# DEVELOPMENT OF YELLOW SEEDED *BRASSICA NAPUS* L. THROUGH INTERSPECIFIC CROSSES

A Thesis

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by

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# ABSTRACT

Yellow seeded *Brassica napus* was developed by introgressing genes for yellow seed colour from the two mustard species, *B. juncea* and *B. carinata* into rape, *B. napus* through interspecific crosses. This achievement was based on the hypothesis that it should be possible to incorporate genes for yellow seed colour in the A and C genomes of *B. napus* L. (genome AACC), from yellow seeded *B. juncea* Czern. (genome AABB) and yellow seeded *B. carinata* Braun (genome BBCC). The interspecific crossing scheme involved the following steps.

Black seeded, fully pigmented *B. napus* ( genome AACC) was crossed with yellow seeded *B. juncea* (genome AABB), and with yellow seeded *B. carinata* (genome BBCC). The objective of these two interspecific crosses was the introgression of genes for yellow seed colour from the A genome of *B. juncea* and the C genome of *B. carinata* into the A and C genomes of *B. napus*, respectively. The interspecific $F_1$ generations were backcrossed to *B. napus* to shift the genome composition towards *B. napus*, thus eliminating undesirable B genome chromosomes and to improve fertility.

Backcross $F_2$ ($BCF_2$) plants of the (*B. napus* x *B. juncea*) x *B. napus* cross, carrying genes for yellow seed colour from *B. juncea* in their A genome, were crossed with $BCF_2$ plants of the (*B. napus* x *B. carinata*) x *B. napus* cross, carrying genes for yellow seed colour from *B. carinata* in their C genome. The objective of this intercrossing was to combine the A and C genome yellow seeded characteristics of the two backcross populations into one genotype.

The $F_2$ generation of the $BCF_2$ intercrosses was grown in the field, and $F_2$ plants were individually harvested. Seed colour of $F_2$ plants was visually rated. The hypothesis was confirmed with the identification of 91 yellow seeded plants from the 4858 inspected plants and that the interspecific crossing and selection scheme was successful in introgressing the genes for yellow seed colour from *B. juncea* and *B. carinata* into *B. napus*. It is

concluded, from the results of this study, that genes for yellow seed colour must be present in the homozygous recessive condition in both the A and C genomes of *B. napus* to achieve yellow seeded forms in this species. Yellow seeded *B. juncea* and *B. carinata* were suitable sources of genes for yellow seed colour, and their introgression into the *B. napus* A and C genomes, respectively, was a successful strategy for developing yellow seeded *B. napus*.

The number of yellow seeded plants, that segregated in six of the 20 $F_2$ families studied, closely approximated a 1:15, two gene segregation ratio which supported the hypothesis of introgression of one seed colour gene each from the A genome of *B juncea* and the C genome of *B. carinata* into the A and C genomes of *B. napus*. It is further concluded that black seed colour in *B. napus* is dominant over yellow seed colour, and that yellow-brown seeded *B. napus* plants are the result of incomplete dominance relationships of brown over yellow in the C genome of *B. carinata*.

The yellow seeded *B. napus* plants developed in this study provide a new source of yellow seededness in *B. napus* which may be utilized in breeding yellow seeded *B. napus* cultivars.

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# 1 INTRODUCTION

Rapeseed is Canada's most important oilseed crop. The two species grown are Argentine or rape (*Brassica napus* L.) and Polish or turnip rape (*B. rapa* L. syn. *B. campestris*). Canola quality cultivars have been developed in both species, and these are presently grown in Canada as sources of edible oil and high quality meal for animal feed. The oil of canola cultivars is low in erucic acid (<2 % of total fatty acids) and the meal has a low glucosinolate content (<30 μmoles of aliphatic glucosinolates per one gram of oil-free meal). The development and introduction into commercial production of canola quality rapeseed was the basis for the establishment of the canola industry in Canada.

Further seed quality improvements can be achieved in canola through the development of yellow seeded forms. This has already been accomplished in turnip rape where yellow seeded cultivars such as Tobin and AC Parkland are widely grown. Yellow seeded cultivars of turnip rape have higher seed oil, meal protein and lower fibre contents than brown seeded cultivars (Stringam *et al.* 1974; Jönsson, 1977; Hutcheson, 1984). The non-pigmented seed coat of yellow seeded types was thinner, lower in crude fibre, and higher in protein and oil contents than the seed coat of brown seeded types, and was found to be also more digestible in swine rations (Slominski and Campbell, 1991). In addition, the embryo within yellow seeds was heavier and larger than in brown seeds (Stringam *et al.* 1974).

Comparative studies of yellow and brown seeded mustard in *B. juncea* (L.) Czern. (Woods, 1980) and in *B. carinata* A. Braun (Getinet, 1986) have revealed that, in both species, yellow seeded forms had higher oil and protein and lower fibre contents than brown seeded forms. In mustard, yellow seeded forms occur naturally.

