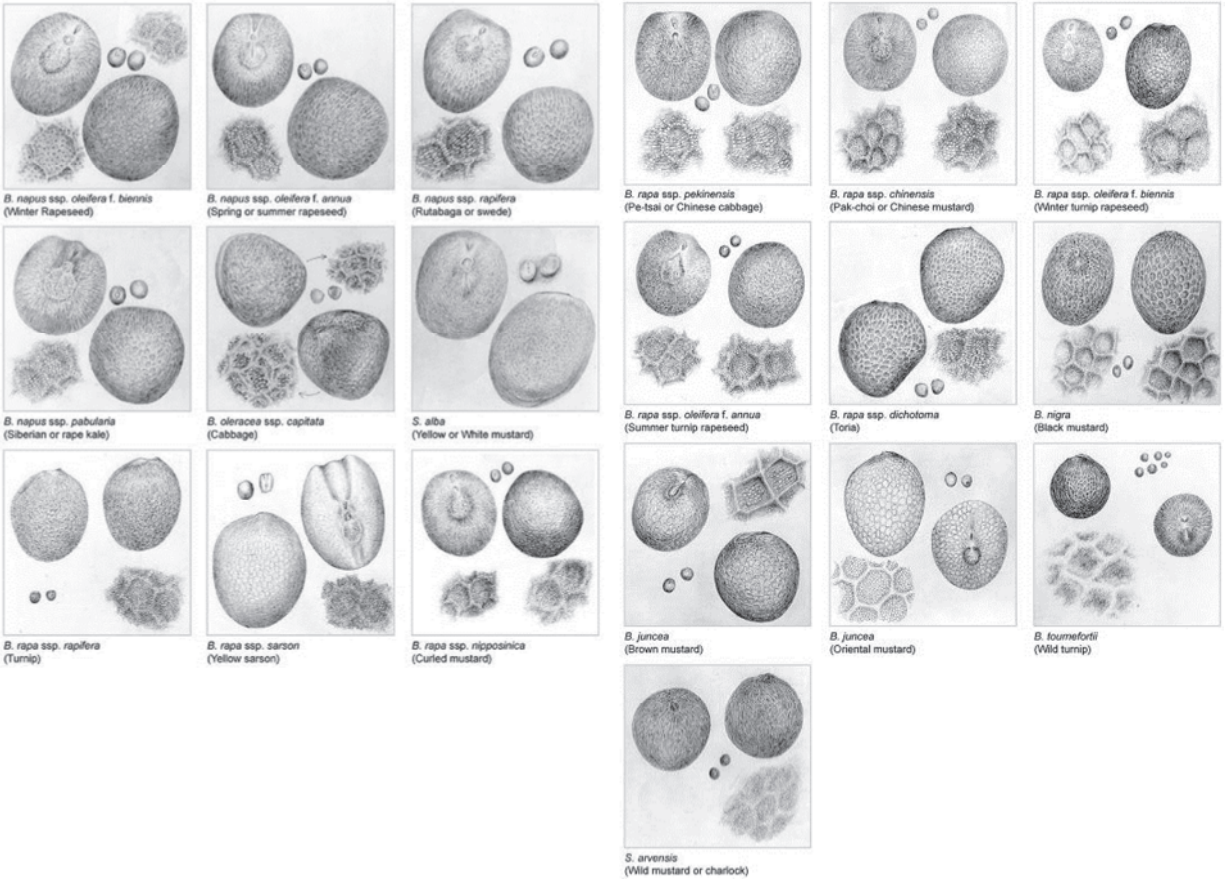
Note: * The Indian cultivar Pusa Bold has larger than normal seed at about 5.3 g/1 000.

Sources: Bengtsson et al. (1972; Elliott et al. (2008); Rakow and Rode (2009); Rakow et al. (2009); Ohio State University (2009); Heather and Sieczka (1991); George (2009).

Vaughan and Whitehouse (1971) investigated and described the seed surface and general features of some 200 Brassicaceous species including shape, colour, mucilage production and hilum characteristics. Koul, Nagpal and Raina (2000) also examined the seed surface architecture of 78 accessions from the 3 subtribes – Brassicinae, Raphaninae and Moricandiinae – at both low magnification (x80) as well as the fine structure using a scanning electron microscope (x640, x1260). They noted that the seed coat patterns at high magnification were generally species-specific. However, significant seed coat

pattern variations were found at the intraspecific level among the *Brassica* diploids, *B. rapa*, (two types), *B. nigra* (one type) and *B. oleracea* (two types), with the patterns of *B. rapa* and *B. oleracea* resembling each other. The seed coat patterns in most of the amphidiploids were intermediate to their putative parents, although one *B. carinata* and one *B. napus* accession exhibited patterns of their respective *B. nigra* and *B. rapa* parents. Thus, employing seed coat reticulations for species identification is not foolproof, but it provides a good starting point to identify the adventitious presence of foreign species in commercial seed lots.

Figure 3.38. **Distinguishing *Brassica* species by their seed coat characteristics**



Notes: The small seeds shown in each compartment are about three times their natural size. The greatly enlarged surface detail is not drawn to scale but a relative proportion is maintained throughout.

Source: USDA.

The fruit of major *Brassica* crops is a glabrous silique, which is 4-5 mm wide and can be over 10 cm long, with 2 rows of seeds lying along the edges of the replum (false septum, an outgrowth of the placenta). A silique normally contains 10-30 seeds. Three to four weeks after the flower opens, the silique attains its full diameter and length. When ripe, the silique has a tendency to dehisce and shatter, dispersing its seed. Species and varieties differ in their susceptibility to shattering. The physical forces of silique hitting silique or other plant parts causes a separation of the valve walls from the placenta, starting at the pedicel end and working toward the unattached end. The exposed

seeds attached to the placenta are soon dislodged by wind action. Threshing operations easily separate the seed from the intact siliques.

All commercially grown *Brassica* crops, as well as weedy species, tend to shatter their seed when ripe. However, the ease or degree of shattering varies among species. Within the oilseed crops, *B. napus* has the greatest tendency to shatter its seed, with *B. rapa* intermediate and *B. juncea* the least. Breeding work is developed to transfer the shatter-resistant characteristic from *B. juncea* to *B. napus* (Wang, Ripley and Rakow, 2007). The vegetable *Brassica* species follow a similar pattern. However, with the high-value F_1 seed of *B. oleracea* hybrids and the relatively small fields used for seed production, every precaution, sometimes including hand harvesting, is taken to ensure little or no seed is lost. Pod shatter is rare in the closely related *S. alba* (yellow or white mustard) species, but some loss of intact ripe pods, due to wind or mechanical action, does occur at harvest.

Seed that falls to the ground can be dispersed by wind and water as well as by birds and other animals. Because the seed is small and round it is difficult to prevent some loss during transportation of farm equipment from field to field, or from field to bin and from bin to its ultimate destination. Significant losses can occur from truck containers of uncovered oilseed rape due to the wind vortex caused by the movement of the truck. The faster the truck goes, the greater the loss. The distribution of seed from the truck vortex will depend on seed size and the direction and velocity of the wind prevailing at the time of loss. For spring *B. napus*, the distance such seed will travel at various wind speeds has been calculated (Table 3.11), although for average spring and winter *B. napus* seed, which is larger and heavier than that used to calculate the table, the wind-borne dispersal distance would be reduced.

Table 3.11. **Estimated dispersal distances of spring *B. napus* seed released from transport vehicles at various heights above adjacent fields**

Height (metres)	Wind speed in km/h					
	10	20	30	40	50	60
	Horizontal dispersal in m					
1.0	1.4	2.7	4.1	5.5	6.8	8.2
2.0	2.1	4.1	6.2	8.3	10.3	12.4
3.0	2.6	5.1	7.7	10.2	12.8	15.3
4.0	3.1	6.2	9.3	12.3	15.4	18.5
5.0	3.6	7.2	10.8	14.4	18.0	21.6
6.0	4.1	8.2	12.4	16.5	20.6	24.7
7.0	4.6	9.3	13.9	18.5	23.2	27.8
8.0	5.1	10.3	15.4	20.6	25.7	30.9

Note: 1. Estimates based on small seeds of spring *B. napus*, calculated to weigh 2.2 mg with a diameter of 1.8 mm, that are the most likely to become air borne and travel the farthest.

Source: Hertz (1999).

Seed viability, longevity and dormancy, germination, seedling establishment

Well-developed, fully mature *Brassica* oilseeds may remain viable for at least 25 years if dry seed is refrigerated in sealed containers (Ellis et al., 1994). As of 2009, seed of oilseed *Brassica*, harvested in 1977 and stored in manila envelopes at -20°C in the Saskatoon AAFC Seed Bank, had retained its high germination (Downey, personal communication). Viability of seed lost during harvest is an important factor in

determining the presence and amount of volunteer plants and populations in subsequent crops. Harvest losses can be substantial and the survival and persistence of this seed is greatly influenced by environment, seed dormancy as well as crop and field management.

Contribution of *B. napus* harvest losses to persistence

Harvest losses in the United Kingdom, when the winter *B. napus* crop is straight combined under ideal conditions, ranged from 2% to 5%, but under unfavourable harvest environments could amount to 50% (Price et al., 1996). Pekrun et al. (1998) placed these losses between 200-300 kg/ha or about 5 000-7 000 seeds/m^2. Lutman et al. (2005) in the United Kingdom and Gruber, Pekrun and Claupein (2004) in Germany recorded average harvest losses of 3 000-3 500 seeds/m^2. Similarly, French studies estimated harvest losses to be between 1.5% and 8.5% of the average yield. This calculates to 50-300 kg/ha of seed remaining on the field after harvest or 1 100-6 700 seeds/m^2 (CETIOM, 2000; Messéan et al., 2007). In Canada, Gulden, Shirtliffe and Thomas (2003a) reported that spring *B. napus*, harvest losses averaged 5.5%, or about 3 590 seeds/m^2, while Légère et al. (2001) estimated the losses at 2 000/m^2. Similarly, Warwick et al. (2003) reported spring *B. napus* harvest losses averaging 5.5%, or about 3 590 seeds/m^2. Salisbury (2002) estimated Australian losses would be similar to those found in Canada. However, a vast majority of the seed remaining in the field after harvest will not survive the first year. The *Brassica* oilseed density of the seed bank in western Canada is reported to drop ten fold in the first year and to decline slowly thereafter, due to replenishment of the seed bank by uncontrolled volunteer plants. However, where post-harvest tillage is shallow and delayed and volunteers in subsequent crops are controlled, very few plants are found four years after a spring *B. napus* crop (Gulden, Shirtliffe and Thomas, 2003b).

Seed dormancy

Seed dormancy can play an important part in determining the amount and persistence of volunteer *Brassica* plants in subsequent crops. There are two main types of seed dormancy: primary and secondary. Primary dormancy is when seed germination is prevented during the seed maturation process and for some time after the seed has been removed from its parent (Karssen, 1980/81; Hilhorst and Toorop, 1997). To overcome primary dormancy, a period of after-ripening is usually required. Secondary dormancy is a reduction in seed germinability that develops after the seed is separated from the parent plant and may, in some cases, be induced prior to the complete alleviation of primary dormancy. Primary dormancy does not occur in ripe seeds of any of the cultivated *Brassica* oilseed, vegetable or condiment crops. For seed certification status, these crops require a minimum germination of at least 90%. However, during seed maturation, germination percentages may be low in spring and winter *B. napus* but increase with maturity (Finkelstein et al., 1985) to where at harvest no primary dormancy occurs (Schlink, 1995). However, secondary dormancy can be induced in *B. napus* and cultivated *B. rapa* under certain conditions (Hails et al., 1997; Pekrun, Lutman and Baeumer, 1998; Adler et al., 1993). An exception to the rule occurs in the weedy forms of *B. rapa*, where primary dormancy is present as a recessive trait in weedy *B. rapa*. Thus, crossing between weedy and cultivated *B. rapa*, as well as between weedy *B. rapa* and *B. napus*, will produce seed that does not exhibit primary dormancy (Linder, 1998; Landbo and Jorgensen, 1997; Adler et al., 1993).

The main factors contributing to secondary dormancy of *B. napus* seed are elevated temperatures, darkness, osmotic stress and limited oxygen (Gulden, Thomas and Shirtliffe, 2004; Pekrun et al., 1997). Studies in Europe (Pekrun, Potter and Lutman,

1997; Gruber, Pekrun and Claupein, 2004) and China (Momoh et al., 2002) suggested that genotypes differ in their predisposition to undergo secondary dormancy. Indeed, it has been clearly shown that genotype is the principal factor controlling its potential in *B. napus* (Gulden, Thomas and Shirtliffe, 2004; Pekrun et al., 1997; Gruber, Emrich and Claupein, 2009; Gruber, Pekrun and Claupein 2004). Gulden, Thomas and Shirtliffe (2004) found seed size was of secondary importance, with large seed more likely to undergo secondary dormancy, while maturity and pre- and post-harvest environment had little influence. The occurrence of secondary dormancy is reduced by alternating temperatures (Pekrun, Potter and Lutman, 1997; Momoh et al., 2002), while cold stratification readily releases secondary dormancy as does exposure to continuous light (Schlink, 1995). Exogenous applications of gibberellic acid (0.2 mg l^{-1}) will also reverse secondary dormancy (Pekrun, Lutman and Baeumer, 1998).

In Germany, Gruber, Pekrun and Claupein (2004) evaluated the persistence and secondary dormancy in the seed of four winter oilseed rape varieties. They found that of the 3 000-3 500 seeds/m^2 lost during harvest, 60-75% of that seed either died or was scavenged within a few months. Similar levels of seed disappearance were observed by Gruber, Pekrun and Claupein (2003) when investigating the effect of different tillage treatments on seed persistence. Six months after harvest, no seed of the variety Artus could be detected in the soil seed bank while the other three varieties – Bristol, Liberator and Capital – respectively contributed 4.3%, 9.3% and 11% of their lost seed to the seed bank. Laboratory tests for the presence of secondary dormancy closely corresponded to that observed in the field. Gruber, Emrich and Claupein (2009) also laboratory tested seed from over 40 varieties for their tendency to undergo secondary dormancy. The seed was harvested from one site for three years and a second site for two years. They found that, over several years, varieties consistently ranked high, medium or low in percentage of seed exhibiting secondary dormancy. However, the rate of secondary dormancy varied significantly with harvest years, dry years having the lowest incidence. They concluded variety rank, rather than the actual percentage of secondary dormancy, should be used to characterise a variety. Thus, selection for varieties without secondary dormancy could be easily achieved and would greatly reduce the incidence of *B. napus* volunteers in subsequent crops. It should probably be made mandatory for all new *B. napus* varieties to be free of the secondary dormancy trait.

At shallow burial depths, *B. napus* and closely related species exhibit low seed bank persistence (Schlink, 1995; Pekrun and Lutman, 1998; Sparrow, Knight and Conn, 1990; Gulden, Shirtliffe and Thomas, 2003a). At 10 cm depth Gulden, Thomas and Shirtliffe (2004) found seed-bank populations shifted from a germinable to an ungerminable state and no seedling recruitment was observed. Masden (1962) reported that 1% of buried *B. napus* seed germinated after five years, and that trace amounts of *B. rapa* seed emerged after ten years. Schlink (1998) and Lutman, Freeman and Pekrun (2003) found that approximately 1% of *B. napus* seed in undisturbed soil could survive for ten years. Jørgensen, Pavlo Hauser and Bagger Jørgensen (2007), sampling a deep soil layer, identified viable seeds of a variety sown in the field 17 years earlier. In Canada, Beckie and Warwick (2010) reported a small population of volunteers resistant to the herbicide bromoxynil in a field that had not grown oilseed rape since the sowing of a bromoxynil-resistant variety seven years previously. The volunteers persisted in low-lying areas of the field which were too wet to plant or spray with herbicides between 2001 and 2007. No volunteers were detected in either 2008 or 2009. There is general agreement that secondary dormancy will be induced in a significant percentage of deeply buried *B. napus* seed.

Persistence

Very few seeds of oilseed rape survive in the seed bank compared with their wild relatives (Chadoeuf, Darmency and Maillet, 1998). Most seeds of the cultivated *Brassica* crops, if left on or near the soil surface, will germinate and be killed by frost or cultivation or be eaten by rodents, birds and insects. Nevertheless, a small proportion may not germinate and secondary dormancy may be induced, particularly if the seed is buried. Studies in Europe with winter *B. napus* found that when seeds were buried immediately after seed shed, 30% of the seed bank survived one winter compared to only 0.1% when seeds were left on the undisturbed soil surface (Pekrun and Lutman, 1998). Similarly for spring *B. napus* in western Canada, Gulden, Shirtliffe and Thomas (2003a) found spring seedlings, from fall-sown seeds buried at a 1 cm depth, to be only 0.1-1.5% of the original seed bank. In Canada, oilseed rape is typically grown on the same land once in four years with most of the volunteers occurring in the year following oilseed rape production. However, volunteers can occur four to five years after production (Légère et al., 2001; Simard et al., 2002; Beckie and Owen, 2007). Harker et al. (2006) found that if first-year volunteers were prevented from producing seed, the densities of volunteers in subsequent years were reduced to levels that would not require herbicidal intervention. Surveys in southern Australia by Baker and Preston (2008), where zero and minimum till are practiced, found zero germination of seed sampled from fields 3.5 years after the last *B. napus* harvest. But in Germany, Förster and Diepenbrock (2002) reported more than 0.5 plants/m^2 of winter *B. napus* three years after the last oilseed rape harvest. However, no information on timing or type of post-harvest cultivation was provided. In France, two conventional oilseed rape varieties, one of which was dwarf, were planted on fields that had grown three different HR varieties three to eight years before (Messéan et al., 2007). The percentage of GM HR seed occurring in the harvest of the conventional varieties was determined. HR seed from two of the GM varieties never exceeded 0.9% of the conventional harvested seed. However, one GM variety that was grown five years previous made up 4-18% of the conventional harvest, with the highest values occurring in the seed harvested from the dwarf variety. Since all oilseed rape volunteers were removed from the rotation crops in the intervening years, the volunteers must have arisen from dormant seed in the seed bank. The results illustrate the importance of breeding varieties without the secondary dormancy trait, not only for GM varieties, but more generally for the production of pure seed stocks and segregation of specialty oil types.