All presently grown cultivars of *B. napus* are black seeded. No naturally yellow seeded forms of *B. napus* exist. It can be assumed that yellow seeded *B. napus*, if it could be developed, would also have higher oil and protein and lower fibre contents than black seeded *B. napus*. It is therefore attractive to plant breeders to develop yellow seeded

*B. napus* through interspecific transfer of genes for yellow seed colour from turnip rape and the two mustard species. These attempts have been partially successful (Hou-Li *et al.* 1983; Bechyne and Aslam, 1986; Bechyne, 1987; Chen *et al.* 1988). However, colour of many of these yellow seeded selections was unstable and often reverted to brown or black. Many breeding institutions throughout the world are presently involved in developing yellow seeded *B. napus*, because of the expected higher oil and protein and lower fibre contents which would make such seed more valuable.

Past studies on the inheritance of seed colour have been directed toward *B. rapa* (Stringam, 1980; Schwetka, 1982; Hutcheson 1984), although inheritance of seed colour in *B. juncea* was studied by Vera *et al.* (1979); Aslamyousuf and Bechyne (1983a); Anand *et al.* (1985); Dhillon *et al.* (1986) and *B. carinata* by Yousuf (1982) and Getinet *et al.* (1987). Seed colour inheritance studies in *B. napus* (Shirzadegan, 1986; Hou-Li and Yong-Tong, 1987) were complicated by the fact that no true breeding yellow seeded *B. napus* forms were available for the investigations. These inheritance studies indicated that brown or black seed colour was dominant over yellow, and that only a few major genes control seed pigmentation in *Brassica* species.

The objective of the present study was to investigate the feasibility of transferring genes for yellow seed colour through interspecific crosses from the mustard species of *B. juncea* and *B. carinata* in *B. napus* with the objective to develop yellow seeded *B. napus*.

# 2 LITERATURE REVIEW

## 2.1. Cytogenetic Relationships Among *Brassica* Species

An interspecific hybridization programme must be based on detailed knowledge of the origin, evolution, and cytogenetic relationships of *Brassica* species. Such knowledge allows the formulation of crossing schemes that makes possible the transfer of genes of interest from related species.

The somatic chromosome number of *B. rapa* was originally determined by Takamine (1916) as 2n=20. Karpechenko (1922, 1929) established the chromosome of *B. nigra* Koch., *B. oleracea* L., *B. carinata* and *B. juncea* as 2n=16, 2n=18, 2n=34, and 2n=36, respectively. Morinaga (1928) determined the chromosome number of *B. napella* (2n=36) which was later found to be conspecific with *B. napus* (Olsson, 1945). Cytogenetic relationships among these six *Brassica* species were elucidated by the studies of Morinaga (1928, 1929a, b, c, 1931, 1933, 1934). He assigned the letter "A" to the *B. rapa*, "B" to the *B. nigra* and "C" to the *B. oleracea* genome. Based on his serial cytogenetic investigations on species hybridizations, Morinaga (1934) put forward the hypothesis that the six *Brassica* species are interrelated. He proposed that *B. rapa* (A, n=10), *B. oleracea* (C, n=9) and *B. nigra* (B, n=8) are elementary diploid species and the three secondary polyploid species originated from crosses between these three elementary diploid species. It is now well established that the *B. napus* amphidiploid (AC, n=19) arose from an interspecific cross between *B. rapa* and *B. oleracea*, *B. juncea* (AB, n=18) evolved in a similar way from a cross between *B. rapa* and *B. nigra*, and *B. carinata* (BC, n=17) arose from a cross between *B. oleracea* and *B. nigra*. This hypothesis was verified by U (1935) who was able to synthesize *B. napus*. He also crossed *B. napus* with *B. rapa*, *B. oleracea*, *B. juncea* and *B. carinata* and on the basis of crossability, he diagrammatically presented *Brassica* relationships in the form of triangle which is now known as the Triangle of U (Figure 1).

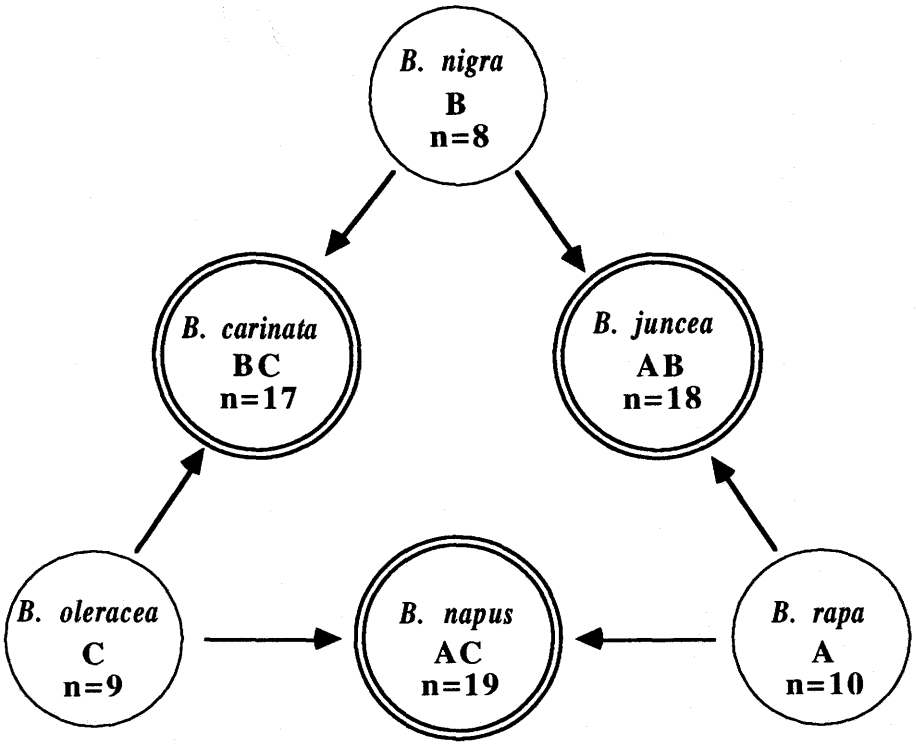


**Figure 2.1 Triangle of *Brassica* species relationships (after U, 1935)**

The findings of Morinaga and U were further confirmed by the synthesis of *B. napus* from *B. rapa* and *B. oleracea* by Frandsen (1947), Mizushima (1950a, b), Hoffmann and Peters (1958), Hosoda (1953), Olsson (1960b), Prakash and Raut (1983), Akbar (1987) and Chen *et al.* (1988, 1989). The amphidiploid origin of *B. juncea* was verified through artificial synthesis of this species based on crosses between *B. rapa* and *B. nigra* by Ramanujam and Srinivasachar (1943), Frandsen (1943), Mizushima (1950a, b), Olsson (1960a), Grabiec (1967a, b), Srinivasachar (1965), Prakash (1973a,b), Nurain (1979) and Mattson (1988). *B. carinata* was synthesized by Frandsen (1947), Mizushima (1950a, b), Pearson (1972) and Sarla and Raut (1988).

The three allotetraploid species are chromosomally balanced, show regular formation of bivalents in meiosis, homologous chromosome pairing and disomic inheritance (Prakash and Hinata, 1980).

## 2.2. Genome Relationships Between A, B and C Genomes

The karyotypes of the *B. rapa*, *B. nigra* and *B. oleracea* genomes revealed close genomic interrelationships. Richharia (1937a, b) investigated the somatic chromosome morphology of *B. rapa* and *B. oleracea* and recognized six to seven types of chromosomes based on their size and primary constrictions. He assigned the genomic formula ABCDDEEFFF to *B. rapa* and ABBBCDDEF to *B. oleracea*. As the *Brassica* chromosomes have certain limitations in cytological studies due to their extremely small size, he tried to confirm the morphology from the secondary chromosome pairing. These studies supported the six basic chromosome theory. Sikka (1940) confirmed the allotetraploid origin of *B. juncea* and *B. napus* by comparing their somatic chromosome constitution using chromosomal size with those of the three elementary species. He observed that *B. juncea* exhibited the sum constitution of *B. rapa* and *B. nigra* chromosomes. Similarly *B. napus* was observed to have the sum of *B. rapa* and *B. oleracea* chromosomes.

The close relationship of the A, B and C genomes were demonstrated by Mizushima (1950a, b) through his studies of chromosome paring in interspecific hybrid plants. He observed 0-4 quadrivalents in a synthetic *B. carinata* (BBCC) obtained from the *B. oleracea* x *B. nigra* cross, after colchicine doubling of the BC hybrid chromosome. He ascribed the four quadrivalents as due to allosyndesis between the B and C genomes as a consequence of partial homology. He observed up to nine bivalents in the ABC triploid derived from *B. carinata* (BBCC) x *B. rapa* (AA). Similarly in the ABC triploid of *B. napus* (AACC) x *B. nigra* (BB), the frequency of bivalents was 7-10. In the ACC triploid of *B. napus* x *B. oleracea*, he found up to six trivalents. These conjugations led him to the conclusion that there are six allosyndetic pairs between the A and C genomes. The partially homologous chromosome constitutions in *Brassica* species were further confirmed by Röbbelen (1960) through studies on the morphology of pachytene chromosomes of the three elementary *Brassica* species. Six basic types of chromosomes were recognized in all

three species on the basis of absolute length, symmetry of arms and the shape of the heterochromatic centromere region. He proposed genetic constitutions of the three genomes: AABCDDEFFF for *B. rapa*, ABCDDEFF for *B. nigra* and ABCCDDEEF for *B. oleracea*, and confirmed that these are the secondarily balanced polyploids originated from a common prototype with the basic chromosome number of six (x=6).