In the United Kingdom, Lutman et al. (2005) recorded a large average harvest seed loss (3 575 seeds/m^2) from four *B. napus* winter varieties grown in multiple-site, multi-year trials. Within six months, the number of seeds present declined by an average of 63%, with a slower decline recorded at 18 and 30 months. Appreciably more seeds were found on sites that were ploughed immediately after harvest compared to sites where cultivation was delayed by about four weeks. These data support the recommendations of Pekrun et al. (1998) and Gulden, Shirtliffe and Thomas (2003a) that cultivation of *B. napus* stubble should be avoided for several weeks after harvest. Regression models applied to the Lutman et al. (2005) data predicted that it would take an average of nine years to reduce the seed in the soil bank by 95%. However, other studies (Lutman, Freeman and Pekrun, 2003) indicate that the 95% reduction would occur in three to four years. Indeed, Beismann and Roller (2003) in Germany reported that no viable *B. napus* seeds could be found in soil sample cores taken from sites where transgenic plots were sown five and six years before.

Studies in the United Kingdom and Canada with winter and spring forms of *B. napus* indicate that seed bank persistence is less in lighter than heavier, clay containing, soils

(López-Granados and Lutman, 1998; Gulden, Thomas and Shirtliffe, 2004). In general, if post-harvest tillage is delayed and volunteers are controlled in the intervening years, the evidence indicates the presence of volunteers from the seed bank decline by at least 90% by the fourth year (Lutman, Freeman and Pekrun, 2003: Gulden, Shirtliffe and Thomas, 2003b; Baker and Preston, 2008). Failure to follow the above-recommended practices can extend the presence of seed bank volunteers by several years (Lutman et al., 2005).

Linder and Schmitt (1995) assessed the persistence, in field and greenhouse trials, of GM *B. napus* lines with elevated levels of stearate and laurate fatty acids in their seed oils. They concluded the risk of persistence of the high stearate and high laurate genotypes, compared with their parental non-GM types, was low. No interspecific hybrid seed could be obtained from hand-crossing GM high stearate *B. napus* × wild *B. rapa*. Greenhouse trials using seed from the high laurate *B. napus* × *B. rapa* cross indicated that such hybrids "will not possess seed bank dynamics promoting reproduction".

Genetics

Relevant detailed genetic information

Cytology

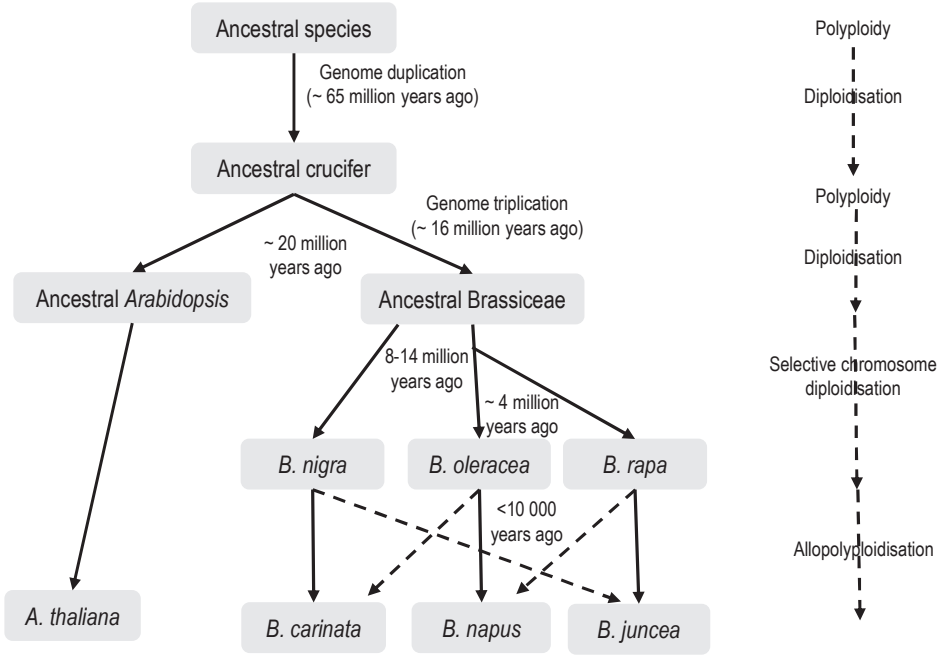
Mitotic metaphase chromosomes of the Brassicaceae are very small. Conventional cytological protocols condense *Brassica* meiotic chromosomes to tiny rods or dot-like shapes. Their small size, lack of distinctive cytological features and the difficulties of pachytene investigations make cytological identification of individual chromosomes almost impossible. Although the small chromosome size of the Brassicaceae family has limited the direct cytology approach, the sequencing of the *Arabidopsis thaliana* (The *Arabidopsis* Genome Initiative 2000), *B. rapa* (Wang, 2010; The *B. rapa* Genome Sequencing Project Consortium, 2011), *B. oleracea* and *B. napus* genomes (Bayer CropScience, 2009) are providing a much clearer picture of species interrelationships. 2014 saw the culmination of a major effort worldwide to generate "reference" annotated *Brassica* genome sequences, and some are available online for *B. napus*, *B. oleracea* and *B. rapa*. From 2015, the focus is on a range of "re-sequencing" efforts (The Multinational Brassica Genome projet, 2015).[5]

Comparative mapping, using more than 20 linkage maps for *B. oleracea*, *B. rapa*, *B. nigra*, *B. napus* and *B. juncea*, has contributed greatly to the understanding of chromosome homology and colinearity (Lysak and Lexer, 2006). In addition, great strides have been made in determining the extent of genome colinearity, and rates and modes of evolution in the Brassicaceae family. Comparative cytogenetic studies now employ a wide array of techniques including, among others, rDNA probes, nucleolus organizer regions (NORs), variation in centromeric satellite repeats, genome *in situ* hybridisation (GISH), fluorescence *in situ* hybridisation (FISH), combined with bacterial artificial chromosomes (BAC FISH) and large-scale comparative chromosome painting (CCP). Such techniques have helped to unravel the genomic evolution of *A. thaliana*, *B. oleracea*, *B. rapa*, *B. juncea* and *B. napus* as well as the time frame in which the species arose.

Research into the genome microstructure of the Brassicaceae species indicates the family originated from an ancestral karyotype that evolved after the monocot/dicot split. The ancestral karyotype had a basic chromosome number of x=4 and underwent a genome duplication some 65 million years ago (Mya) followed by diploidisation (Song,

Osborn and Williams, 1988a; Rana et al., 2004; see Figure 3.39). From this progenitor, the ancestral Brassicaceae form evolved with x=8 chromosomes (Lysak et al. 2006). This was followed by the divergence about 20 Mya of the ancestral genera of *Arabidopsis* and tribe Brassiceae. Genome triplication via allohexaploidy occurred about 14-16 Mya (Lysak and Lexer, 2006), followed by diploidisation and chromosome number reduction resulting in the evolution of the ancestral Brassiceae karyotype with x=6 chromosomes (Lysak et al., 2005; Yang et al., 1999). It is estimated that the separation of the Nigra and Rapa/Oleracea lineages took place about 7.9 Mya (Lysak et al., 2005). The *B. oleracea* and *B. rapa* divergence is estimated to have occurred about 4 Mya (Inaba and Nishio, 2002), with the interspecific crosses, forming *B. napus*, *B. juncea* and *B. carinata*, taking place less than 10 000 years ago (Song, Osborn and Williams, 1988a; Rana et al., 2004; Lysak et al., 2005).

Figure 3.39. **Illustration of major events in the evolution of selected *Brassica* species and *Arabidopsis thaliana***



Note: The dotted lines indicate the species believed to be the maternal parent in the interspecific cross.

Source: Modified from Song, Osborn and Williams (1988a; 1988b); Rana et al. (2004).

A slightly different scenario of the polyploidy events in the evolution of the Brassiceae genomes has been put forward by Mun et al. (2009), following a *B. rapa* and *A. thaliana* genome-wide comparative analysis. They suggest that a whole genome duplication (WGD) occurred twice, once about 55-63 Mya and again at 23-30 Mya, between the existence of an ancient ancestral species and the evolution of the ancestral Brassicaceous karyotype. They suggest that the second WGD resulted in the divergence of *Arabidopsis* from the *Brassiceae* linage about 13-17 Mya. This was followed by a whole genome triplication in the Brassiceae about 11-12 Mya with the divergence of *B. rapa* from *B. oleracea* taking place about 8 Mya. Their data also suggest that the allopolyploidisation that resulted in the species *B. napus* occurred only 0.7-1 Mya.

Genome mapping of *B. rapa* and *B. oleracea* has shown the gross organisation of their genomes to be highly collinear (Lagercrantz and Lydiate, 1996) but their genome size and complexity differ. The genome size of *B. rapa* is *ca.* 500 Mb compared to the much larger and more complex genome of *B. oleracea* at *ca.* 600 Mb (Arumuganathan and Earle, 1991). Comparative studies have shown that within the amphidiploids species, *B. napus*, *B. juncea* and *B. carinata*, the chromosomes within the respective putative diploid genomes have remained more or less intact (Parkin et al., 1995; Sharpe et al., 1995; Axelsson et al., 2000). DNA sequence data indicate that the A genome of *B. rapa* and the C genome of *B. oleracea* are very closely related while *B. nigra*, with its B genome, is from an earlier divergent lineage (Mizushima, 1972; Song, Osborn and Williams, 1988b; Prakash and Chopra, 1991). Song et al. (1995) reported there was rapid genome change after polyploidisation in *B. napus* and *B. juncea*, which suggests that the micro-structural changes observed in the *Brassica* lineage happened shortly after genome duplication, followed by a slow but ongoing rate of change (Rana et al., 2004).

The techniques of fluorescence *in situ* hybridisation (FISH) facilitates the integration of genetic and physical chromosome maps as it allows chromosomal location of labelled DNA probes to be directly determined (Snowdon et al., 2007). Since molecular markers can now be ordered and physical distances measured, it is possible to construct molecular karyotypes and distinguish individual chromosomes of the A and B genomes that make up *B. napus* (Fukui et al., 1998; Armstrong et al., 1998; Snowdon et al., 2002). Snowdon, Lühs and Friedt (2007) provides a consensus genetic linkage map of molecular markers for *B. napus* where linkage groups (LGs) N1-N10 correspond to the *B. rapa* A genome LGs of A1-A10, and LGs N11-N19 correspond to *B. oleracea* C genome LGs of C1-C9.

Nuclear genome size

The genome size of the *Brassica* diploids (approximately 500-700 Mbp) are more than four times that of the related Brassicaceous species *A. thaliana* (approximately 157 Mbp; see Table 3.12). The gene content of *A. thaliana* is believed to be very similar to *Brassica* diploids with more than 87% sequence identity in the coding regions (Parkin et al., 2005). Although it is believed that the diploid *Brassica* evolved through a common hexaploid ancestor (Parkin et al., 2005), the necessary genome triplication would be insufficient to explain the differences in genome size. Therefore, this important difference in genome size is likely to reflect a different rate of non-coding DNA accumulation.

Possible extent of repetitive or non-coding DNA sequences

Transposable elements (TEs) constitute a major fraction of non-coding DNA in plant species. Good estimates of TE distribution and density are presently only available for the *B. oleracea* genome, based on a partial draft genome sequence (Zhang and Wessler, 2004). Class 1 (retro) elements were the most abundant TE class with long terminal repeat (LTR) and non-LTR elements comprising the largest fraction of the genome. However, several families of class 2 (DNA) elements have amplified to very high copy numbers in *B. oleracea* compared to *A. thaliana* and have contributed significantly to genome expansion. Approximately 20% of the *B. oleracea* genome was estimated to be composed of class 1 and class 2 TEs.

Table 3.12. **Ploidy level, chromosome number, genome size and map length of *A. thaliana* and *Brassica* species of "Triangle of U 1"**

Species	Ploidy level	Chromosome number	1C nuclear DNA[2] content (Mb)[3]	Observed map length (cM)[4]
A. thaliana	2	10	157	437 and 501
B. nigra	2	16	634-765	855
B. oleracea	2	18	696-765	820-1 738
B. rapa	2	20	528-784	1 455
B. carinata	4	34	1 280-1 548	–
B. juncea	4	36	1 070-1 500	2 073
B. napus	4	38	1 127	1 441-1 765

Notes: 1. The Triangle of U is a theory about the evolution and relationships between members of the plant genus *Brassica* (*Source:* Wikipedia, the Free Encyclopedia). 2. The C value refers to the haploid DNA content of the species. 3. Data adopted by Lysak and Lexer (2006) compiled from Bennett and Leitch (2004) and Johnston et al. (2005). 1 pg = 980 Mb. 4. From Lysak and Lexer (2006), choice based on map marker coverage.

Lim et al. (2005) describe the morphology and molecular organisation of heterochromatin domains in the interphase nuclei and mitotic and meiotic chromosomes of the ten chromosomes of *B. rapa*, using DAPI staining and FISH of rDNA and pericentromere tandem repeats. They characterised the centromeric repeat sequences, which fell into two classes, CentBr1 and CentBr2, occupying the centromeres of eight and two chromosomes, respectively. The centromere satellites encompassed about 30% of the total chromosomes, particularly in the core centromere blocks of all the chromosomes. Interestingly, centromere length was inversely correlated with chromosome length.

Main genetic diversity or variability

Considerable genetic diversity has been found within the six cultivated *Brassica* species using nuclear restriction fragment length polymorphisms (RFLP) markers (Song, Osborn and Williams, 1988b). These results suggested that: 1) *B. rapa* and *B. oleracea* have multiple centres of origin; 2) *B. nigra* originated from one evolutionary pathway whereas *B. rapa* and *B. oleracea* came from another pathway; and 3) amphidiploid *B. napus* and *B. juncea* arose from different combinations of diploid morphotypes, indicating polyphyletic origins may be a common mechanism for the natural occurrence of amphidiploids in *Brassica*.

The genetic diversity within *B. napus* is considerably less than that found within either of the diploid ancestral species. This is probably a result of *B. napus* being a relatively modern species, fixed as a product of human civilisation and with no truly wild populations. Most of the diversity within *B. napus* has been introduced from its diploid progenitors. Variation in the A genome has been increased by natural *B. napus* × *B. rapa* crosses whereas variation in the C genome is more limited. Recent molecular marker analysis has identified more extreme genetic variation in exotic vegetable and fodder genotypes as well as newly resynthesised *B. napus* lines (Snowdon and Friedt, 2004 for a review). In *B. juncea* the A genome is mostly conserved and the C genome is significantly changed, more so than the considerably altered C genome in *B. carinata*. Similar genetic information, with much duplication, is contained in all three genomes (Slocum, 1989; Slocum et al., 1990; Chyi, Hoenecke and Sernyk, 1992; Jackson et al., 2000; Parkin, Sharpe and Lydiate, 2003). However, the chromosomal organisation and the genetic distribution within the genome is different (Truco et al., 1996). New high throughput and very informative simple sequence repeat (SSR) and single nucleotide

polymorphism (SNP) molecular markers are now being used routinely to expedite the introduction of novel genetic variation in *Brassica* breeding programmes.

Maternal and/or paternal inheritance of organelle genomes

Analysis of the chloroplast DNA of the cultivated diploid *Brassica* species, and their close relatives, divided the subtribe Brassicinae into two ancient evolutionary lineages (Warwick and Black, 1997), the "Nigra" lineage, which contained the diploid *B. nigra* and the related wild mustard *Sinapis arvensis*, and the "Rapa/Oleracea" lineage, which contained the diploid progenitors of *B. napus* (Figure 3.31). There has been little work studying the origins of the cultivated amphidiploids *B. carinata* or *B. juncea*. However, studies of organellar and nuclear DNA of *B. napus* and related species suggested that a species closely related to *B. montana* gave rise to the cytoplasm of both *B. rapa* and *B. oleracea* (Song and Osborne, 1992). The same study and an earlier study on chloroplast evolution in amphidiploid *Brassica* species (Palmer et al., 1983) suggested that oilseed rape (*B. napus*) evolved from multiple hybridisations between *B. oleracea* and the closely related n=9 species, *B. montana* and *B. rapa*. Some of these lineages may have been subject to introgression from post-hybridisation with their diploid progenitor.

Self- incompatibility, "S" alleles

Self-incompatibility (SI) occurs in many flowering plants and is one of the most important systems to prevent inbreeding (Takayama and Isogai, 2005). SI is defined as the inability of plants to produce functional gametes to effect fertilisation upon self-pollination or when crossed with certain relatives (De Nettancourt, 1971). Although the amphidiploid *Brassica* species, *B. napus*, *B. juncea* and *B. carinata* are largely self-pollinating (autogamous), the diploid species, with some exceptions, are self-incompatible and are obligatory out crossers. Among *Brassica* species and their close relatives, 50 out of 57 species are self-incompatible (Hinata, Isogai and Isuzugawa, 1994). The self-/non-self recognition in most species is controlled by a single locus, termed the "S locus" that inhibits the self pollen from penetrating the style when the same S-allele specificity is expressed by both the pollen and pistil. In the *Brassica* incompatibility system, over 30 *B. rapa* alleles and 50 *B. oleracea* alleles have been identified: S_1, S_2, S_3....S_{50+} (Nou et al., 1993; Ockendon, 2000). Self-compatible (S_f) alleles are also known.

Among angiosperms there are two major types of physiological SI systems: gametophytic (GSI) and sporophytic (SSI) (Briggs and Knowles, 1967). In a GSI system, the pollen reaction is controlled by the genotype of the individual pollen grain, i.e. a plant heterozygous at the S-locus would produce two possible types of pollen with each microspore receiving one of the two possible S-alleles. However, in the SSI system that is present in the Brassicinae, all pollen released by a plant has the same phenotype with respect to the compatibility reaction, regardless of the genotype of the individual pollen grain. The S-locus consists of at least three tightly linked transcriptional units arranged in pairs, with one functioning as the female determinant and the other the male. This multi-gene complex at the S-locus is inherited as one segregating unit so the gene complexes are called "*S*-haplotypes". Self-/non-self recognition operates at the level of protein-protein interaction of the two determinants (Takayama and Isogai, 2005). When the SI system is activated in a Brassicinae species, a recognition reaction occurs between the papilla cells of the stigma and the pollen (Hinata and Nishio, 1980).

There are three highly polymorphic genes involved in the SI response. The two female determinants consist of the S-locus glycoproteins (SLGs) and the S-locus receptor kinase (SRK). SRK consists of an SLG-like extracellular domain, a transmembrane domain and an intercellular serine/threonine domain. SLG and SCR expression occurs just before the flower opens, primarily in the stigma papilla cells. They also exhibit allelic sequence diversity (Takayama and Isogai, 2005). The male determinant genes named *SP11* (*S-locus protein 11*) or *SCR* (*S-locus cysteine rich*) code for the secretion of small, cystine-rich proteins, *SP11/SCR*, in anther tapetum cells and gametophytically in the microspores (Takayama et al., 2000). These genes are tightly linked and behave as a single Mendelian locus, displaying multiple allelic versions (Takayama and Isogai, 2003). The SI-response occurs when stigma and pollen share at least one allele. Upon pollination, SP11, carried in the pollen coat, penetrates the papilla cell wall and binds with SRK. The binding induces autophosphorylation of SRK starting a signalling cascade that causes the rejection of self-pollen by preventing hydration and further development of the pollen tube (Takayama and Isogai, 2005). SLGs are not present or active in all members of the mustard family (Kusaba et al., 2001). If there is a compatible reaction, the papilla cells provide moisture for pollen germination; however, with self-pollination, the absorption of water and germination are disrupted (Dickinson, 1995) and a callus deposition may occur at the attachment site (see Hinata, Isogai and Isuzugawa, 1994 for a review). If the incompatible pollen is able to germinate, the pollen tube growth is slowed or inhibited due to the inability of the pollen tube to grow through the papilla cell wall.

For vegetable crops, the National Vegetable Research Station at Wellesbourne, England, maintains a collection of all known S alleles together with their internationally accepted nomenclature (Dickson and Wallace, 1986). The genotypes of most self-incompatible *Brassica* plants will be heterozygous at the S locus, since cross-fertilisation is mandated by the self-incompatibility specificities of the S alleles present. Dominant and recessive interactions occur between *S*-haplotypes (Thompson and Taylor, 1966). The interaction is complex with the *S*-haplotypes classified as class I or class II, based on the nucleotide sequences of *SGL* and *SRK* alleles (Nasrallah, Nishio and Nasrallah, 1991). The class I *S*-haplotypes are normally dominant over class II *S*-haplotypes in the pollen. The S allele specificities of the pollen and the stigma can be co-dominant, which occurs more frequently than the dominance/recessive. Dominance/recessive relationships occur more frequently in the pollen than the stigma and are not identical for *S* alleles between the stigma and pollen (Watanabe and Hinata, 1999). Among the *SP11*/SCR alleles in Class I S-haplotypes, the dominance relationship is non-linear whereas Class II *S*-haplotypes exhibit linear dominance (Takayama and Isogai, 2003; Hatakeyama et al., 1998). The molecular mechanism of the dominance relationship in the stigma is an active area of investigation and is not fully understood (Takayama and Isogai, 2003; Fujimoto et al., 2006). Selfed seed of most incompatible plants can be obtained through bud pollination i.e. applying pollen to the stigma one to four days before the flower opens since the SGLs and SRK are not expressed until just prior to the flower opening (Takayama and Isogai, 2005). Various other methods have been utilised to overcome the SI system including stigma mutilation, stigma treatment with various organic acids, solvents, oils and ionic solutions, thermally aided pollination as well as elevated carbon dioxide treatment and momentary high temperature application (Hinata, Isogai and Isuzugawa,1994).

The SI system of *S*-haplotypes has been used by vegetable breeders to capture heterosis by producing top cross, double or three-way F_1 hybrids. However, from the

perspective of intra- and interspecific outcrossing in the field, it has been noted that the incidence of interspecific crossing in mixed species populations is likely to increase as the number of plants in the self-incompatible species decreases, due to scarcity of pollen of the same species and increasing pollen competition from other nearby species.

Although nearly all the mono-genomic *Brassica* species are self-incompatible, the natural amphidiploids species – *B. napus*, *B. juncea* and *B. carinata* – are all self-compatible (Takahata and Hinata, 1980). Okamoto et al. (2007) note that interspecific crosses between *B. rapa* and *B. oleracea* are difficult to make and, when the chromosome complement is doubled, produce self-incompatible amphidiploids plants (Beschorner, Plümper and Odenbach, 1995; Nishi, 1968). They suggest that a single mutation in a dominant *S*-haplotype could result in a self-compatible *B. napus* plant that could reproduce itself through the production of self seed. Amphidiploid plants without such a mutation would be forced to cross with one or the other diploid parent and rapidly be assimilated into one or the other parent species. Fujimoto et al. (2006) provide evidence for such mutations in *B. rapa* and *B. oleracea.*

Interspecific hybridisation and introgression

Introduction

With the introduction of genetically modified (GM) *B. napus*, the potential for inserted genes to transfer and introgress into related Brassicaceae species has been the subject of much speculation and research. There are many conditions which have to be met for such an event to occur. First, the cross of interest must occur. However, crossing success depends on a series of preconditions that include physical proximity of the parents, pollen movement and longevity, synchrony of flowering, breeding system of the parents, flower characteristics, pollen-style compatibility and competitiveness of foreign pollen. If all these pre-fertilisation conditions are met, the next series of hurdles include sexual compatibility, embryo-endosperm imbalance as well as hybrid fertility and viability in nature. In addition, the hybrid must have sufficient fitness to backcross with the recipient parent producing fertile progeny through several generations. For example, Wei and Darmency (2008) found crosses between male sterile *B. napus* and *B. juncea*, *B. nigra*, *H. incana* and *R. raphanistrum* produced only small seed, resulting in poor seedling establishment of the hybrids under field conditions. Even if all the conditions are met, introgression will not occur unless there is pairing between a chromosome of the recipient parent and a donor parent chromosome segment that carries the inserted gene. Gene transfer cannot occur in nature if any one of these requirements is not met. However, it has been speculated that strong selection pressure over many backcross generations could result in the transgene existing in a stable strain carrying an extra chromosome pair (Chèvre et al., 2001).

Modern researchers have overcome many of the natural barriers to interspecific and intergeneric crosses within the tribe Brassiceae. Techniques such as ovule, ovary and embryo culture, as well as protoplast fusion have produced hybrids that would otherwise fail due to sexual barriers. Success has also been achieved by crossing induced polyploids from one or both parents. Such techniques have been used to try to integrate important agronomic or quality traits from a foreign species into a cultivated crop. However, success using such techniques is no indication that the same result could occur through sexual crossing in nature.

The development of male sterile *B. napus* parental lines, for the production of commercial varieties, has also provided a means to investigate intraspecific, interspecific and intergeneric crossing on a field scale, without pollen competition. The results have shown that where male sterile plants were used, the frequency of interspecific crosses was significantly higher as indicated in the following species cross reports below. Thus, the presence of male sterile *B. napus* plants in commercial fields was seen as increasing the incidence and/or risk of unwanted species hybrids.

Some of the first developed hybrid *B. napus* varieties used a seed-production system termed "synthetic hybrids". Commercial production fields growing such hybrids consisted of about 80-90% male sterile hybrid plants with the remaining fully fertile plants (10-20%) providing the pollen cloud necessary to fertilise the male sterile plants in the rest of the field. Fortunately, this "synthetic hybrid" system has been replaced with new systems that reverse the ratio of fully fertile to male sterile plants in commercial hybrid fields. Today only a small percentage (15-20%) of male sterile plants may occur as off-types in these hybrid varieties. Such plants would be saturated with pollen from the surrounding *B. napus* plants, thus greatly reducing the risk of pollination by a foreign pollen source.

Chèvre et al. (2004) identified 14 species related to *B. napus* to which gene introgression from *B. napus* could be of concern to oilseed rape growing countries in Europe and North America. The reports of interspecific and intergeneric sexual crossing attempts between these species and *B. napus* are summarised in Table 3.13. Each species cross is discussed in the following paragraphs.

Warwick, Francis and Gugel (2009) have compiled a complete list of reports on interspecific and intergeneric hybridisation within the Brassicaceae that includes studies that use sexual as well as special techniques to effect a cross.

Table 3.13. **Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression**[1]

Interspecific cross	Sexual cross	Field cross	Seeds/ cross	BC ♂	BC ♀	Potential		References
						Natural cross	Introgression	
Brassica napus								
B. napus × *B. carinata*	Y	NR	8		Y	L	L	U (1935); Roy (1980, 1977); Alam et al. (1992); Gupta (1997); Rashid, Rakow and Downey (1994); Fernandez-Escobar et al. (1988); Sacristan and Gerdemann (1986); Navabi et al. (2010)
B. carinata × *B. napus*	F, Y,	NR	<1		Y	L	L	
B. napus × *B. juncea*	Y	Y	4	Y	Y	H	H	Bing, Downey and Rakow (1991); Bing et al. (1996); Alam et al. (1992); Frello et al. (1995); Jørgensen (1999); Jørgensen et al. (1998); GoshDastidar and Varma (1999); Choudhary and Joshi (1999); Kirti et al. (1995); Davey (1959); Sharma and Singh (1992); Heyn (1977); Roy (1984, 1980); Dhillon et al. (1985); Shpota and Podkolzina (1986); Sacristan and Gerdemann (1986); Wei and Darmency (2008)
B. juncea × *B. napus*	Y	Y	0.54	Y	Y	H	H	
B. napus × *B. fruticulosa*	Y	NR	0.008			VL	VL	Heyn (1977); Plümper (1995); Siemens (2002); Salisbury (2002)
B. fruticulosa × *B. napus*	Y	NR	F			VL	EL	
B. napus × *B. maurorum*	Y					EL	EL	Bijral et al. (1995)
B. maurorum × *B. napus*								
B. napus × *B. nigra*	Y	Y	0-0.09	Y	F	L	L	Bing, Downey and Rakow (1991);This et al. (1990); Brown and Brown (1996); Struss, Bellin and Röbbelen (1991); Daniels et al. (2005); Wei and Darmency (2008)
B. nigra × *B. napus*		F	0.01	F	F	VL	L	
B. napus × *B. oleracea*	Y	NR		Y	Y	VL	VL	Gupta (1997); Ford et al. (2006)
B. oleracea × *B. napus*	Y	Y				VL	VL	
B. napus × *B. rapa*	Y	Y	Many	Y	Y	H	H	Bing, Downey and Rakow (1991); Bing et al. (1996); Brown and Brown (1996); Gupta (1997); Jørgensen and Andersen (1994); Landbo and Jørgensen (1997); Mikkelsen, Jensen and Jørgensen (1996); Vijayakumar et al. (1994); Metz et al. (1997); Choudhary and Joshi (1999); Daniels et al. (2005)
B. rapa × *B. napus*	Y	Y	Many	Y	Y	H	H	
B. napus × *B. tournefortii*	Y	NR	0.69			L	L	Nagpal et al. (1996); Gupta (1997); Lokanadha and Saria (1994); Liu, Landgren and Glimelius (1996); Salisbury (2002)
B. tournefortii × *B. napus*	F					VL	VL	
B. napus × *D. catholica*	Y	NR	NR	NR	NR	VL	VL	Bijral and Sharma (1998)
D. catholica × *B. napus*	NR	NR	NR	NR	NR	EL	EL	
B. napus × *D. muralis*	Y	NR	0.28			L	VL	Bijral and Sharma (1996a)
D. muralis × *B. napus*	NR	NR						
B. napus × *Eruca sativa*	Y	NR				L	VL	Bijral and Sharma (1996b)
Eruca sativa × *B. napus*	NR	NR						
B. napus × *Erucastrum gallicum*	Y	F	0.1	Y	Y	VL	VL	Lefol, Seguin-Swartz and Downey (1997); Batra, Shivanna and Prakash (1989); Warwick et al. (2003)
Erucastrum gallicum × *B. napus*	F	F	0			VL	VL	
B. napus × *H. incana*	Y	Y	2	Y	Y	H	L	Chadoeuf, Darmency and Maillet (1998); Lefol, Danielou and Darmency (1996) ; Lefol et al. (1995, 1991); Eber et al. (1994); Chèvre et al. (1996); Kerlan, Chèvre and Eber (1993) ; Kerlan et al. (1992); Wei and Darmency (2008)
H. incana × *B. napus*	Y	Y	2×10^{-5}		Y	H	L	

Table 3.13. **Interspecific and intergeneric sexual crossing attempts, degree of success and potential for gene introgression[1]** (*cont.*)

Interspecific cross	Sexual cross	Field cross	Seeds/ cross	BC ♂	BC ♀	Potential		References
						Natural cross	Introgression	
B. napus × *R. raphanistrum*	Y	Y	Y	Y	Y	H	L	Baranger et al. (1995); Chadoeuf, Darmency and Maillet (1998); Darmency, Lefol and Fleury (1998); Eber et al. (1994); Lefol, Seguin-Swartz and Downey (1997); Rieger et al. (1999); Chèvre et al. (1998, 1997a); Wei and Darmency (2008)
R. raphanistrum × *B. napus*	Y	F	$10^{-4,-8}$	Y	Y	VL	VL	
B. napus × *R. sativus*	Y	NR	0.6	NR	NR	VL	EL	Gupta (1997); Ammitzbøll and Jørgensen (2006)
R. sativus × *B. napus*	NR	F	0	NR	NR	VL	EL	
B. napus × *S. alba*	Y	NR	Y			VL	EL	Bijral, Sharma and Kanwal (1993); Ripley and Arnison (1990); Mathias (1991); Lelivelt et al. (1993); Chèvre et al. (1994); Brown et al. (1997); Sridevi and Saria (1996)
S. alba × *B. napus*	F	NR				EL	EL	
B. napus × *S. arvensis*	Y	F	0.18			L	VL	Bing, Downey and Rakow (1991);Moyes et al. (2002); Inomata (1988); Brown et al. (1996); Sweet et al. (1997); Eastham and Sweet (2002); Daniels et al. (2005); Lefol, Danielou and Darmency (1996)
S. arvensis × *B. napus*	Y	F	F	F		EL	EL	
B. napus × *D. erucoides*	NR	NR		Y		VL	VL	Ringdahl, McVetty and Sernyk. (1987)
D. erucoides × *B. napus*	Y	NR						

Notes: Y = successful cross by hand pollination or in the field; F = cross attempted but failed; NR= not reported. Probability of crossing in nature and/or gene introgression H = high, L = low, VL = very low, EL = extremely low.

B. napus – Raphanus raphanistrum

R. raphanistrum is an economically damaging weed with a worldwide distribution but its range is limited to areas with acid soils. Hand crosses between *B. napus* and *R. raphanistrum* have produced reciprocal hybrids with a higher number of hybrids obtained with *B. napus* as the female (Kerlan et al., 1992: Chèvre et al., 1996). In France, when *R. raphanistrum* served as the female, only three hybrids have been identified, even though tens of thousands of seeds were examined (Eber et al., 1994; Baranger et al., 1995; Chèvre et al., 2000, 1998, 1997b; Darmency, Lefol and Fleury, 1998; Darmency and Fleury, 2000). Chèvre et al. (2000) estimated the hybridisation frequency to be 10^{-7} to 10^{-5} while Australian and Canadian studies reported respective frequencies of 4×10^{-8} (Rieger et al., 2001) and 3×10^{-5} (Warwick et al., 2003).

Guéritaine, Bazot and Darmency (2003) found that under field conditions the F_1 hybrid emergence was lower and slower and seedling survival significantly less than both parents. A six-year UK monitoring programme of natural populations of *R. raphanistrum* growing near fields of HR *B. napus* showed no evidence of intergeneric crossing (Eastham and Sweet, 2002). Similarly in the United Kingdom, Daniels et al. (2005) found no *R. raphanistrum* × *B. napus* plants or progeny when they sampled *R. raphanistrum* plants growing in or near four fields sown to glufosinate resistant *B. napus*. Further, no hybrids were found in a Swiss survey (Thalmann, Guadagnuolo and Felber, 2001). When *R. raphanistrum* was the female, no hybrids were found in any of these studies. The frequency of hybridisation can vary depending on the *B. napus* parental variety and the population source of *R. raphanistrum.* When *B. napus* male sterile plants were used as females, the frequency of hybrids was greatly increased, ranging from <0.2% (Chèvre et al., 2000; 1996) to as high as 90% in Danish and French field trials (Eber et al., 1994; Baranger et al., 1995; Ammitzbøll and Jørgensen, 2006). These findings would be of concern if the use of synthetic hybrids became standard, as the vast majority of plants in commercial oilseed rape fields would be male sterile. However, as indicated earlier, this hybrid system has now been phased out.

In the *B. napus* by *R. raphanistrum* cross, the majority of the F_1 hybrids had half the chromosomes of each species (ACRr, 2n=28) while one hybrid had all the chromosomes of *R. raphanistrum* and half the *B. napus* chromosomes (RrRrAC, 2n=37) (Chèvre et al., 2000). Thus, the fertility of the hybrids is very low (Baranger et al., 1995; Chèvre et al., 1998, 1996; Darmency, Lefol and Fleury, 1998; Pinder et al., 1999; Thalmann, Guadagnuolo and Felber, 2001; Warwick et al., 2003). However, Rieger et al. (2001) reported two fertile amphidiploids hybrids with a genome complement of AACCRrRr, 2n=56. Chèvre et al. (2000) also reported four fertile amphidiploids but questioned their genetic stability due to the presence of univalents and multi/quadrivalents at meiosis. The fitness of F_1 hybrids produced on *B. napus* male sterile plants was assessed in the field by Guéritaine, Bazot and Darmency (2003). They found that the hybrids were slower to emerge and less likely to survive than either parent, particularly when subjected to crop competition. The hybrids also flowered later than either parent, which limited the opportunities for backcrossing to *R. raphanistrum.* It should also be noted that if crossing between these species were to occur, it would most likely take place in a field of oilseed rape. Thus, most of the crossed seed would be harvested and only a very small proportion of the original hybrid seed would remain (Rieger et al., 2001). The few surviving hybrids would germinate among *B. napus* volunteers with backcrosses to *B. napus* much more likely than with wild radish.

When *B. napus* herbicide resistant (HR) hybrids were surrounded by *R. raphanistrum* plants in the field, the seed set was less than one seed per hybrid plant (Darmency, Fleury and Lefol, 1995). Despite the low fertility and poor fitness of the hybrids, the fertility and fitness of the backcross progeny improved with each backcross generation but the percentage of HR plants decreased (Chèvre et al., 1999, 1998, 1997b; Darmency, Lefol and Fleury, 1998; Benabdelmouna et al., 2003; Guéritaine, Bazot and Darmency, 2003). In each generation the progenies were selected for herbicide tolerance and only HR plants advanced to the next backcross (BC). None of the HR plants in the BC_3 to BC_5 had the chromosome number of *R. raphanistrum* (2n=18) indicating that no genomic introgression had occurred (Chèvre et al., 1998; Guéritaine et al., 2002). Backcrossing to *R. raphanistrum* was continued up to BC_7 followed by random mating and selection pressure in generations (G) G8 through G11 (Al Mouemar and Darmency, 2004). Root tip cytology of HR G9 plants established that all 32 plants were either carrying extra chromosomes or, as indicated by the non-Mendelian segregation of the progeny, did not have the HR gene stably introgressed into the *R. raphanistrum* genome. The authors concluded that "the prospect of stable introgression of herbicide tolerance to wild radish in nature seems remote".