Interspecific hybrids resulting from crosses among the three elementary *Brassica* species exhibit variable degrees of chromosome pairing (Prakash and Hinata, 1980). Because such crosses are readily accomplished, a comparison of the elementary diploid species, amphihaploid hybrids, and their derived amphidiploids can be used to estimate the meiotic behaviour. The degree of homology between the A, B and C genomes was further revealed by the study of chromosome pairing in different types of hybrids derived from species hybridizations. Busso (1985) observed no differences in the chromosome pairing between hybrids of A.AC ( *B. rapa* x *B. napus)* and AC.A (*B. napus* x *B. rapa*), in both cases trivalents appeared. A similar configuration was observed in the BC.A hybrid (*B. carinata* x *B. rapa*), but trivalents did not occur in the A.BC (*B. rapa* x *B. carinata*) hybrid. This led him to conclude that the B genome plays little part in the chromosome pairing.

The degree of homology between the A, B and C genomes was further confirmed by cytological studies of interspecific hybrid plants (Attia and Röbbelen, 1986). It was found that A and C genomes are more closely related than either is to B. The conclusion is based on the fact that AC amphihaploids always show higher preferential pairing and a more regular meiotic behaviour than either AB or BC amphihaploids. They observed 5.9 to 8.7 bivalents per pollen mother cell (PMC) in AC amphihaploids derived from *B. rapa* x *B. oleracea* with a mean of 7.3 bivalents per PMC, while in BC amphihaploids (*B. nigra* x *B. oleracea*) the average chromosome pairing was 1.9 per PMC with 13 out of 17 chromosomes were left unpaired. In AB amphihaploids (*B. rapa* x *B. nigra*), the average number of bivalents was 4.4. The low degree of pairing observed in amphihaploids with the B genome

was considered to cast doubt on the hypothesis of a common ancestor for all three species. It was also suspected that *B. nigra* might carry a genetic system which suppressed homologous pairing in *Brassica*. Phylogenetic studies would be essential to assess how much of the pairing that occurres between chromosomes of the different genomes, is in fact allosyndetic. To discriminate between auto- and allo-syndetic pairing a "triploid test" can be utilized in which a digenomic hybrid combing two homologous genomes and a third non-homologous one is analyzed for its chromosome pairing in PMC's. Chromosome paring in trigenomic haploids, and consequently the involvement of pairing between B and the other two genomes was confirmed (Busso *et al.* 1987). Six trigenomic haploids, A.BC, BC.A, B.AC, AC.B, C.AB, and AB.C were derived from interspecific crosses followed by embryo rescue. In the hybrid plants, more than half of the 27 chromosomes were involved in bivalent pairing and six to ten bivalents were observed in 50-93.3% of PMC's. These results indicated the inability of the B-genome to affect the level of pairing between homologous chromosomes of the A and C genomes. No cytoplasmic effects on the regulation of pairing and no genetic factor(s) for suppression of pairing were associated with the B genome. Studies on digenomic triploids, synthesized through embryo rescue, came to the same conclusion (Attia *et al.* 1987). In the triploid hybrid of the BC.B (*B. carinata* x *B. nigra*) two B genomes of *B. nigra* paired with each other to form 8 bivalents in 71% of the PMC's whereas the tendency of allosyndetic pairing between the B and C genomes was very low (5.5%). The triploid hybrid of C.BC (*B. oleracea* x *B. carinata*) exhibited 9 bivalents and 8 univalents indicating preferential pairing of the 9 C genome chromosomes and no chromosome pairing between the C and B genomes. On the other hand, a complete pairing of A genome chromosomes in the A.AC (*B. rapa* x *B. napus*) and AC.A (*B. napus* x *B. rapa*) hybrids also resulted in a high frequency for allosyndetic pairing between A and C genomes expressed by the formation of one or more trivalents in over 50% of all PMC's examined.

## 2.3. Interspecific Crosses Among Species with Genome "A" in Common

### 2.3.1. Crosses between *B. napus* and *B. rapa*

Interspecific crosses between *B. napus* and *B. rapa* have been relatively easy to carry out but crosses have always been more successful when *B. napus* has served as the female parent (U, 1935; Mizushima, 1950a; Grabiec, 1967a, b; Lammerink, 1970; McNaughton, 1973; Johnston, 1974; Mackay, 1977; Heyn, 1977; Salam and Downey, 1978; Olsson and Ellerström, 1980; Tang, 1982; Gowers, 1982; Aslamyousuf and Bechyne, 1985; Banga, 1986; Chang and Tai, 1986; Wahiduzzaman, 1987; Bing, 1991). Mackay (1977) and Wahiduzzaman (1987) reported that seed set in the interspecific cross between *B. napus* and *B. rapa* was equal to that obtained in intraspecific crosses within these two species. Röbbelen (1966) harvested fewer seeds and observed poor pollen germination and pollen tube growth of *B. napus* pollen when *B. rapa* was used as female parent. The lower rate of hybrid seed production in the reciprocal cross *B. rapa* x *B. napus* was reported by Labana *et al.* (1983), and Rousselle and Eber (1983). Nevertheless, Lammerink (1970), Shiga (1970), Heyn (1977), Yamagishi and Takayanagi (1982) and Bing (1991) reported that seed set per pollinated flower was similar in both directions of the crosses between *B. rapa* and *B. napus*.