B. napus – B. rapa

B. rapa, a widespread weed of cultivated and disturbed lands, is also grown as a vegetable and oilseed crop. The weedy type differs from the cultivated oilseed form only in the primary seed dormancy trait. Plant breeders of *B. rapa* and *B. napus* have known for many years that these two species readily cross in nature and they were not surprised that natural interspecies gene flow was demonstrated in several countries, including Denmark (Landbo, Andersen and Jørgensen, 1996; Hansen et al., 2001), Canada (Warwick et al., 2003; Beckie et al., 2003; Yoshimura et al., 2006), the United Kingdom (Daniels et al., 2005; Allainguillaume et al., 2006), the United States (Halfhill et al., 2002) and the Czech Republic (Bielikova and Rakousky, 2001).

Normally the highest hybrid frequencies occur when individual, self-incompatible plants of *B. rapa* are present in *B. napus* fields (Jørgensen et al., 1996). In the field, more hybrids are produced on *B. rapa* plants than on *B. napus* plants (Jørgensen and Andersen, 1994; Hauser, Jorgensen and Ostergard, 1997; Jørgensen et al., 1998), primarily due to their respective self-incompatible and self-compatible breeding systems. However, in reciprocal hand crosses, more hybrids per cross are found when *B. napus* is the female (Downey, Klaasen and Stringham, 1980). Natural interspecific hybridisation between *B. rapa* and *B. napus* varies widely, depending on the environment under which the plants develop and the design of the experiment, particularly the ratio of *B. rapa* to *B. napus* plants. In Danish trials, up to 95% hybrids were found in *B. rapa* progeny (Mikkelsen, Jensen and Jørgensen, 1996), while in New Zealand Palmer (1962) reported a range of 10-88%. In contrast, others in Canada (Bing, Downey and Rakow, 1991) and England (Wilkinson et al., 2000) found less than 1% hybridisation. In Canadian field experiments (two in the east and one in the west), *B. rapa* plants were grown at various positions within and alongside HR *B. napus* plots. Approximately 7% of the harvested *B. rapa* seed was found to be triploid hybrids (AAC, 2n=29) (Warwick et al., 2003). Similarly, in commercial *B. napus* fields containing sparse populations of weedy *B. rapa*, the hybrid frequency was approximately 13.6%. However, the frequency of hybrids from weedy *B. rapa* growing in a harvested corn field with HR *B. napus* volunteers was only 0.023% (Warwick et al., 2003). In New Zealand field studies with ratios of *B. rapa* to *B. napus* plants of 1:400 and 1:1, the hybrid frequencies ranged from 2.1% to 0.06% with the total

for the experiment of 0.46% (Jenkins, Conner and Frampton, 2001). A study of *B. rapa* populations growing outside *B. napus* fields in the United Kingdom found few hybrids (0.4-4.5%) in 7% of the populations, and no hybrids in the remaining 93% (Scott and Wilkinson, 1998).

Hybridisation also occurs with *B. napus* as the female; however, most of the hybrid seed that is formed will be removed from the field at harvest. Any hybrids that volunteer the following year are almost certain to be surrounded by *B. napus* volunteers. Thus, any backcrosses will quickly revert to *B. napus* form and chromosome number.

Compared to the parent species, natural interspecific hybrids have reduced fertility and poor seed set, averaging two to five seeds per pod (Jørgensen and Andersen, 1994). The survival rate of hybrid seedlings is also low, with <2% survival (Scott and Wilkinson, 1998), reducing the rate of introgression (Jørgensen et al., 1996; Sweet et al., 1999b). Interspecific vegetative and reproductive competition strongly impacts the relative and absolute fitness of the hybrids (Hauser et al., 2001). When Mikkelsen, Jensen and Jørgensen (1996) sowed interspecific hybrids within a *B. napus* population, no *B. rapa* × hybrid BC progeny were found among 2 000 offspring raised from 30 *B. rapa* plants. Further, the hybrids lacked primary seed dormancy (Linder, 1998). This may explain why Landbo and Jørgensen (1997) found interspecific hybrids in feral *B. rapa* populations, but no hybrid seed in the seed banks at those sites. Introgression of HR transgenes from *B. napus* to *B. rapa* has occurred in Europe (Jørgensen, 1999; Hansen et al., 2001; Norris and Sweet, 2002). However, no evidence of introgression was found in seed samples taken from *B. rapa* plants in the field, indicating there may be selection pressure against backcross individuals (Norris and Sweet, 2002).

The rate of introgression of a *B. napus* trait into the *B. rapa* genome will greatly depend on the selection pressure exerted on the gene (Scott and Wilkinson, 1998; Sweet et al., 1999a; Snow and Jørgensen, 1999). The introgression of a gene into the *B. rapa* genome might be slowed by positioning it in the C genome of *B. napus* but the findings of Stewart, Halfhill and Warwick (2002), where 12 independent *B. napus* transformations distributed across both the A and C genomes all generated backcrosses at similar rates, suggests this theory may not be valid. Leflon et al. (2006) found that the transmission rate of the C chromosomes depended on which C chromosome was involved, and that a gene carried on a C chromosome is less likely to be transferred in a *B. rapa* background than if it was on an A chromosome. The presence of an introgressed HR gene in *B. rapa* did not increase its fitness or weediness relative to conventional non-GM *B. rapa* including glufosinate resistant BC_3 hybrids (Snow, Andersen and Jørgensen, 1999) or BC_2F_2 glyphosate hybrids (Warwick, 2007). It should be kept in mind that if introgression of an R gene does occur, the resulting HR *B. rapa* plant(s) can be controlled with other herbicides or cultivation. In Canada, with 16 years of experience growing millions of hectares of HR *B. napus* each year, no significant agronomic problems with HR *B. rapa* have been encountered (Beckie et al., 2006).

B. napus – Hirschfeldia incana

H. incana is an important weed in some European countries and eastern Australia, but not in Canada or the Indian sub-continent. Hand crosses between *B. napus* and *H. incana* produced 1.3 and 3.1 hybrids per 100 pollinations when *H. incana* and *B. napus*, respectively, were used as the female (Kerlan et al., 1992). In the field, when male sterile *B. napus* was used as the female, 1.9 hybrids were recorded per pollinated flower (Eber et al., 1994). However, in three years of field trials, isolated *H. incana* plants

growing in *B. napus* plots only produced 0.6 hybrid seeds per plant. Most F_1 plants had reduced fitness with seedling emergence over three years being <1% (Chadoeuf, Darmency and Maillet, 1998). However, some hybrids were at least as competitive as the wild parent (Eber et al., 1994; Lefol, Fleury and Darmency, 1996).

When F_1 plants were backcrossed to *H. incana* and only HR progeny were selected for further backcrossing, fewer seeds were produced in each generation. BC_3 produced only one seed with no viable seeds obtained in BC_4 (Darmency and Fleury, 2000). It is suggested that a *H. incana* gene inhibits homeologous pairing, resulting in an expulsion of *B. napus* chromosomes (Kerlan et al., 1992; Lefol, Fleury and Darmency, 1996). Thus, although interspecific F_1 hybrids will frequently occur in areas where *H. incana* is prevalent, their persistence will be short and the possibility of gene introgression from *B. napus* remote.

B. napus – B. juncea

B. juncea is primarily a crop plant grown in China, the Russian Federation and on the Indian sub-continent as a major source of edible oil, and in Canada and a few other countries as a condiment crop. However, it is present as a weed in parts of Europe and Australia. Since *B. juncea* (AABB) and *B. napus* (AACC) have a common genome, the chance of interspecific crossing is enhanced. In Canadian co-cultivation experiments, Bing et al. (1996) identified five interspecific hybrids in seed harvested from 469 *B. napus* plants and 3 out of 990 plants when *B. juncea* was the female. Jørgensen et al. (1998) noted that as the ratio of *B. juncea* to *B. napus* plants increased from 1:3 to 1:15, the hybridization frequency on *B. juncea* plants decreased from 2.3% to 0.3%. Warwick (2007) reported gene flow from HR *B. napus* to neighbouring fields of *B. juncea* at a rate of 0.245% at the adjacent *B. juncea* field border and 0.030%, 0.021% and 0.005% at 50 m, 100 m, and 200 m, respectively.

The viability of F_1 pollen is reported to be low (18-26%) (Frello et al., 1995; Choudhary and Joshi, 1999; GoshDastidar and Varma, 1999), but spontaneous backcrossing with improved fertility has been reported (Alam et al., 1992; Bing, Downey and Rakow, 1991; Bing et al., 1996; Jørgensen, 1999). Given this background of results, the introgression of genes from *B. napus* could be expected to occur where these two species are widely grown.

B. napus – Sinapis arvensis

S. arvensis is a serious weed in all oilseed rape growing countries. In a five-year study of *S. arvensis* growing in and around GM *B. napus* crops in the United Kingdom, Sweet et al. (1997) and Norris et al. (unpublished, cited in Eastham and Sweet, 2002) failed to detect any hybridisation with *S. arvensis*. Also in the United Kingdom, Daniels et al. (2005) tested 60 768 progeny from 818 *S. arvensis* plants, growing in or close to 23 glufosinate resistant *B. napus* fields. No resistant plants were found in the parents or their progeny. Similarly, Warwick et al. (2003) found no interspecific hybrids among 43 828 *S. arvensis* progeny from plants growing in HR *B. napus* fields in western Canada. Bing et al. (1996) also found no hybrids in Canadian co-cultivation experiments involving the assessment of 7 500 *S. arvensis* seeds. Similar results were reported from UK trials where 9 688 *S. arvensis* seedlings were screened (Moyes et al., 2002) and in France, Lefol, Danielou and Darmency (1996) found no hybrids among the 2.9 million *S. arvensis* seeds tested. However, when male sterile or emasculated *B. napus* plants were pollinated with *S. arvensis* pollen, either naturally or artificially, a small number of hybrids were obtained. Chèvre et al. (1996) found 0.18 hybrids per 100 pollinations while

Lefol, Fleury and Darmency (1996) detected 6 hybrids in 50 000 seeds analysed. In hand crosses using *S. arvensis* females from different UK and French populations, Moyes et al. (2002) detected one completely sterile hybrid. No such hybrid had previously been reported without embryo rescue or ovule culture (Inomata, 1988; Kerlan et al., 1992; Bing et al., 1996, 1991; Chèvre et al., 1996; Lefol, Fleury and Darmency, 1996). All hybrids produced were weak, largely or completely sterile, and unlikely to survive in nature (Moyes et al., 2002). None of the hybrids were able to backcross to *S. arvensis*.

Daniels et al. (2005) identified a single plant in the United Kingdom that they believed to be a *S. arvensis* × *B. napus* hybrid. It was growing in a patch of *S. arvensis* plants adjacent to a field that had grown a crop of glufosinate resistant *B. napus* the previous year. The hybrid classification was based on a null reaction to the application of glufosinate to a single leaf followed by a positive DNA test for the glufosinate resistance gene. However, only morphological characteristics were used to classify the plant as a *S. arvensis* × *B. napus* hybrid. The lack of any information on chromosome number and/or markers, and in the light of previous studies, the question remains as to whether the plant was indeed a *S. arvensis* × *B. napus* hybrid rather than another interspecific cross such as *B. rapa* × *B. napus*. In the words of the report's reviewer "such a finding needs to be interpreted with caution."

Despite the one hybrid produced by Moyes et al. (2002) on an emasculated *S. arvensis* plant, there is general agreement among researchers that the possibility of gene flow between *B. napus* and *S. arvensis* is extremely low (Moyes et al., 2002) to non-existent (Downey, 1999a; 1999b).

B. napus – Raphanus sativus

R. sativus is a vegetable crop in many parts of the world, but when grown for seed it can escape from cultivation and colonise disturbed sites such as roadsides, fields and coastal sand dunes (Snow, Uthus and Culley, 2001). Daniels et al. (2005) reported flowering of *R. sativus* plants could coincide with either winter or spring *B. napus*. In *R. sativus* plants growing in or near a field of glufosinate resistant *B. napus* in the United Kingdom, Daniels et al. (2005) found no *R. sativus* × *B. napus* hybrids. Further, progeny from the sampled *R. sativus* plants were all susceptible to glufosinate. Hybrids between *B. napus* and *R. sativus* have been obtained in several studies with the aid of ovule culture or embryo rescue (Lelivelt et al., 1993; Paulmann and Röbbelen, 1988; Sundberg and Glimelius, 1991; Metz, Nap and Stiekema, 1995; Takeshita, Kato and Tokumasu, 1980) and also by hand pollination (Gupta, 1997). All artificially produced hybrids were male sterile. However, in natural crosses Ammitzbøll and Jørgensen (2006) obtained an average of 0.6 seeds per pod when male sterile *B. napus* plants were used as the female and a radish cultivar as the pollen parent. Huang et al. (2002) in hand crosses also produced many hybrids on Ogura male sterile plants. All seeds produced proved to be F_1 triploid hybrids with low pollen fertility (0-15%). It is highly probable that the presence of radish cytoplasm in the male sterile *B. napus* parent greatly facilitated *R. sativa* pollen penetration of the stigma. Further studies need to be carried out with this cross since *R. sativa* crosses easily with *R. raphanistrum* (Snow, Uthus and Culley, 2001).

B. napus – Erucastrum gallicum

E. gallium is a self-compatible, annual or winter annual with very small seeds. It is a minor weed of cultivated fields and waste places in many oilseed rape growing countries. Batra, Shivanna and Prakash (1989) obtained three hybrids from the cross *E. gallicum* ×

B. napus using embryo rescue. Lefol, Seguin-Swartz and Downey (1997), using reciprocal hand crosses, obtained one slow-growing *B. napus* × *E. gallicum* F_1 hybrid with pollen viability of 28%. Indications were that the F_1 would not survive in competition with a *B. napus* crop. No seed was produced when *E. gallicum* served as the female parent. The F_1 hybrid was backcrossed in all combinations and many seeds were obtained when *E. gallicum* was the male and a few when *B. napus* was the female. Backcross seed from the hybrid produced plants identical to *E. gallicum*, suggesting that the *B. napus* chromosomes were lost. A survey of 22 000 seedlings of *E. gallicum* from western Canadian *B. napus* fields yielded no hybrids, indicating that the possibility of hybridisation between *B. napus* and *E. gallicum* is very low ($<5 \times 10^{-5}$) (Warwick et al., 2003).

B. napus – B. nigra

B. nigra is a minor weed and an occasional crop in warmer, shorter day-length locations of oilseed rape growing regions. Interspecific hand crosses between *B. napus* and *B. nigra* have been difficult to obtain, with some success using oilseed rape as the female (Davey, 1959; Heyn, 1977; Diederichsen and Sacristan, 1988; Nishiyama, Sarashima and Matsuzawa, 1991; Bing, Downey and Rakow, 1991; Bing et al., 1996; Kerlan et al., 1992; Struss, Quiros and Röbbelen, 1992; Zhu, Struss and Röbbelen, 1993). The F_1 hybrids were moderately to highly sterile but a few F_2 and BC seeds were obtained (Bing, Downey and Rakow, 1991; Zhu, Struss and Röbbelen, 1993). Using controlled crosses hybridization levels were extremely low (Raybould and Gray, 1993; Scheffler and Dale, 1994). In the cross *B. napus* × *B. nigra*, Brown and Brown (1996) observed the pollen tubes of *B nigra* were short and twisted with only a few penetrating the style. No hybrids were found in natural crosses when *B. nigra* was the female (Bing, Downey and Rakow, 1991; Leckie, Smithson and Crute, 1993; Daniels et al., 2005).

B. napus – B. oleracea and *Brassica* vegetables

Gene flow from oilseed rape to *B. napus* vegetables (Swedes, rutabaga, Siberian kale) is possible since they are all within the same species. Similarly, gene flow to *B. rapa* vegetables (e.g. turnip, Chinese cabbage, etc.) is possible since they have the A genome in common. However, *B. napus* and *B. rapa* vegetables are not considered weedy. In addition, they are generally harvested prior to flowering.

Hand crosses between *B. napus* and *B. oleracea* have been successful but at a very low frequency (Chiang, Chiang and Grant, 1977) and natural crosses have only been successful with the assistance of embryo rescue (Ayotte, Harney and Souza Machado, 1987; Takeshita, Kato and Tokumasu, 1980; Quazi, 1988; Habman et al., 2010). However, amphidiploid F_1 hybrids were fertile and readily backcrossed to either parent (Sundberg and Glimelius, 1991; Kerlan et al., 1992; Chèvre et al., 1996).

No spontaneous hybrids between *B. napus* and *B. oleracea* were found in two UK surveys of wild *B. oleracea* populations (Scheffler and Dale, 1994; Wilkinson et al., 2000). However, a later UK survey of two wild *B. oleracea* populations, growing within 25 m of *B. napus* fields, identified one triploid F_1 hybrid and nine introgressants based on flow cytometry and crop-specific microsatellite markers (Ford et al., 2006). The fertility of these plants has not been reported.

B. napus – Sinapis alba

S. alba is commercially grown as a condiment crop but weedy forms occur in the Mediterranean region and in some countries where *S. alba* is used as a green manure crop. The cross *B. napus* × *S. alba* is difficult to make even with hand pollination, usually requiring embryo or ovule culture (Ripley and Arnison, 1990; Mathias, 1991; Bijral, Sharma and Kanwal, 1993; Lelivelt et al., 1993; Chèvre et al., 1994; Brown et al., 1997; Sridevi and Saria, 1996). No field crosses have been reported (Daniels et al., 2005) and the possibility of such an occurrence is very low.

B. napus – Other weedy species

Hand crosses have been made in enclosed environments between *B. napus* and a number of weedy species within the tribe Brassiceae (e.g. *B. fruticulosa*, *B. tournefortii*, *B. maurorum*, *Diplotaxis muralis*, *D. tenuifolia*, *Rapistrum rugosum*, *Eruca sativa*) while protoplast fusion and embryo or ovule rescue have produced F_1 plants in *B. napus* crosses with *B. oxyrrhina*, *B. barrelieri*, *B. elongata*, *B. gravinae*, *B. souliei* and *Diplotaxis tenuisiliqua*. No field interspecific or intergeneric hybrids have been reported between *B. napus* and the above species (Salisbury, 2002).