Cytogenetic studies on $F_1$ hybrids of this cross revealed regular meiotic behaviour. Ten bivalents and nine univalents were observed at metaphase I. Ten Bivalents resulted from the pairing of both A genome chromosomes of *B. rapa* and *B. napus* while nine univalents of the C genome remained unpaired (U, 1935; McNaughton, 1973; Aslamyousuf and Bechyne, 1985; Chang and Tai, 1986). As expected, $F_1$ plants had low pollen production and fertility, but always some seeds were produced. U (1935) observed 10% average fertility in the $F_1$ plants derived from a *B. rapa* x *B. napus* cross. Mackay (1977) and Wahiduzzaman (1987) observed good pollen production of $F_1$ hybrids of *B. napus* x *B. rapa*. Although pollen viability appeared to be less than 60%. He also harvested 3.5 seeds/pod from $F_1$ hybrid plants. McNaughton (1973) reported that $F_1$ hybrid plants from

*B. napus* x *B. rapa* were almost entirely sterile and only one seed was produced from 220 self pollinated buds. Heyn (1977) reported that seed production on $F_1$ hybrids of *B. napus* x *B. rapa* ranged from 5 to 40 seeds per plant. Salam and Downey (1978) reported similar results with some plants being completely sterile. Bing (1991) reported a range in pollen viability from 0-25% in most of the $F_1$ plants and harvested 4.3 seeds per pollinated flower. Salam and Downey (1978) reported that more backcross seed was produced when $F_1$ plants of the *B. napus* x *B. rapa* cross were used as females in backcrosses with *B. rapa*. Seedling mortality and hybrid sterility was much greater in backcrosses than in $F_2$ progenies. Fertility increased in $F_3$ to $F_5$ generations. However, no seed was obtained from $BC_1F_2$ plants. Meiotic chromosome counts of $F_2$ and $BC_1F_2$ progenies indicated that plants with higher chromosome numbers appeared more frequently than those with lower chromosome numbers. Mackay (1977) also used $F_1$ hybrids as pollen parents in backcrosses with *B. napus*. Chromosome counts of the backcross hybrids of *B. napus* x (*B. napus* x *B. rapa*) suggested that gametes of n = 10, 11, 17, 18, and 19 were at an advantage as indicated by the higher than random frequencies of 2n=29, 30, 36, 37 and 38 chromosome plants. It was suggested that competition among pollen grains at the stigmatic surface or in the style resulted in less effective fertilization by aneuploid pollen with chromosome numbers of n = 12, 13, 14, 15 and 16.

*B. rapa* and *B. napus* are important oilseed crops and both can be readily hybridized. Plant breeders have taken advantage of this fact to combine the desirable characteristics of these two species into one genotype. Several techniques were used to transfer desirable traits through interspecific crosses between *B. rapa* and *B. napus*. Olsson and Ellerström (1980) found that plants with *B. napus* morphology and fertility were obtained by interpollinating plants of the allotriploid hybrid (AAC) or backcrossing to *B. rapa* and *B. napus*. Mizushima (1950a, b) and McNaughton (1973) created hexaploid *B. napocampestris* L. (AAAACC) by doubling the chromosomes of interspecific hybrids (AAC) from the *B. napus* (AACC) x *B. rapa* (AA) cross.

Johnston (1974) used the B. *napocampestris* concept to transfer genes for resistance to clubroot (*Plasmodiophora brassicae* Wor.) from *B. rapa* into forage rape (*B. napus*). Interspecific hybrid (AAC) plants were produced by pollinating the susceptible *B. napus* var. Nevin with pollen from the *B. rapa* var. Waaslander, which was resistant to several races of this fungus. Resistant plants were obtained after two generations of backcrossing to *B. napus*. These plants were morphologically identical to *B. napus*, the recurrent parent and had a *B. napus* chromosome complement.

Resistance to clubroot was also transferred into *B. napus* from *B. rapa* by crosses between *B. rapa* and *B. napus* followed by selection of resistant *B. napus* like plants in generations following the cross. Lammerink (1970) observed no major fertility barriers between the *B. napus* and *B. rapa*. *B. napus* plants, highly resistant to clubroot races B, C and E were selected in high yielding $BC_1F_3$ progenies from the cross of resistant *B. rapa* and susceptible *B. napus* and backcrossed to *B. napus*. Gowers (1982) also followed a similar approach to develop clubroot resistant *B. napus*.