Ecology

Interactions in natural and agricultural ecosystems

Glucosinolates and their ecological interaction

Virtually all plants of the Brassicaceae produce sulphur compounds called glucosinolates (Kjaer, 1960). Although there are some 250 of these allelochemicals that occur in 16 botanical families of the order Brassicales (Verkerk et al., 2009), only about 20 are commonly found in *Brassica* species (Sarwar and Kirkegaard, 1998). A single species will usually contain significant amounts of 4 different glucosinolates but a single plant may contain as many as 15 different glucosinolates. They are present in varying amounts in all tissues of the plant and directly or indirectly impact their biological environment (Brown and Morra, 1997). They are the source of the flavour and odour of the *Brassica* vegetables and the hot component in mustards. The kind and quantity of glucosinolate varies within and among species and even between stages of plant development as well as between plant parts e.g. cotyledon, leaf, root, flower buds and seed. The highest concentration of glucosinolates is normally found in flower buds and seeds.

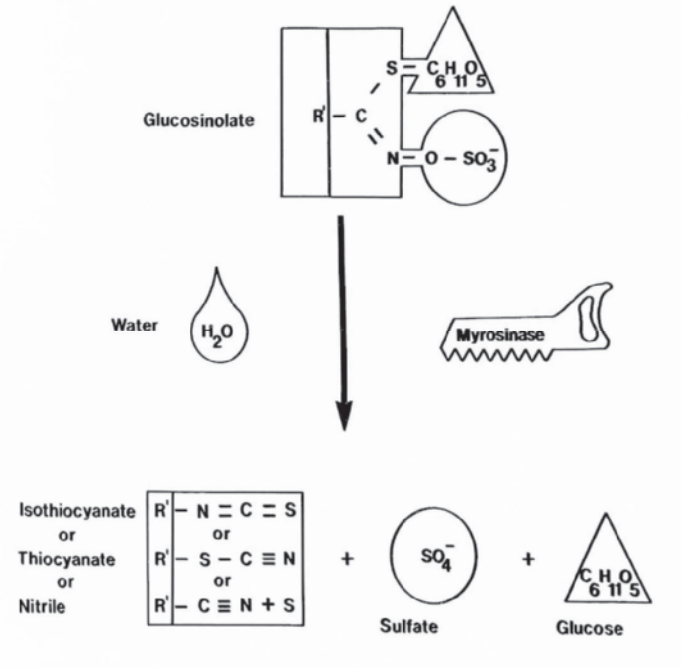
All glucosinolates have the same basic structure consisting of a β–D-thioglucose group, a sulphonated oxime group and a side chain "R", derived from one of the amino acids, methionine, phenylalamine, tryptophane or a branched-chain amino acid (Figure 3.40). Glucosinolates accumulate in plant cell vacuoles. They can be broken down (hydrolyzed) by the enzyme myrosinase which is located separately in the idoblast cells. When plant cells are crushed or broken, and moisture is present, the glucosinolates and myrosinase are released and the enzyme catalyses the hydrolysis of the glucosinolates into glucose, sulphate and thiocyanates, isothiocynates and nitriles plus sulphur (Figure 3.40). The intact glucosinolates have little biological activity but their thiocyanate and isothiocynate breakdown products have broad biocidal activity (Brown and Morra, 1997).

The glucosinolates serve as an advance-prepared system of protection that is activated only when plant tissue is damaged by a disease or insect attack. The destruction of the plant cells results in the hydrolysis of the glucosinolates by the myrosinase enzyme, thus releasing the volatile isothiocyanates that have a wide spectrum of anti-microbial effects and act as attractants or repellents to some insects and herbivores (Vašák, 2002; Brown and Morra, 1997; Fenwick et al., 1983).

Brassica crops are also used as biofumigants based on the release of the bioactive isothiocyanates in the soil when seed meal amendments or green manure are incorporated, or *Brassica* crops are used in the rotation (Brown and Morra, 1997). It is also suspected that the volatile isothiocyanates, from residue of *Brassica* crops, result in inhibitory effects on some subsequent crops (see the section on "Allelopathy").

The glucosinolates also impact on the health and nutrition of animals and humans as well as the quality and usefulness of products from *Brassica* crops. These aspects are discussed in the following section.

Figure 3.40. **Glucosinolate chemical structure and enzymatic breakdown products formed in broken *Brassica* plant cells with moisture**



Damaging insects

Brassica species are important components of temperate climate ecosystems. They provide forage for many insects as well as wild life. The complex of insects that feed upon the Brassicas is one of the important factors limiting the production of commercial *Brassica* crops (Ekbom, 1995; Lamb, 1989). Brassicaceous plants produce a family of sulphur compounds called glucosinolates, whose breakdown products are attractants and stimuli for feeding and oviposition but, on the other hand, act as deterrents or toxins for herbivores not adapted to plants of the Brassicaceae. A list of insects important to *Brassica* plants is given in Table 3.A1.1 of the annex. Some of the more important insects are discussed below.

Phyllotreta spp. – Flea beetles

Flea beetles feed on spring-sown seedlings and in some years the second generation may attack green foliage and pods in the fall. Several species of flea beetles occur in different *Brassica*-growing areas of the world. Damage by these small beetles is characterised by feeding holes in cotyledons and first true leaves and is most severe under warm, dry conditions. Some Brassicaceous species (e.g. *Sinapis alba*, *B. villosa*) avoid damage due to the presence of hairs (trichomes) on cotyledons, leaves and stems. Attempts at biological control have not been successful, but research is underway to develop *B. napus* plants expressing large numbers of trichomes as a means of defence (Gruber et al., 2006). The primary control measure is insecticidal seed dressings.

Psylliodies chrysocephala – Cabbage stem flea beetle

This beetle is one of the most important pests of oilseed rape in Europe (Ekbom, 1995). Eggs, laid by adults in the soil at the base of seedlings, produce larvae that eat into leaf stocks and later into the stem and base of the biennial plants, where the larvae overwinter. Feeding damage results in weakened plants, resulting in reduced yield and winter kill. Control is dependent upon insecticide sprays.

Ceutorhynchus spp. – Stem weevils

Both *C. napi* and *C. quadridens*are are important pests in continental Europe. The weevils overwinter as adults and lay their eggs on leaf petioles of overwintered *Brassica* plants. The larvae eat into and feed in the stems resulting in weakened and broken plants. Insecticide sprays are used for control.

Aphid species

Three species of aphids can be of economic importance on Brassicaceous plants (Ekbom, 1995). *Lipaphis erysimi* and *Brevicoryne brassicae* prefer Brassicaceae hosts while *Myzus persicae* is polyphagous. On the Indian sub-continent *L. erysimi* is a very serious pest capable of reducing oilseed mustard yields by 50%. In temperate zones, *B. brassicae* is a common pest of vegetable Brassicas and occasionally of oilseed crops. Suction feeding causes a direct loss of vigour and yield. *M. persicae* also causes indirect damage as a vector of beet western yellow virus (BWYV) (Hill et al., 1991). Insecticide sprays can be used for control but care must be taken not to kill beneficial insects present during flowering.

Lepidoptera species

The lepidopteron pests occur sporadically and can have more than one generation per year. The eggs are laid on the leaves where the larvae feed. In Canada, a second generation of the diamondback moth (*Plutella xylostella*) may also attack pods of oilseed rape. The diamondback and *Pieris brassicae* (the large cabbage white butterfly) are also important pests of vegetable crops, where their leaf damage affects market value. Chemical control is applied where populations warrant.

Meligethes species

Pollen beetles are important pests of both spring and winter oilseed rape in Europe. Adult beetles move onto the crop from unrelated early flowering plants to feed on pollen from open flowers and to lay eggs in unopened buds. The larvae emerge and eat the

stamens, causing buds to abort. The final instar larvae fall to the ground and pupate with the new generation emerging in July into August (Ekbom, 1995). Pyrethroids are the most commonly used chemical control.

Ceuthorhynchus assimilis – Seed pod weevil

This weevil occurs in both Europe and North America. It has one generation per year, emerging from over-wintering sites in the late spring to feed on the crop. The main damage is done to the seed pods. The adults make small holes in the pods to feed on the seeds within and to lay eggs. The larvae eat their way out of the pods, drop to the ground and pupate. The weevil has only recently invaded the oilseed rape growing area of western Canada. Control is by chemical sprays.

Dasineura brassicae – *Pod midge*

The pod midge is a European pest that uses the small holes in the pods made by the seed pod weevil for oviposition. The larvae eat the developing seed and cause the pods to open, losing their seed. The larvae over-winter in cocoons in the soil, pupate in the late spring and fly to the plants to oviposition, living only a few days (Ekbom, 1995). Early spraying for the pollen beetle can provide control of pod midge and other pod pests.

Beneficial insects

The interaction between bees, both farmed and wild, and *Brassica* plants are mutually beneficial. The bees aid fertilisation and receive nectar and pollen in return. Where grown, oilseed rape and mustard provide productive bee pasture while the fertilising activities of the bees are essential for the production of hybrid seed and tend to increase seed yields of commercial fields.

Animal interaction

Succulent *Brassica* plants attract many foraging animals including rabbits, rodents and deer to name a few. Winter oilseed rape is an important winter pasture for wild deer and other animals. Ruminant animals, both wild and domestic, under certain circumstances, can become ill from grazing kale or winter oilseed rape crops (Marquard and Walker, 1995). The toxic compound responsible is dimethyldisulphide that arises from the breakdown products of glucosinolates and S-methylcysteine sulphoxide (SMCO), also known as the kale anaemia factor (Maxwell, 1981). Birds often feed on fall-germinating seedlings and on the developing seed in the pod.

Soil microbial interaction

The genetic makeup of crop plants can influence the composition of the soil microbial community in which they grow. However, the interaction between plants and their residues with the soil microflora is not well understood (Dunfield and Germida, 2004). The soil microbial communities associated with the growing of conventional spring oilseed rape (both *B. napus* and *B. rapa*) and transgenic HR *B. napus* were investigated in western Canada plot trials. The soil microflora in the plots of the glyphosate resistant variety Quest differed significantly from that found in both conventional and transgenic glufosinate resistant varieties, particularly at the flowering stage. However, although the microbial diversity was altered, the effects varied by test site and plant growth stage. In addition, the change in the microbial community was temporary as no differences were

found the following spring (Dunfield and Germida, 2004, 2003, 2001: Siciliano and Germida, 1999).

Allelopathy

There have been numerous reports of inhibitory affects by *Brassica* residues on the following planting of pasture, cereal and oilseed crops (Campbell, 1959; Bell and Muller, 1973; Rice, 1984; Mason-Sedun, Jesspo and Lovett, 1986; Horricks, 1969; Vera, McGregor and Downey, 1987). The allelopathic effects include germination inhibition, reductions in root growth, plant height, dry weight, tiller number and seed yield. Species involved in the inhibition included marrow stem kale (*B. oleracea*), oilseed and turnip rape (*B. napus*, *B. rapa*) and condiment and black mustard (*B. juncea* and *B. nigra*). The inhibiting compound(s) are leached by water from dead or decaying stems and leaves of *Brassica* vegetation. The compound(s) appear to reside in the upper soil layer for a short period and then dissipate. Mason-Sedun, Jesspo and Lovett (1986) compared the effect of water extracts from dry residues of four *Brassica* species on coleoptile growth of common wheat (*Triticum aestivum*). All residues significantly reduced grain yield, plant dry weight, plant height and tiller production, with the greatest level of inhibition resulting from *B. juncea* residues followed by *B. nigra*, *B. napus* and *B. rapa*.

Laboratory studies indicated that when stored, dry residues became less toxic over time. Waddington and Bowren (1978) found that rapeseed residue was no more toxic to barley, bromegrass or alfalfa than comparable amounts of wheat residue. Normally *Brassica* residue will have been rained on well before seeding, resulting in no inhibition. Indeed, there is good evidence that cereal crops are more productive following oilseed rape than another cereal (Almond, Dawkins and Askew, 1986). Vera, McGregor and Downey (1987) suggested that the primary cause of the observed inhibition in western Canada may be the release of a chemical compound from volunteer oilseed rape seedlings that are killed by cultivation at seeding time. The chemical was thought to be the indole glucosinolate, glucobrassicin, present in high concentrations in tissues of young seedlings (Röbbelen and Thies, 1980).

Pathogens

The *Brassica* crops and their wild allies are subject to a broad range of pathogens and adverse conditions or disorders associated with non-infectious causes. Although many of the *Brassica* species have many diseases in common, there are also significant differences in susceptibility among and within species. The *Compendium of Brassica Diseases* (Rimmer et al., 2007) provides an authorative and practical reference guide to disease problems in *Brassica* crops the world over. Colour plates and text describe the infectious diseases caused by fungi, oomycetes, bacteria, mollicutes, viruses and nematodes. In addition, non-infectious disorders such as those related to environmental effects, herbicide injury and nutritional deficiencies are also described. The American Phytopathological Society (APS) also provides a listing by common and scientific name of known *Brassica* diseases and conditions at its website as reproduced in Table 3.A1.2 in the annex (APS, 2001).

Of the many *Brassica* field crop diseases listed in Table 3.A1.2, three stand out as particularly troublesome as they are pandemic and have the potential to cause major crop injury: blackleg or stem canker (*Leptosphaeria maculans*); Sclerotinia stem rot (*Sclerotinia sclerotiorum*); and clubroot (*Plasmodiophora brassicae*). To date there are few control measures for these pathogens that are fully effective and economical.

Varieties with single race resistance have been developed, but the multi-race pathogenicity of these fungi has made it difficult to breed varieties with long-lasting resistance. However, it is anticipated that with the location of resistance genes on marker-saturated genome maps, breeders will be able to bring together multiple resistance genes from both within and outside the genus that will provide long-lasting disease resistance.

Breeding improved varieties

Introduction

The objective of all plant breeding programmes is to produce plants of greater value to the producer, the industry and the consumer. The objective is achieved by building on past advances, through the incorporation of desirable traits that impart increased yield, pest resistance, superior quality and/or utility to new varieties. To accomplish the task, many related disciplines are essential including genetics, biotechnology, agronomy, cytology, chemistry, pathology, entomology, physiology and statistics. Within the biotechnology component, gene transfer and the production of transgenic varieties has attracted public attention but the discipline is much broader and includes, among others, tissue culture, protoplast fusion, dihaploid production, gene identification and cloning.

The essential requirement for success is genetic variation for the trait or traits of interest. The breeder will normally search for the desired trait within adapted genotypes and then the crop's world germplasm collection. If it is not present within the species but present in a related species, interspecific and intergeneric crosses and/or protoplast fusion may be attempted. If those approaches fail, induced mutation may be explored. Generally gene transfer, because of regulatory hurdles, is the last resort.

Valuable, new gene-controlled traits are added with each improved variety. The breeder evaluates the need and the genetic variability available and stacks desirable traits, be they large or small advances, into the genetic base that previous breeders have built. Gene stacking is the very essence of plant breeding. Breeding techniques vary with the crop being bred and its mode of pollination and reproduction. Among the commercial *Brassica* crops, both self-compatible and self-incompatible species are present so that a wide array of techniques are employed, as described below, depending on the species and the trait or traits to be introduced.

The application of conventional genetic manipulation in plants can have major beneficial impact on the nutritional quality and quantity of the world's food supply. A very successful example, described below, is the conversion of *Brassica* oilseed crops from a problematic commodity to the high-quality productive crop we now define as canola.

Lipids not only make our food taste better but are required dietary ingredients. They are essential cell membrane components, regulating cell permeability and are responsible for vitamin transport as well as the starting point for hormone biosynthesis. Oils and fats are predominantly (~98%) triacylglycerols (TAGs) that consist of a three-carbon chain with fatty acids attached to each carbon. The fatty acid composition of an oil determines its value, use and nutritional worth.

Oils from *B. juncea*, *B. rapa* and later *B. napus* have been part of the Asian diet for centuries, but in Europe and the Americas they are relatively recent edible oil additions. Prior to and during the Second World War, rapeseed oil was primarily used as a lubricant for steam engines and as a lamp oil, but following the war, *B. napus* and *B. rapa* oils

became an important constituent of margarine. Researchers became interested in the nutritional value of *Brassica* seed oils because they differed from most other edible oils in having a high percentage of long carbon chain monoenoic fatty acids, eicosenoic (C20:1) and erucic (C22:1) (Table 3.14).

Small animal feeding studies in the late 1950s and throughout the 1960s indicated that the nutritional value of rapeseed oil could be substantially improved if the long chain fatty acids could be reduced to <5% of the fatty acid total (Kramer et al., 1983). Breeding and selection within the world's germplasm was successful in developing plants of *B. napus* (Stefansson, Hougen and Downey, 1961), *B. rapa* (Downey, 1964) and later *B. juncea* (Kirk and Oram, 1981) that produced oils with less than 2% erucic acid. This oil was found nutritionally superior to the high erucic oil (Kramer et al., 1983) and proved to be an excellent liquid and salad oil, as well as a suitable ingredient for margarine and shortening manufacture. This new natural oil is called "canola oil" in most countries of the world and is defined as oils from *B. napus*, *B. rapa* or *B. juncea* containing less than 2% erucic acid of the fatty acid total. The genetic blocking of the biosynthesis of eicosenoic and erucic acids resulted in an increased percentage of oleic and linoleic acids (Table 3.14).

Table 3.14. **Fatty acid composition of rapeseed, canola, soybean, sunflower and linseed oils**

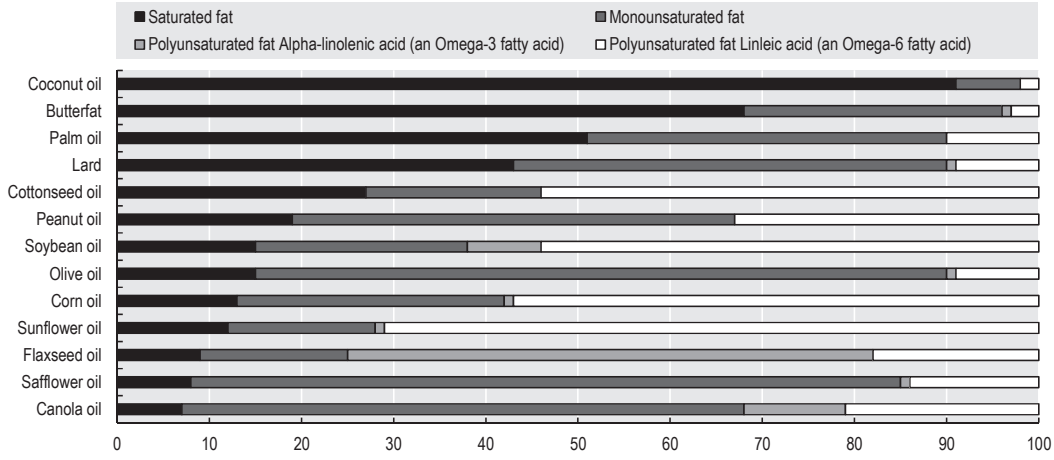
Fatty acid	Symbol[1]	Rapeseed	Canola	Soybean	Sunflower	Linseed
		Fatty acid composition (%)				
Palmitic	C16:0	4.0	4.7	11.5	7.5	7.0
Stearic	C18:0	1.5	1.8	3.5	4.5	4.0
Oleic	C18:1	17.0	61.5	23.0	16.0	20.0
Linoleic	C18:2	13.0	21.0	43.0	71.0	17.0
Linolenic	C18:3	9.0	11.0	8.0	1.0	52.0
Eicosenoic	C20:1	14.5	<1.0	0.0	0.0	0.0
Erucic	C22:1	41.0	<1.0	0.0	0.0	0.0

Note: 1. The first number denotes the number of carbon atoms in the fatty acid chain and the second numbe, the number of double bonds in the chain.

When nutritionists recommended that dietary intake of saturated fat be reduced, the nutritional value of canola oil gained widespread recognition since it contains the lowest level of saturated fatty acids of any edible oil (Grundy and Denke, 1990; Gurr, 1992; Hu et al., 1997; see Figure 3.41). Further, in 1985 Mattson and Grundy reported on the nutritional desirability of the so-called "Mediterranean diet", pointing out the health advantages of oils with a low level of saturates and high content of oleic acid. The fatty acid composition of canola oil met or exceeded the nutritional requirements of a superior edible oil, with the lowest saturate content (6-7%) of any edible oil and a high (58-60%) level of oleic (18:1n-9) that reduces the undesirable low-density lipoproteins (LDLs) without reducing the desirable high-density lipoproteins (HDLs).

Plant breeders have now developed varieties that produce canola oils with less than 3% α-linolenic acid which improves the oxidative stability of the oil and reduces the development of unpleasant flavours and cooking odours (Scarth, Rimmer and McVetty, 1995; Scarth et al., 1988; Eskin et al., 1989; Przybylski et al., 1993).

Figure 3.41. **Canola oil compared to other edible vegetable oils as to total saturated fat content and other fatty acids**



Source: Analyses conducted by POS Pilot Plant Corporation, Saskatoon, Canada, data courtesy of Canola Council of Canada.

More recently plant breeders have combined the low linolenic trait with a reduced level of linoleic acid to provide an oil with over 70% oleic acid (Table 3.15; Downey, 1996). The high oleic acid level further increases the oil's stability so that little or no hydrogenation of the oil is required, which would otherwise result in undesirable trans fatty acids. Canola varieties that produce this latter fatty acid composition now occupy about 10% of Canada's oilseed rape growing area.

Table 3.15. **Fatty acid composition of canola and specialty *B. napus* varieties grown in Canada**

Oil type	Fatty acid composition (%)							
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1
Canola	4.7	<1.0	1.8	61.5	21.0	11.0	<1.0	<1.0
High erucic	2.0	<1.0	2.0	13.0	12.0	9.0	7.0	54.0
Low linolenic	4.0	<1.0	2.0	64.0	27.0	2.0	1.0	<1.0
High oleic	4.0	<1.0	2.0	75.0	9.0	8.0	<1.0	<1.0

Oil extraction of *Brassica* oilseeds yields about 40% oil and some 60% high protein meal. The meal is used as a high-quality protein supplement in diets for animals, poultry and fish. Unfortunately, the plant translocates and concentrates the glucosinolates in the seed. As a result, rapeseed and mustard can contain over 120 mg/g of glucosinolates per whole seed. This high concentration of glucosinolates, and their breakdown products, greatly limited the amount of traditional rapeseed meal that could be fed to non-ruminant animals, such as swine and poultry. Glucosinolates and their breakdown products reduced the palatability of the meal but, more importantly, they interfered with the iodine uptake by the thyroid gland and are active goitrogens. Feeding rapeseed meal to non-ruminant animals frequently resulted in poor feed efficiency and weight gains as well as reproductive difficulties (Bell, 1993). Thus, the amount of seed that could be processed was determined by the limited size of the meal market.

A partial solution was the inactivation of the myrosinase enzyme as the first step in the oil extraction process but enzymes in the animal gut, although less efficient, were also able to hydrolyse the glucosinolates. The answer to this problem was to breed plants with little or no glucosinolates in their seed.

Analytical advances in the 1960s allowed breeders to identify plants with only 10-12 µmoles of aliphatic glucosinolate per gram oil free meal. These plants were crossed with low erucic acid varieties to produce "double low" or "canola quality" varieties of *B. napus* (Stefansson, 1983), *B. rapa* (Downey and Rakow, 1987) and *B. juncea* (Love et al., 1990). The reduction in glucosinolate levels allowed canola meal to be fed at maximum economic levels to non-ruminants and canola meal became the preferred protein supplement for dairy cattle. Canola is defined as seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa* or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30.0 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl, or 2-hydroxy-4-pentenyl glucosinolate, per gram of air-dried, oil free solid (Canola Council of Canada).

Breeding methods

The amphidiploids, *B. napus*, *B. juncea* and *B. carinata*, are largely self-pollinating with the self-pollinated progeny exhibiting very little, if any, loss in vigour. Thus, methods developed for highly inbred crops, such as the cereal grains, have been adapted for these partially outcrossing species. In the oilseed forms of these species, complete homozygosity is normally not the objective, although varietal distinctness, uniformity and stability are still a requirement. However, with cole crops and hybrids, high levels of homozygosity are required.

Regardless of the breeding technique employed, success is dependent upon the identification of suitable parents that, when crossed, will yield progenies that express the desirable traits of both parents.

Mass selection

This early plant-breeding technique relied on the identification and harvesting of seed from the most productive or desirable plants within a population for sowing in the following year. The system is one of population improvement based on plant phenotype and is best suited to self-fertilised crops and where gene action is additive. It lacks the efficiency of present-day techniques, but a variation is used today to preserve the identity of established varieties whereby off-types are removed from elite lines and breeder seed plots.

Pedigree method

In the past, most *B. napus* and *B. juncea* commercial varieties were developed using the pedigree method. Crosses are made between parents exhibiting the traits to be combined and the F_1s are selfed or intercrossed. The progeny are selfed or allowed to interpollinate and selection of the best F_4 rows is done within the best F_3 families. By the $F_{5\text{-}6}$, the vast majority of loci will be homozygous and the characteristics of the breeding line are fixed.

The pedigree method may be modified in various ways depending on the inheritance of the trait or traits being introduced or combined. The method is well suited to the mainly self-pollinating species *B. napus* and *B. juncea*, because the seed multiplication rate, unlike cereal grains, is high (*ca.* 1 000:1). In the self-incompatible *Brassica* vegetables and oilseed *B. rapa*, inbreeding leads to a rapid loss in vigour and reduced fertility. However, it is sometimes used to produce inbred lines destined for the

production of hybrid vegetables. Some cauliflower varieties are exceptions, being natural self-pollinators that do not exhibit the usual vigour and fertility losses.

Single seed descent

As with the pedigree method, the first step in single seed descent (SSD) breeding is the careful choice of parents for hybridisation. However, unlike the pedigree method, selection is not practiced until a high degree of homozygosity is reached. The object is to advance generations as rapidly as possible and subsequently select among the randomly derived lines. The size of the segregating population is kept at a manageable level by planting only one randomly chosen single seed from each plant in the previous generation.

Since the degree of homozygosity is not as critical in *B. napus* and *B. juncea* as it is in cereals, this method has not been widely used in *Brassica* breeding programmes.

Backcross method

The backcross method is designed to introduce one or more specific trait(s) into an otherwise highly desirable parent or variety. The donor parent, containing the trait(s) to be incorporated, is crossed onto plants of an adapted, desirable, recurrent parent. Depending on the inheritance of the trait(s) and the ease or efficiency of selection, the F_1 or selected BCF_1 plants will be backcrossed to the recurrent parent. By the fourth to sixth backcross, the genetic makeup of the recurrent parent is expected to have been reconstituted with the new trait incorporated. However, linkage between the desirable trait and one or more undesirable characteristics may require selection within large populations to identify plants or lines with an uncoupled linkage.

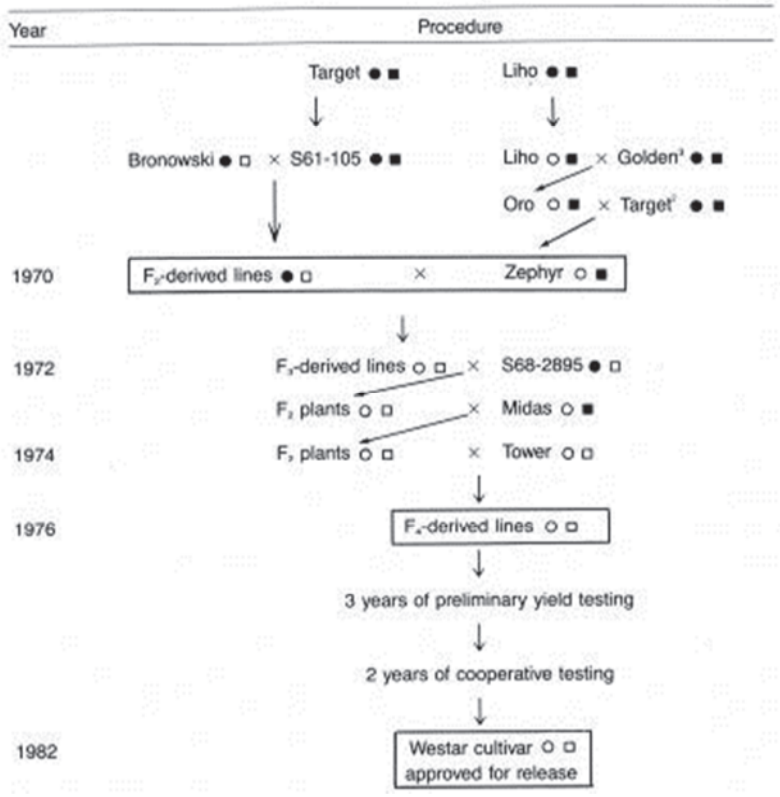
Frequently in the self-pollinating species, only one or two backcrosses are made followed by pedigree selection.

Figure 3.42 illustrates the combined use of the backcross and pedigree methods.

In the self-incompatible species, backcrossing can also be effective for the incorporation of specific traits. However, crosses in oilseed *B. rapa* need to be made with sufficient numbers of recurrent parent plants to ensure that heterozygosity of the self-incompability alleles of the recurrent parent is maintained in the backcross generations. To overcome this potential problem, the "recurrent selection" breeding system is widely used.

Backcrossing is also effective in the self-incompatible vegetable species. Dickson and Wallace (1986) outline a complete backcross breeding programme for cabbage improvement.

Figure 3.42. **Breeding scheme combining the backcross and pedigree selection systems to develop a low erucic, low glucosinolate variety with high seed and oil yield**



Notes: This breeding scheme uses agronomically superior parents that contribute either high (●) or low (○) erucic acid levels and high (■) or low (□) glucosinolate content.

Source: Downey and Rakow (1987).

Recurrent selection method

This method is standard procedure for improving populations of self-incompatible species. Any type of segregating population may be a candidate for improvement. Normally open-pollinated seed is harvested at random from individual plants within the population, and a progeny row or rows sown from each plant. However, some seed from each plant is held in reserve. The progeny rows are evaluated and the best performing identified. An equal amount of the reserve seed from the best single plants, based on the performance of their progeny, is bulked. The first cycle of recurrent selection is complete when the new seed composite is sown in an isolation plot, and the second cycle begins with the harvesting of random single plants within the new composite. A bulk sample from the remaining plants can be harvested and planted in replicated trials to measure the response to selection. Recurrent selection is continued as long as it is anticipated that there will be a reasonable response to selection.

With every additional trait under selection, the intensity of selection increases exponentially, thus it is difficult to improve a population for several traits simultaneously. This constraint is overcome by having specialised composites for different traits that are brought together after the original objectives for each have been met.

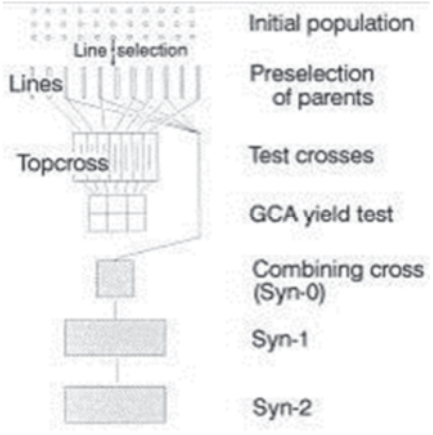
Synthetic varieties

Allard (1960) defines a synthetic variety as one "that is maintained from open pollinated seed following its synthesis by hybridisation in all combinations among a number of selected genotypes". This method, which is widely used in breeding forage crops, is also effective for the breeding of oilseed *B. rapa* (Falk et al., 1994). Equal amounts of seed from varieties or recurrent lines that arise from widely different gene pools are mixed and sown in Syn.-0 isolation plots. Seed harvested from the Syn.-0 plot constitutes the Syn.-1 generation. Syn.-1 seed from a two component synthetic will consist of 25% from each parental genotype and 50% hybrid seed. Thus, if the parental lines are good combiners, a significant amount of heterosis can be captured.

The method (Figure 3.43) has also been explored in *B. napus* (Becker, Löptien and Röbbelen, 1999) but breeding programmes in this species are now directed to F_1 hybrid varieties.

Normally, despite the high multiplication rate (1 000: 1), there is insufficient Syn.-1 seed for commercialisation so that Syn.-1 seed is sown to provide commercial Syn.-2 seed. This procedure has been used in Canada to produce the first commercial *B. rapa* synthetic varieties, Hysyn 100 and Hysyn 110. Because of the large number of genotypes within the parental lines, there is very little loss in heterosis between the Syn.-1 and Syn.-2 generations (Falk and Woods, 2003). If the market is very large a Syn.-3 generation could be added.

Figure 3.43. **Breeding scheme for development of commercial synthetic varieties of oilseed *Brassica* crops**



Source: Becker, Löptien and Röbbelen (1999).

Diallel and polycross methods

In vegetable crops, uniform maturity, head size and appearance are critical to the success of a variety and seed yield is of secondary importance. Further, the numbers of parents that make up a variety are few and the market price of seed is substantially greater than the commodity oilseed crops. Thus, breeding methods used for vegetables can be more intensive than the large population breeding methods used in oilseed improvement programmes. For example, if a deleterious trait is controlled by a recessive gene, it is difficult to completely eliminate it from a self-incompatible plant population.

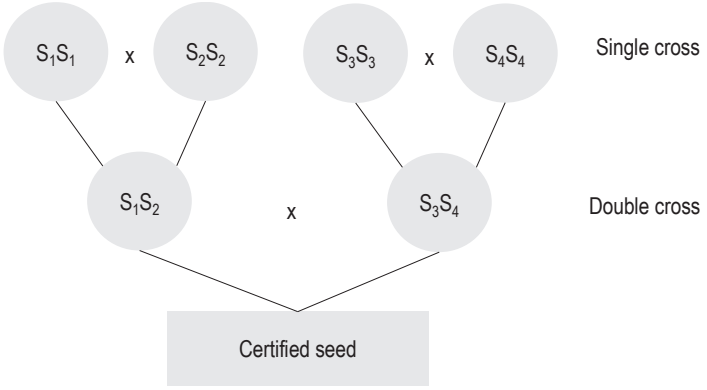
However, within a small population of potential elite parents, diallel crossing, i.e. hand crossing each parent with all other potential parents, followed by progeny assessment, can eliminate the heterozygous parent(s). Although labour intensive, this technique is suitable for most vegetable Brassicas because individual plants (parents) can be vegetatively maintained over many generations. Vegetative propagation also makes possible the use of the polycross breeding method used to identify desirable parents with good general combining ability. In this method, parental clones are space planted in a field design that assures each parent is equally exposed to pollen from all the other parents in the nursery. Progeny evaluation then identifies the best parents for inter-pollination to produce seed of a new variety.

Hybrid varieties

The vigour, yield and uniformity advantages associated with hybrids in both oilseed and vegetable *Brassica* crops have been demonstrated by many breeders. The main constraint to their commercial exploitation has been an effective pollen control-fertility restoration system. Vegetable breeders have utilised the variations in SI alleles, which control the self-incompatible system, to produce single and double cross hybrids. Kuckuck (1979) illustrates how lines, selected for general combining ability and specific S alleles, are programmed to produce double-cross cabbage hybrids (Figure 3.44).

The self-incompatible parent can be maintained through bud pollination, micro propagation or by overcoming the SI barrier by exposing flowering plants to high CO_2 concentrations. Nuclear male sterility in oilseed rape has also been used commercially in China but the segregating male fertile progeny have to be removed by hand (Fu et al., 1997), thus making the system expensive in many regions.

Figure 3.44. **Self-incompatability scheme for breeding cabbage hybrid seed production**



Source: Kuckuck (1979).

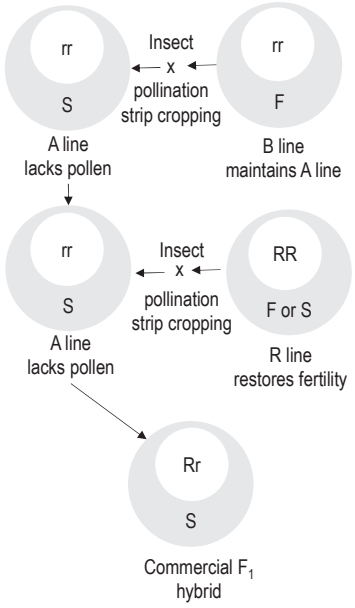
The most practical and efficient system is that of cytoplasmic male sterility (CMS). More than 17 different male sterile forms have been investigated in *Brassica* species (Stiewe et al., 1995; Prakash et al., 1995). Only a few have been developed to the commercial stage, but varietal development programmes worldwide are rapidly moving to the use of CMS-restorer systems for hybrid seed production. The CMS systems are based on genetic miscommunication between cytoplasmic mitochondria and nuclear genes, resulting in the disruption of normal anther and/or pollen development. There are three components to the system: the A line, carrying the cytoplasmic mitochondrial genome that results in male sterility, the B line that is fully fertile and maintains the

A line, and the R line with a nuclear gene that restores fertility. The R line should be highly heterotic to the A line to produce a high yielding, fully fertile F_1 commercial crop (Figure 3.45).