Mackay (1977) reported the transfer of self-incompatible alleles from *B. rapa* into self-compatible *B. napus* in order to facilitate hybrid seed production in *B. napus*. Self-compatible cultivars Emerald and Nevin of *B. napus* were crossed with *B. rapa* and $F_1$ hybrid plants (as female) were backcrossed to *B. napus*. Self-incompatible plants of *B. napus* with 38 chromosomes were recovered from backcross progenies.

### 2.3.2. Crosses between *B. napus* and *B. juncea*

The fact that *B. napus* and *B. juncea* share the A-genome makes it possible to cross these two species. Greater success has been achieved when *B. juncea* was used as the female parent in crosses with *B. napus* than in crosses with *B. napus* as female. Roy (1977) reported wide variations in seed set in different crosses between *B. napus* as the female and *B. juncea* as the male. In some cases, he observed well developed pods, and a comparatively good seed set of 10 to 52% (per 100 pods formed) while in some crosses no seed was produced even though very well developed pods were produced. In the reciprocal combinations of *B. juncea* x *B. napus* he obtained 60 to 100% seed set in some cases, but pods without seed in others. He reported that problems associated with interspecific crosses were cross-incompatibility and hybrid sterility. Roy (1980) found that seed set can be increased with identification and use of cross compatible parents. Labana *et al.* (1983) reported that seed set, germination of hybrid seed and pollen fertility were always greater in the *B. juncea* x *B. napus* than in reciprocal crosses. Dhillon *et al.* (1985) obtained good seed set in *B. juncea* x *B. napus* crosses but failed to produce any hybrid seed when *B. napus* was used as female parent. Similar results were obtained by Rousselle and Eber (1983). Gerdemann and Sacristán (1986) applied ovule and embryo culture techniques to overcome the cross incompatibility in interspecific crosses. They obtained nine plants from 100 ovules cultured *in vitro* from the *B . napus* x *B. juncea* cross. Bajaj *et al.* (1986) also using ovule culture, obtained 40-42 plants per 100 cultured ovules. Wahiduzzaman (1987) observed extremely low seed set when *B. napus* was used as female parent in crosses with *B. juncea* in spite of well developed pods. *B. juncea* x *B. napus* showed a high cross-compatibility and he harvested 1.2 seeds per pod. He found that seed set was increased when $F_1$ hybrids were backcrossed to *B. napus*. Bing (1991) obtained 4.3 seeds per pollinated bud from *B. juncea* x *B. napus* crosses and observed 0-25% pollen viability in $F_1$ plants.

*B. napus* and *B. juncea* share the A genome. There are expected to have complete homology and produce ten bivalents. The B genome of *B. juncea* and the C genome of *B. napus* have partial homology. Prakash and Chopra (1988) observed 10-14 bivalents and 9-17 univalents in metaphase-I in hybrids plants of *B. juncea* and *B. napus*. Any bivalents greater than 10 would be the result from non-homologous pairing of chromosomes from the B and C genomes.

*B. juncea* and *B. carinata* species are known to posses genes for resistance to diseases, drought, seed shattering and are also a source of yellow seed colour genes. The possibility of the transfer of these characters to *B. napus* could be anticipated on the basis of evolutionary relationships existing between the *Brassica* species (U 1935). Many breeders have attempted to hybridize these species in an effort to transfer these characters from *B. juncea* to *B. napus* through interspecific hybridization. Roy (1978) studied disease reaction in $F_1$ and $F_2$ generations in the crosses between *B. juncea* and *B. napus*. He reported that gene(s) for adult plant resistance to blackleg (*Leptoshaeria maculans*) were present in the A genome of *B. juncea*. While gene(s) for complete resistance (seedling plus adult plant) were located in the B genome of *B. juncea* and *B. carinata*. He successfully transferred blackleg resistance from *B. juncea* to *B. napus* through an interspecific cross between *B. napus* and *B. juncea*, however, he found that the juncea type resistance appeared to be inherited as a major gene or genes (Roy (1984).

Sacristán and Gerdemann (1986) attempted to transfer blackleg (*Phoma lingam*) resistance from *B. carinata* and *B. juncea* to *B. napus* through interspecific hybridization. They found that both *B. napus* x *B. juncea* and *B. napus* x *B. carinata* hybrids had the same high level of resistance as the respective resistant parent, but transfer of resistance behaved differently in the two hybrids. In *B. napus* x *B. juncea* hybrids, resistance was transferred to about half of the plants in the first backcross progeny, whereas the resistance of the *B. napus* x *B. carinata* hybrids was almost totally lost in the first backcross. They suggested that these differences could be due to the fact that a) the resistance gene in

*B. juncea* was located in the A-genome, b) the recombination between genomes B and C (*B. juncea* cross) was more frequent than between genomes B and A (*B. carinata* cross), c) the reversion to the normal chromosome number of *B. napus* occurred more rapidly in backcrosses with *B. carinata* hybrids than with *B. juncea* hybrids.