China developed the first *B. napus* commercial CMS system, known as the Polima system (Fu et al., 1997). However, the Polima system is rapidly being replaced in western breeding programmes with the *ogu*-INRA system, the Male Sterile Lembke (MSL) system and others under development (Stiewe et al., 1995; Prakash et al., 1995; Downey and Rimmer, 1993).

A transgenic pollen control-restorer system, developed by Plant Genetic Systems and commercialised by Bayer CropScience, is in widespread use in Canada and the United States. Details of how this system functions are outlined by Downey and Rimmer (1993).

Figure 3.45. **Production system for cytoplasmic male sterile hybrid seed of oilseed rape**



Note: The small circle represents the nucleus showing the fertility restorer genes r and R and the larger circle the cell with cytoplasm containing fertile (F) or sterile (S) mitochondrial genes.

Source: Modified from Buzza (1995).

Improvement through "interspecific hybrids" and "cybrids"

Interspecific and intergenomic crosses are important options for the introduction of desired traits that are not available, or cannot be found, within the primary gene pool of a crop species. Normally such crosses are difficult to make. As noted previously, there are many natural barriers, both pre- and post-fertilisation, that protect the integrity of a species. Further, even if such crossing is successful, chromosome pairing and alien gene introgression into the genome of the target species must occur.

However, in the Brassicaceae, a number of desirable nuclear genes from different genera and species have been transferred to targeted crop species. A list of traits that have been transferred to *B. napus*, *B. juncea* and/or *B. oleracea* from other Brassicaceae species is presented in Table 3.16 (Prakash et al., 2009).

The development of protoplast fusion technology has been highly successful in circumventing the natural sexual barriers that separate the Brassicaceae species and genera. The technology has the potential to access desirable genes present in distant relatives (Glimelius, 1999; Christey, 2004; Navrátilová, 2004; Liu, Xu and Deng, 2005). Prakash et al. (2009) have compiled a list of intertribal somatic hybrids in the Brassiceae and the desirable traits to be transferred (Table 3.17). Additional intergenomic hybrids have been produced but failed to establish in soil e.g. *Camelina sativa* + *B. carinata* (Narasimhulu et al., 1994), *C. sativa* + *B. oleracea* (Hansen, 1998) and *Barbarea vulgaris* + *B. napus* (Fahleson, Eriksson and Glimelius, 1994).

With some exceptions, the somatic hybrids so far obtained have exhibited a high degree of sterility and/or morphological abnormalities that have limited their use. However, the importance of somatic hybridisation is not so much the direct use of the resulting amphidiploids, containing both parental genomes, but rather to utilise the somatic hybrids as a bridge to transfer desirable traits to target species (Glimelius, 1999).

Cell fusion not only brings together the nuclear contents of both parents but also combines the cytoplasm and organellar content of fused cells. Frequently, to improve the outcome, the nucleus of one parent is eliminated by X-ray, centrifugation or chemical treatment before fusion but the fused cell contains the cytoplasm of both parents. The resulting plant is termed a "cybrid". This technique allows cytoplasmic substitution which frequently results in cytoplasmic male sterility (CMS). Cell fusion among the Brassicaceae, where the cytoplasm of both parents are combined, can also generate novel cytoplasmic variability, bringing about organellar reassortment and DNA rearrangement, which is not possible using sexual hybridisation.

Chloroplast segregation is independent of mitochondrial segregation and while mitochondrial recombination has been frequently observed in the Brassiceae (Glimelius, 1999), recombination is rarely found in the chloroplasts. It is also rare to have a mixture of the two chloroplasts occurring in the same hybrid. In general, the chloroplasts are usually contributed by crop species. This may occur because many of the fusions are with the allopolyploid crop species that contribute large numbers of chloroplasts per cell (Butterfass, 1989).

Table 3.16. **Introgression of nuclear genes conferring desirable traits to *Brassica* crops**

Trait	Donor species	Recipient species	Reference
Yellow seed coat	*B. rapa*	*B. napus*	Chen and Heneen (1991)
	B. juncea/B. carinata	*B. napus*	Rashid, Rakow and Downey (1994)
	B. carinata	*B. napus*	Qi et al. (1995)
	B. rapa/B. carinata	*B. napus*	Meng et al. (1998)
	B. rapa/B. juncea	*B. napus*	Rahman (2001); Potapov and Osipova (2003)
CMS fertility	*Raphanus sativus*	*B. napus* CMS (*Ogu*)	Heyn (1977); Rousselle et al. (1985)
Restoration	*Raphanus sativus*	*B. napus* CMS (*Kosena*)	Sakai et al. (1996)
	B. juncea	*B. napus* CMS (*Polima*)	Fan, Tai and Stefansson (1985)
	B. tournefortii	*B. napus* CMS (*Tour*)	Stiewe and Röbbelen (1994)
	Trachystoma ballii	*B. juncea* CMS (*Trachy*)	Kirti et al. (1997)
	Moricandia arvensis	*B. juncea* CMS (*Moricandia*)	Prakash et al. (1998)
	Erucastrum canariense	*B. juncea* CMS (*Canariense*)	Prakash et al. (2001)
		B. napus CMS (*Canariense*)	Banga et al. (2003)
	Enarthrocarpus lyratus	*B. rapa* CMS (*Lyratus*)	Deol et al. (2003)
		B. juncea/B. napus CMS (*Lyratus*)	Banga, Deol and Banga (2003)
Chlorosis removal	*Raphanus sativus*	*B. napus* CMS (*Ogu*)	Paulmann and Röbbelen (1988)
Beet cyst nematode	*Sinapis alba*	*B. napus*	Lelivelt et al. (1993)
Resistance	*Raphanus sativus*	*B. napus*	Lelivelt and Krens (1992); Voss, Snowdon and Lühs (2000); Peterka et al. (2004): Budahn et al. (2006)
Club root resistance	*B. napus*	*B. oleracea* var. *capitata*	Chiang, Chiang and Grant (1977); Chiang et al. (1980)
	B. rapa	*B. napus*	Gowers (1982)
Blackleg resistance	*B. juncea*	*B. napus*	Roy (1984); Dixelius (1999); Sacristan and Gerdemann (1986)
	B. nigra	*B. napus*	Struss et al. (1996); Chèvre et al. (1997b, 1996); Plieske, Struss and Röbbelen (1998); Dixelius (1999)
	Arabidopsis thaliana	*B. napus*	Bohman, Wang and Dixelius (2002); Ogbonnaya et al. (2003); Saal et al. (2004)
	Sinapis arvensis	*B. napus*	Snowdon et al. (2000); Winter et al. (2003)
	Coincya monensis	*B. napus*	Winter et al. (2003)
	B. rapa	*B. napus*	Chèvre et al. (2003)
Alternaria leaf spot	*Sinapis alba*	*B. napus*	Primard et al. (1988)
Resistance	*Diplotaxis erucoides*	*B. napus*	Klewer et al. (2003)
	Sinapis alba	*B. oleracea*	Sigareva, Ren and Earle (1999)
Black rot resistance	*B. juncea*	*B. napus*	Tonguc and Griffiths (2004)
Soft rot resistance	*B. oleracea* var. *italica*	*B. oleracea*; *B. rapa* subsp. *pekinensis*	Ren, Dickson and Earle (2001a, 2001b)
Altered oil quality	*Orychophragmus violaceus*	*B. napus*	Hu et al. (2002); Hua and Li (2006)
Earliness	*B. rapa*	*B. napus*	Shiga (1970); Namai, Sarashima and Hosoda (1980)
	B. oleracea	*B. napus*	Habman et al. (2010)
Low erucic acid	*B. juncea*	*B. carinata*	Getinet et al. (1994)
Low glucosinolate	*B. napus*	*B. carinata*	Getinet et al. (1994)

Source: Prakash et al. (2009).

Table 3.17. **Intertribal somatic hybrids in Brassiceae for the integration and incorporation of desirable traits into *Brassica* crops**

Somatic hybrid	Desirable trait for introgression	Reference
Arabidopsis thaliana (*n*=5) + *B. nigra*	Resistance to flea beetles, cold tolerance, short life cycle	Siemens and Sacristan (1995*)
Arabidopsis thaliana (*n*=5) + *B. oleracea*	Plastome transformation	Nitovskaya and Shakhovskyi (1998); Yamagishi and Nakagawa (2004); Nitovskaya et al. (2006a)
Arabidopsis thaliana (*n*=5) + *B. rapa*	Experimental demonstration	Gleba and Hoffmann (1980, 1979)
Arabidopsis thaliana (*n*=5) + *B. juncea*	Phosphinothricin resistance	Ovcharenko et al. (2004)
Arabidopsis thaliana (*n*=5) + *B. napus*	Herbicide resistance, Blackleg resistance	Bauer-Weston et al. (1993*); Forsberg, Landgren and K. Glimelius (1994*); Forsberg et al. (1998); Yamagishi et al. (2002*)
	Transposable element *Spm/dSpm*	Ovcharenko et al. (2005*)
Armoracia rusticana (*n*=16) + *B. oleracea*	Clubroot resistance	Navrátilová et al. (1997)
Barbarea vulgaris (*n*=8) + *B. oleracea*	Cold tolerance	Ryschka et al. (1999)
Barbarea vulgaris (*n*=8) + *B. rapa*	Cold tolerance	Oikarinen and Ryöppy (1992)
Barbarea vulgaris (*n*=8) + *B. napus*	Cold tolerance	Fahleson, Eriksson and Glimelius (1994)
Barbarea stricta (*n*=8) + *B. rapa*	Cold tolerance	Oikarinen and Ryöppy (1992)
Camelina sativa (*n*=20) + *B. oleracea*	Alternaria resistance	Hansen (1998); Sigareva and Earle (1999)
Camelina sativa (*n*=20) + *B. carinata*	Alternaria resistance	Narasimhulu et al. (1994)
Capsella bursa-pastoris (*n*=16) + *B. oleracea*	Resistance to flea beetles, alternaria blight	Nitovskaya et al. (1998); Sigareva and Earle (1999)
Crambe abyssinica (*n*=45) + *B. napus*	High erucic acid content, insect resistance	Wang, Sonntag and Rudloff (2003*); Wang et al. (2004*)
Lepidium meyenii (*n*=32) + *B. oleracea*	Glucosinolate content	Ryschka, Klocke and Schumann (2003)
Lesquerella fendleri (*n*=6) + *B. napus*	High lesquerolic acid content, drought tolerance *Lesquerella* chloroplasts	Skarzhinskaya, Landgren and Glimelius (1996**); Skarzhinskaya et al. (1998) Schröder-Pontoppidan et al. (1999); Nitovskaya et al. (2006b)
Lunaria annua (*n*=14) + *B. napus*	High nervonic acid content	Craig and Millam (1995)
Matthiola incana (*n*=7) + *B. oleracea*	Oil quality	Ryschka et al. (1999)
Orychophragmus violaceus (*n*=12) + *B. napus*	High linoleic and palmitic acid content Phosphinothrin resistance Chlorosis correction	Hu et al. (2002* ,1999) Sakhno et al. (2007) Vasilenko et al. (2003)
Thlaspi perfoliatum (*n*=21) + *B. napus*	High nervonic acid content	Fahleson et al. (1994**)
Thlaspi caerulescens (*n*=7) + *B. napus*	Zinc and cadmium tolerance	Brewer et al. (1999)
Thlaspi caerulescens (*n*=7) + *B. juncea*	High metal accumulation	Dushenkov et al. (2002)

Note: * Denotes asymmetric hybrids; ** Both asymmetric and symmetric hybrids identified.

Source: Prakash et al. (2009).

Biotechnology in Brassica *breeding*

Introduction

Although the above breeding procedures have been very effective in combining important agronomic and nutritional traits in superior cultivars, the process of identifying the desired genotype in genetically stable, uniform and high-yielding varieties takes many years. Further, the small chromosome size plus their lack of distinctive features have been an additional limitation on the selection of superior genotypes. However, beginning in the mid- to late 1980s, developments in tissue culture, embryo rescue, cell fusion, molecular markers and genetic mapping have not only reduced the time from cross to market but have given breeders powerful tools to quickly identify and assemble desirable traits in a single genotype. In addition, these biotech tools have greatly expanded the size and variation of the available gene pool, well beyond species boundaries.

Doubled haploid breeding

The doubled haploid (DH) breeding technique is now widely used in *B. napus* and *B. juncea* breeding programmes (Ferrie and Keller, 2004). This breeding tool not only eliminates the several generations needed to attain genetic stability and uniformity in breeding lines, but also significantly reduces the size of populations needed to find a desired genotype. For example, in *B. napus*, two genes code for the level of the fatty acid erucic in the seed oil, and an additional six genes code for the content of glucosinolates in the seed. Thus, when making a high by low cross, to produce progeny that have both low erucic acid and low glucosinolate (double low or canola quality), large segregating populations must be examined since the desired genotype must have all eight genes in the recessive state.

Table 3.18 illustrates the DH technique's increased selection efficiency, particularly when the selected plants are completely homozygous individuals that can be used directly as pure breeding varieties or as hybrid parents.

Table 3.18. **Minimum population size required to select the least frequent homozygote at 95% probability**

Number. of genes	Minimum F_2 population	
	Diploid	Haploid
1	11	5
2	47	11
4	766	47
5	3 067	95
6	12 269	191
7	49 077	382
8	**196 259**	**766**
10	3 123 923	3 067

Source: Rajhathy (1976).

The technique involves inducing large numbers of immature pollen grains (microspores) from *Brassica* species to develop into plants with the gametic or half the somatic chromosome number. Such plants are termed haploids and are sterile. By applying colchicine to the developing haploid plant, cell division is temporarily arrested, bringing about chromosome duplication. The result is a doubled haploid (DH) or

dihaploid plant that is fully fertile and totally homozygous. Thus, complete homozygosity is reached in a single generation, and all seeds arising from self-fertilisation of that plant will be genetically identical. It is this single step to homozygosity that reduces the number of generations and time required to develop a new variety or hybrid parent. However, in a breeding programme, large populations of DH lines must be generated and evaluated since no prior selection has taken place. DH lines are usually derived from F_1 donors, although the use of F_2 and F_3 donor plants allows for more recombination and some preselection.

Molecular markers and their application

Marker-assisted selection and chromosome mapping came into general use in the 1980s with the development of restriction fragment length polymorphisms (RFLP) techniques that resulted in the first linkage maps for *B. oleracea* (Slocum et al., 1990), *B. rapa* (Song et al., 1991) and *B. napus* (Landry et al., 1991). This technique was important in identifying genomes and their chromosomes, locating genes and qualitative trait loci (QTLs), which are DNA regions containing a gene or genes that regulate traits of agronomic or quality interest.

The discovery of the polymerase chain reaction (PCR) by Mullis and Faloona (1987) resulted in new types of genetic markers such as amplified fragment length polymorphisms (AFLPs) that are more sensitive than RFLPs and simultaneously detect various polymorphisms in different genomic regions.

Additional marker systems have since been added to the toolbox including: random amplified polymorphic DNAs (RAPDs); sequence tagged sites (STS); simple sequence repeats (SSRs) or microsatellites and single nucleotide polymorphisms (SNPs). Breeders use these molecular markers to produce densely marked chromosome maps that can then be used to: 1) characterise germplasm and its genetic variability; 2) estimate the genetic distance between gene pools, inbreds and populations; 3) detect and locate QTLs and monogenic traits of interest; 4) select genotypes based on the presence or absence of specific markers; 5) identify useful candidate genes for sequencing (for more detailed information on genome mapping and molecular breeding in *B. napus*, see Snowdon, Lühs and Friedt, 2007; Snowdon et al., 2007). The marker systems differ in their ease of use, cost and other characteristics. It is expected that the SNPs system will become the marker system of preference, dispite its initial high cost, due to its ease of use, low cost per analysis and high level of reproducibility (Korzun, 2003).

Comparative genomic gene identification

The distantly related and intensively studied species *Arabidopsis thaliana* provides information that is highly relevant for gene isolation and characterisation in *Brassica* crops. However, the genomes of *Brassica* species are much more complex (Snowdon, Lühs and Friedt, 2007).

A comprehensive comparative RFLP linkage map of *A. thaliana* and *B. napus* genomes indicated the 5 *Arabidopsis* chromosomes could be allocated to a minimum of 22 conserved, duplicated and rearranged blocks throughout the *B. napus* genome (Parkin et al., 2005).

Such information highlights the complexity of genome rearrangements between the two species, but also the great potential the model genome offers for comparative genetic analysis of the *Brassica* crops (Snowdon, Lühs and Friedt, 2007).

TILLING technique

The technique of TILLING (targeted induced local lesions in genomes) can be used to identify a series of mutations (alleles) in a target gene by heteroduplex analysis (McCallum et al., 2000). This method combines a standard technique of mutagenesis with a chemical mutagen such as ethyl methanesulfonate (EMS),[6] with a sensitive DNA screening technique that identifies single-base mutations (also called point mutations) in a target gene.

This technique is available from the Canadian TILLING Initiative (CAN-TILL) at the University of British Columbia on a fee-for-service basis. The CAN-TILL facility is currently developing a large-scale mutant population for *Brassica napus* as part of a Genome Canada project and has completed projects on *B. oleracea* and *Arabidopsis thaliana*. *B. rapa* TILLING services are available from RevGenUK in the United Kingdom (John Innes Centre).

Gene transfer

The transfer of a gene(s) from an unrelated species is undertaken only when the desired trait cannot be found or induced by traditional methods. Because of the huge costs and time required to comply with multiple regulations in multiple countries, only those traits that have a potentially large and valuable market are considered for commercial exploitation.

Notes

1. The authority for the scientific names used in this chapter is given in Tables 3.2 and 3.3. The nomenclatural authority for genus and species names not listed in the tables will be included in the text where they first appear.
2. Autosyndesis is defined as the pairing of completely or partially homologous chromosomes during prophase of the first meiotic division.
3. This section is drawn from Wang, Guan and Zhang (2007).
4. This section is drawn from Downey, Klaasen and Stringham (1980); Dickson and Wallace (1986).

5 This citation has been added for update in January 2016.

6. Ethyl methanesulfonate (EMS): *http://en.wikipedia.org/wiki/Ethyl_methanesulfonate*.

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Annex 3.A1
Common pathogens and pests

Table 3.A1.1. **Insect, mite and other Brassicacous crop pests and their regional distribution**

Order, genus and species	Common name	Regions affected
Coleoptera		
Acalymma vittatum (F.)	Striped cucumber beetle	North America
Agriotes lineatus (L.)	Lined click beetle	North America, Europe, Russian Federation
Baris laticollis Marsh.	Not reported	Europe
Ceutorhynchus assimillis Payk.	Cabbage seed weevil	North America, Europe
Ceutorhynchus hepaticus Gyll.	Not reported	Europe
Ceutorhynchus napi Gyll.	Rape stem weevil	Europe
Ceutorhynchus obstricus (Marsh.)	Cabbage seedpod weevil	Europe
Ceutorhynchus pallidactylus (Marsh.)	Cabbage stem weevil	Europe
Ceutorhynchus pleurostigma Marsh.	Turnip gall weevil	Europe, North Africa, Russian Federation
Ceutorhynchus rapae (Gyll.)	Cabbage curculio	North America, Europe, Russian Federation
Chaetocnema indica Weise	Not reported	India
Entomoscelis americana Brown	Red turnip beetle	Canada
Listroderes costirostris Schönh.	Vegetable weevil	United States, South America, Europe, Africa, Asia, Australia, New Zealand
Meligethes aeneus F.	Pollen, rape or blossom beetle	North America, Europe, North Africa, Russian Federation, China (People's Republic of)
Meligethes viridescens (F.)	Pollen or blossom beetle	North America, Europe
Phyllotreta aerea Allard	Leaf beetle	North America, Europe, North Africa, Russian Federation, India
Phyllotreta atra F.	Cabbage flea beetle	Russian Federation
Phyllotreta chotanica Duvivier	Striped flea beetle	India, South East Asia
Phyllotreta consobrina (Curtis.)	Turnip flea beetle	No distribution information found
Phyllotreta cruciferae (Goeze)	Crucifer flea beetle	North America, Europe, North Africa, Russian Federation, India
Phyllotreta flexuosa (Ill.)	Not reported	Thailand, Malaysia
Phyllotreta nemorum (L.)	Striped flea beetle	Europe
Phyllotreta striolata (F.)	Cabbage flea beetle	North America, Europe, Russian Federation, India, Asia
Phyllotreta undulata Kutschera	Lesser striped flea beetle	North America, Europe, Australia
Psylliodes chrysocephala L.	Cabbage stem flea beetle	Canada, Europe, North Africa, Russian Federation
Psylliodes punctulata Melsh.	Hop flea beetles	North America
Diptera		
Atherigona orientalis Schiner	Pepper fruit fly	United States, Central and South America, Africa, India, Asia, Australia
Chromatomyia horticola Gour.	Pea leaf miner	Europe, Africa, India, Asia
Contarinia nasturtii (Kief.)	Swede midge	North America, Europe
Dasineura brassicae (Winn.)	Brassica pod midge	Europe
Delia floralis (Fall.)	Turnip maggot	North America, Europe, Russian Federation, China (People's Republic of), Japan
Delia radicum (L.)	Cabbage root fly	North America, Europe, North Africa, Russian Federation, China (People's Republic of)
Liriomyza brassicae Riley	Serpentine leaf miner	Worldwide, except the Russian Federation
Liriomyza bryoniae Kltb.	Tomato leaf miner	Europe, Russian Federation, India, China (People's Republic of), Japan
Phytomyza horticola Gour.	Cruciferous leaf miner	Europe, India, Asia

Table 3.A1.1. **Insect, mite and other Brassicacous crop pests and their regional distribution** (*cont.*)

Order, genus and species	Common name	Regions affected
Phytomyza rufipes Meig.	Cabbage leaf miner	United States, Europe
Homoptera		
Brevicoryne brassicae (L.)	Cabbage aphid	Worldwide
Smynthurodes betae Westw.	Gall-forming aphid, bean root aphid	United States, Europe, Middle East, Australia
Hemiptera		
Aleyrodes proletella (L.)	Cabbage whitefly	Europe
Bagrada hilaris (Burm.)	Painted bug	India, Sri Lanka, Africa, Arabia
Bemisia tabaci (Genn.)	Tobacco whitefly	Worldwide
Eurydema olerace (L.)	Cabbage bug	Turkey, Russian Federation
Eurydema pulchrum (Westw.)	Small cabbage bug	India, Asia
Eurydema rugosum Mots.	Cabbage bug	Russian Federation, China (People's Republic of), Japan
Eurydema species	Orange stink or shield bugs	Europe, North Africa, Russian Federation, India, Asia, Australia
Eurydema ventralis Kolenati	Cabbage bug	Europe, Africa, Russian Federation
Lipaphis erysimi Kltb.	Mustard aphid	Worldwide
Lygus borealis (Kelton)	Not reported	Canada
Lygus elisus Van D.	Pale legume bug	North America
Lygus hesperus Knight	Western tarnished plant bug	North America
Lygus lineolaris (P. de B.)	Tarnished plant bug	North America
Lygus ruqulipennis Popp.	Bishop bug	Canada, Europe, Russian Federation
Murgantia histrionica (Hahn)	Harlequin bug	United States
Myzus persicae Sulz.	Spinach aphid or Green peach aphid	Worldwide
Nysius niger Baker	False chinch bug	India, North America, Caribbean
Nezara viridula (L.)	Green stink bug	Worldwide
Pemphigus populitransversus Riley	Poplar petiolegall aphid	United States
Pseudococcus calceolariae (Mask.)	Scarlet mealybug	United States, Central and South America, Europe, Africa, China (People's Republic of), Australia, New Zealand
Hymenoptera		
Athalia lugens (Klug)	Mustard sawfly	India
Athalia rosae (L.)	Turnip or cabbage leaf sawfly	Europe, Russian Federation, China (People's Republic of), Japan
Lepidoptera		
Acronicta rumicis (L.)	Knotgrass moth	Europe, Russian Federation, India, China (People's Republic of)
Agrotis exclamationis L.	Heart and dart moth	Europe, Russian Federation
Agrotis ipsilon (Hufn.)	Black cutworm	Worldwide
Agrotis orthogonia Morr.	Pale western cutworm	Canada
Agrotis segetum D. & S.	Turnip moth	Europe, Africa, India, China (People's Republic of), Japan
Argyrogramma signata (F.)	Green semi-looper	India, South East Asia
Ascia monuste (L.)	Gulf white cabbage worm	South America
Autographa californica Speyer	Alfalfa looper	North America, Malaysia
Autographa gamma (L.)	Silver Y moth	Europe, North Africa, India, Asia
Autographa nigrisigna (Wlk.)	Beet worm	Russian Federation, India, China (People's Republic of), Japan
Cacoecimorpha pronubana Hbn.	Carnation tortrix	United States, Europe, North Africa, Japan
Chrysodeixis agnata Stgr.	Three-spotted plusia	China (People's Republic of), Japan
Clepsis spectrana (Treit.)	Oblique-banded caterpillar	Europe, Canada

Table 3.A1.1. **Insect, mite and other Brassicacous crop pests and their regional distribution** (*cont.*)

Order, genus and species	Common name	Regions affected
Crocidolomia pavenana (F.)	Large cabbage-heart caterpillar	India, Africa, Asia, Australia
Cydia nigricana F.	Pea moth	Caribbean, Europe, Russian Federation
Diacrisia oblique Wlk.	Jute hairy caterpillar	India, Asia
Elasmopalpus lignosellus (Zell.)	Lesser cornstalk borer	United States, Central America, Thailand
Estigmene acraea Drury	Salt marsh caterpillar	United States, Central America
Euxoa ochrogaster (Gn.)	Red-backed cutworm	North America
'tEvergestis forficalis L.	Crucifer caterpillar	Europe, India, Japan
Evergestis rimosalis (Gn.)	Cross striped cabbageworm	North America
Hadula trifolii (Hufn.)	Clover cutworm	North America, Europe, Africa, Russian Federation, India, China (People's Republic of)
Helicoverpa armigera (Hbn.)	Cotton bollworm	Europe, Africa, India, Russian Federation, South East Asia, Australia, New Zealand
Hellula phidilealis (Wlk.)	Cabbage budworm	Central America
Hellula undalis (F.)	Cabbage webworm	Europe, Africa, India, Asia, Australia, New Zealand
Lacanobia oleracea (L.)	Bright-line brown-eye moth	Europe
Lacanobia suasa D. & S.	Not reported	Europe, Russian Federation
Loxostege sticticalis L.	Beet webworm	North America, Asia, Europe, Russian Federation
Mamestra brassicae (L.)	Cabbage moth	Europe, Russian Federation, India, Asia
Mamestra configurata Wlk.	Bertha armyworm	North and Central America
Noctua pronuba (L.)	Common yellow underwing moth	Europe
Ochropleura flammatra D. & S.	Indian cutworm	India
Peridroma saucia (Hbn.)	Pearly underwing moth	The Americas, Europe, India, China (People's Republic of), Japan
Pieris brassicae (L.)	Cabbage caterpillar	South America, Europe, Russian Federation, India, China (People's Republic of), Japan, Africa
Pieris canidia (Sparrman)	Small cabbage butterfly	China (People's Republic of), South East Asia
Pieris napi (L.)	Green-veined white butterfly	Europe, North Africa, Russian Federation, India, China (People's Republic of), Japan
Pieris rapae L.	Imported cabbageworm or cabbage white butterfly	North and Central America, Europe, North Africa, Russian Federation, India, Asia, Australia, New Zealand
Plutella xylostella L.	Diamondback moth	Worldwide
Pontia daplidice (L.)	Not reported	Russian Federation
Spodoptera exigua (Hbn.)	Beet armyworm	North and Central America, Europe, Africa, Russian Federation, India, Asia, Australia
Spodoptera frugiperda J. E. Smith	Fall armyworm	The Americas
Spodoptera littoralis (Bdv.)	Cotton leafworm	Africa, Middle East
Trichoplusia ni (Hbn.)	Cabbage looper	Worldwide, except Australia and New Zealand
Vanessa cardui L.	Painted lady butterfly	North America, Europe, Africa, Russian Federation, Australia
Xestia c-nigrum (L.)	Spotted cutworm	North and Central America, Europe, Russian Federation, India, Asia
Acari		
Halotydeus destructor (Tucker)	Redlegged earth mite	Australia, New Zealand, South Africa
Tyrophagus putrescentiae (Schr.)	Cereal mite	United States, Central and South America, Europe, Africa, India, China (People's Republic of)
Stylommatomophora		
Arion lusitanicus Mabille	Spanish slug	Europe
Deroceras reticulatum Müll	Grey field slug	North America, Europe, Russian Federation, Australia, New Zealand
Lissachatina fulica (Bowdich)	Giant African land snail	South America, Africa, India, South East Asia

Table 3.A1.1. **Insect, mite and other Brassicacous crop pests and their regional distribution** (*cont.*)

Order, genus and species	Common name	Regions affected
Thysanoptera		
Thrips tabaci Lind.	Onion thrips	Worldwide
Tylenchida		
Meloidogyne ethiopica Whitehead	Not reported	South America, Europe, Africa
Meloidogyne graminicola Golden & Birchfield	Rice root knot nematode	United States, South America, South Africa, India, South East Asia
Pratylenchus neglectus (Rensch) Filipjev & Stekhoven	California meadow nematode	Europe, Russian Federation, Pakistan

Source: Information drawn from CAB International Crop Protection Compendium; Bonnemaison (1965); Lamb (1989); Thomas (1994).

Table 3.A1.2. **Diseases of rapeseed = Canola (*B. napus* L. and *Brassica rapa* L. [= *B. campestris* L.])**

Common name(s)	Scientific name (and synonyms)
Bacterial black rot	*Xanthomonas campestris* pv. *campestris* (Pammel 1895) Dowson 1939 = *Xanthomonas campestris* pv. raphani = *Xanthomonas campestris* pv. aberrans
Bacterial leaf spot	*Xanthomonas campestris* pv. *armoraciae* (McCulloch 1929) Dye 1978
Bacterial pod rot*	*Pseudomonas syringae* pv. *maculicola* (McCulloch 1911) Young, Dye and Wilkie 1978) (Canada, United Kingdom)
Bacterial soft rot	*Erwinia carotovora* (Jones 1901) Bergey et al. 1923 *Pseudomonas marginalis* pv. *marginalis* (Brown 1918) Stevens 1925
Scab	*Streptomyces* spp. *Streptomyces scabiei* corrig. (ex Thaxter 1891) Lambert and Loria 1989 = *Streptomyces scabies* (Thaxter 1891) Waksman and Henrici 1948
Crown gall	*Agrobacterium tumefaciens* (Smith and Townsend 1907) Conn 1942
Fungal diseases	
Alternaria black spot = Dark pod spot (United Kingdom)	*Alternaria brassicae* (Berk.) Sacc. *A. brassicicola* (Schwein.) Wiltshire *A. japonica* H. Yoshii = *A. raphani* Groves and Skolko
Anthracnose	*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz. *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk [teleomorph] *C. higginsianum* Sacc. in Higgins
Black leg = stem canker (United Kingdom)	*Leptosphaeria maculans* (Desmaz.) Ces. and De Not *Phoma lingam* (Tode: Fr.) Desmaz. [anamorph]
Black mold rot	*Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill.
Black root	*Aphanomyces raphani* Kendrick
Brown girdling root rot*	*Rhizoctonia solani* Kühn (Canada) *Thanatephorus cucumeris* (A.B. Frank) Donk [teleomorph]
Cercospora leaf spot	*Cercospora brassicicola* Henn.
Clubroot	*Plasmodiophora brassicae* Woronin
Downy mildew	*Peronospora parasitica* (Pers.: Fr.)Fr.
Fusarium wilt	*Fusarium oxysporum* Schlechtend.: Fr. f. sp. *conglutinans* (Wollenweb.) W.C. Snyder and H.N. Hans
Gray mold	*Botrytis cinerea* Pers.: Fr. *Botryotinia fuckeliana* (de Bary) Whetzel [teleomorph]

Table 3.A1.2. **Diseases of rapeseed = Canola (*B. napus* L. and *Brassica rapa* L. [= *B. campestris* L.])** (*cont.*)

Common name(s)	Scientific name (and synonyms)
Head rot	*Rhizoctonia solani* Kühn *Thanatephorus cucumeris* (A.B. Frank) Donk [teleomorph]
Leaf spot*	*Alternaria alternata* (Fr.: Fr.) Keissl. (Canada) *Ascochyta* spp. (former USSR)
Light leaf spot	*Pyrenopeziza brassicae* Sutton and Rawlinson in Rawlinson et al.(1978) *Cylindrosporium concentricum* Grev. [anamorph]
Pod rot*	*Alternaria alternata* (Fr.: Fr.) Keissl. (Canada) *Cladosporium* sp.
Powdery mildew	*Erysiphe polygoni* DC. *E. cruciferarum* Opiz ex Junell.
Ring spot	*Mycosphaerella brassicicola* (Duby) Lindau in Engl. and Prantl *Asteromella brassica* (Chev.) Boerema and Van Kesteren [anamorph]
Root rot	*Alternaria alternata* (Fr.: Fr.) Keissl. *Fusarium* spp. *Macrophomina phaseolina* (Tassi) Goidanich *Phymatotrichopsis omnivora* (Duggar) Hennebert *Phytophthora megasperma* Drechs. *Pythium debaryanum* Auct. non R. Hesse *P. irregulare* Buisman *Rhizoctonia solani* Kühn *Thanatephorus cucumeris* (A.B. Frank) Donk [teleomorph] *Sclerotium rolfsii* Sacc. *Athelia rolfsii* (Curzi) Tu and Kimbrough [teleomorph]
Sclerotinia stem rot	*Sclerotinia sclerotiorum* (Lib.) de Bary
Seed rot, damping-off	*Alternaria* spp. *Fusarium* spp. *Gliocladium roseum* (Link) Bainier *Nectria ochroleuca* (Schwein.) Berk [teleomorph] *Pythium* spp. *Rhizoctonia solani* Kühn *Thanatephorus cucumeris* (A.B. Frank) Donk [teleomorph] *Rhizopus stolonifer* (Ehrenb.: Fr) Vuill. *Sclerotium rolfsii* Sacc.
Root gall smut*	*Urocystis brassicae* Mundkur (People's Republic of China, India)
Southern blight (leaf, root and seed rot)	*Sclerotium rolfsii* Sacc.
Verticillium wilt*	*Verticillium longisporum* (comb. Nov. Karapappa et al.) (Europe)
White blight*	*Rhizoctonia solani* Kühn (India) *Thanatephorus cucumeris* (A.B. Frank) Donk [teleomorph]
White leaf spot = grey stem (Canada)	*Pseudocercosporella capsellae* (Ellis and Everh.) Deighton = *Cercosporella brassicae* (Faitrey and Roum.) Höhn. *Mycosphaerella capsellae* (Inman and Sivansen) [teleomorph]
White rust = staghead	*Albugo candida (Pers.) Kunze* *= A. cruciferarum (DC.) S.F. Gray* *(Peronospora sp. commonly present in staghead phase)*
Yellows	*Fusarium oxysporum Schlechtend.: Fr.*
Nematodes, parasitic	
Cyst nematode	*Heterodera cruciferae* Franklin *H. schachtii* Schmidt
Lesion nematode	*Pratylenchus* spp. *P. pratensis* (de Man) Filipjev
Root-knot nematode	*Meloidogyne* spp.

Table 3.A1.2. **Diseases of rapeseed = Canola (*B. napus* L. and *Brassica rapa* L. [= *B. campestris* L.])** (*cont.*)

Common name(s)	Scientific name (and synonyms)
Viral diseases	
Crinkle*	genus *Carmovirus*, Turnip crinkle virus (TCV) (former 'Yugoslavia')
Mosaic	genus *Caulimovirus*, Cauliflower mosaic virus (CaMV) genus *Cucumovirus*, Cucumber mosaic virus* (CMV) (Hungary) genus *Comovirus*, Radish mosaic virus (RaMV) genus *Potyvirus*, Turnip mosaic virus (TuMV)
Yellows	genus *Luteovirus*, Beet western yellows virus (BWYV) genus *Cytorhabdovirus*, Broccoli necrotic yellows virus* (BNYV)
Phytoplasmal diseases	
Aster yellows and phyllody	Aster yellows phytoplasma
Miscellaneous diseases and disorders	
Autogenic necrosis	Genetic disorder
Black speck	Physiological
Sulfur deficiency	Sulfur deficiency
Tipburn	Calcium deficiency

Note: * Not known to occur naturally in the United States.

Source: Reproduced from the American Phytopathological Society listing of known *Brassica* pathogens and disorders (2001).

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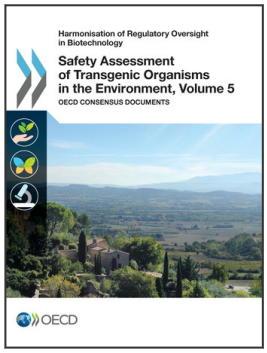

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