Roy and Tarr (1984) obtained one *B. napus* type plant with poor fertility in the $F_2$ generation from the interspecific cross between zero erucic acid forms of *B. juncea* x *B. napus*. The seed oil of this single plant contained 30.4% linoleic, and 8.2% linolenic acid. In the $F_5$, they isolated plants with good fertility, moderate to high linoleic acid and reduced levels of linolenic acid.

Seed shattering is a major problem in *B. rapa* and *B. napus* while *B. juncea* and *B. carinata* were found to have better shattering resistance (Roy, 1977). Prakash and Chopra (1988) attempted to transfer shattering resistance from *B. juncea* into *B. napus*. They found that shattering resistance was transferred through non-homologous chromosome recombinations in the $F_1$ hybrid (AABC) between B and C genomes of *B. nigra* and *B. oleracea*, respectively. The interspecific exchange of genes, resulting in non-shattering recombinants, appeared to be a rare event because only one plant of the desirable phenotype was recovered in the $BC_3$ generation.

Mathias (1985) developed cytoplasmic male sterility (CMS) lines from *B. juncea* x *B. napus* interspecific crosses followed by four backcrosses to *B. napus*. However, no restorer genes were identified. Bartkowiak-Broda *et al*. (1991) attempted the development of CMS double low winter rape (*B. napus*) from crosses with *B. juncea*. Wahiduzzaman (1987) successfully introgressed earliness into *B. napus* by using *B. rapa*, *B. juncea*, *B. carinata* and *B. oleracea* var. *alboglabra* as donor species. Banga (1988) developed a *B. juncea* line in which some B genome chromosomes were replaced with *B. napus* C genome chromosomes. He reported that this line was meiotically stable with normal male and female fertility.

## 2.4. Interspecific Crosses Among Species with Genome "C" in Common

### 2.4.1. Crosses between *B. napus* and *B. oleracea*

Röbbelen (1966) harvested on average one hybrid seed from every third pod of the cross *B. napus* x *B. oleracea* and one seed from every fourth pod formed in the reciprocal cross. He observed that pollen germination and pollen tube growth were greater in the *B. napus* x *B. oleracea* cross than in the reciprocal. Chiang *et al.* (1977) obtained 0.049 hybrids per pollination from the cross *B. napus* x *B. oleracea* and the number of hybrids per pollination was increased 8.9 times when tetraploid *B. oleracea* was used in crosses with *B. napus*. The reciprocal crosses were unsuccessful, regardless of whether diploid or tetraploid *B. oleracea* served as the female parent. They also found that the physiological condition of the female plant and favourable environmental conditions improved the chance for hybrid embryos to develop into viable seeds. Yamagishi and Takayanagi (1982) harvested only 1.3 seeds per pod from the *B. napus* x *B. oleracea* cross due to poor embryo development. Wahiduzzaman (1987) also found that the production of hybrid seeds in the *B. napus* x *B. oleracea* var. *alboglabra* cross was highly dependent on environmental conditions under which the plants were grown as well as on the genotypes used in the crosses. He obtained many seeds from the *B. napus* x *B. oleracea* cross while in the reciprocal direction, very few seeds were produced even though the pods developed normally. He also found that crosses with tetraploid *B. oleracea* were more successful than crosses with diploid *B. oleracea.* Dharmaratne and Hodgkin (1988) found that pollen from *B. oleracea* usually failed to penetrate the stigmatic surface of *B. napus* pistils. They suggested that interspecific incompatibility could be overcome by intensive bud pollination or by the use of cycloheximide. Quazi (1988) applied the embryo rescue technique to avoid abortion and poor embryo development. He obtained 24 plants from 100 cultured embryos in *B. napus* x *B. oleracea* (broccoli and kale) crosses.

*B. oleracea* consists of many important vegetable crops including cabbage, kohlrabi, broccoli, brussel sprout, and cauliflower. In interspecific crosses between *B. napus* and *B. oleracea*, different types of *B. oleracea* were used. Each type has a different growth habit and requires specific growing conditions so that the results of such interspecific crosses are not necessarily comparable.

### 2.4.2. Crosses between *B. napus* and *B. carinata*

Roy (1977) observed a wide variation in seed set in crosses between *B. napus* x *B. carinata* and obtained 0 to 28% seed set (per 100 pods formed) but did not obtain any seed from reciprocal crosses. Rousselle and Eber (1983) reported that fewer embryos developed in the *B. carinata* x *B. napus* than in the reciprocal cross. Sacristán and Gerdemann (1986) obtained 29 plants from 100 embryos cultured from the *B. napus* x *B. carinata* cross. Aslamyousuf and Bechyne (1983b) observed differences in the ability of various genotypes of *B. napus* and *B. carinata* to support the development of interspecific hybrid embryos. They harvested 5 to 173 seeds (per 100 pods formed) from crosses of *B. napus* x *B. carinata* and fewer seeds (3 to 38) from the reciprocal crosses. Wahiduzzaman (1987) also observed varying results depending on genotype and plant growing conditions in *B. napus* x *B. carinata* crosses. In one case, he obtained 71 seeds per 100 pollinations while in another case only one seed was obtained. In the reciprocal direction, very few hybrid seeds were harvested. He suggested that this may be due to pollen discrimination by *B. carinata* as well as embryo and/ or endosperm incompatibility.

*B. napus* and *B. carinata* have complete homology for the C genome which is common in both species and partial homology for the A and B genomes. Aslamyousuf and Bechyne (1983b) observed 1 to 3 trivalents, 9 to 14 bivalents and 7 to 18 univalents in $F_1$ hybrids of *B. napus* x *B. carinata*. They suggested that 1 to 3 trivalents and more than 9 bivalents might be anticipated as a result of pairing of chromosomes between the B and C genomes.

## 2.5. Development of Yellow Seeded *B. napus*

Several approaches have been used to develop yellow seeded *B. napus.* Bechyne (1987) crossed *B. oleracea* with *B. rapa* in an attempt to produce yellow seeded *B. napus*. He crossed *B. rapa* var. yellow sarson with *B. oleracea* var. *acephala* and chromosomes were doubled with colchicine. Only one plant of the resynthesized *B. napus* was obtained which produced yellowish seeds. After repeated generations of selfing and selection for seed colour, improved yellow seeded forms were obtained, but the yellow colour was not as bright and deep as that in the *B. rapa* parent.

Chen *et al.* (1988) resynthesized *B. napus* by crossing black and light brown seeded *B. alboglabra* with yellow seeded forms of *B. rapa*. Chromosomes of the AC interspecific $F_1$ hybrid plants were successfully diploidized with colchicine. The seed colour of resynthesized *B. napus* was either black or light brown depending on the seed colour of the *B. alboglabra* parent used. They found that seed colour was controlled by multiple loci and concluded that the genes for black or brown seeded characteristics mask the expression of the yellow or light brown seeded character. The difficulty in obtaining true yellow seeded forms of *B. napus* appeared to be due to the lack of truly yellow seeded *B. oleracea* genotypes. It was suggested that it might be possible to intogress genes for yellow seed colour from *B. carinata* into *B. oleracea.*

Hou-Li *et al.* (1983) transferred genes for yellow seed colour from *B. rapa* into *B. napus* through interspecific crosses. They observed that seeds of interspecific plants had different shades of yellow colour. The occurrence of yellow seeds in the yellow seeded interspecific derived population was increased from 38 to 100% after seven generations of continuous selfing, sibbing and interline crossing.

Aslamyousuf and Bechyne (1983b) used a black seeded *B. napus* as the female in crosses with yellow seeded *B. carinata*. The $F_1$ hybrid plants were found to exhibit intermediate morphological characters but were morphologically similar to *B. carinata*. Segregation for a few characters was observed in the $F_2$ and three yellow seeded plants were obtained.

Wahiduzzaman (1987) also attempted to transfer the genes for yellow seed colour from *B. rapa*, *B. juncea* and *B. carinata* into *B. napus* through interspecific hybridization. Although the *B. rapa*, *B. carinata* and *B. juncea* parents were yellow seeded, the introgression of the genes for yellow seed colour from these species into *B. napus* was not successful. All *B. napus* like segregants obtained from interspecific crosses were black or brown seeded. This led him to the conclusion that gene introgression for yellow seed colour from these species into *B. napus* is difficult due to complex gene interactions.

## 2.6. Inheritance of Seed Colour in *Brassica* Species

The efficient breeding of yellow seeded cultivars in any *Brassica* species requires an understanding of its inheritance. A number of researchers studied the inheritance pattern of seed colour within *Brassica* species. Mohammad *et al.* (1942) was the first to investigate seed colour inheritance in *B. rapa*. They studied the seed colour in dark reddish brown, reddish brown, yellowish brown and yellow seeded cultivars of *B. rapa*. They found that three independent loci $Br_1$, $Br_2$ and $Br_3$ controlled seed colour. Dominance at each of the three loci would result in dark reddish brown, reddish brown and yellowish brown seed colour, respectively, provided that alleles at the other loci were recessive. The homozygous recessive condition at all three loci would result in a yellow seed. They also observed different shades of yellow colour. They believed that this variation was due to the presence or absence of other modifying genes, besides the major genes conditioning a particular seed colour.

Ahmed and Zuberi (1971) found that reddish brown seed colour in *B. rapa* was controlled by a single dominant gene. Salam and Nessa (1979) also conducted seed colour inheritance studies in *B. rapa* and obtained similar results. However, the occurrence of yellowish brown seed in the yellow phenotypic group possibly indicated the effect of some minor genes.

Stringam (1980) studied the inheritance of seed colour in crosses between yellow sarson and brown seeded Canadian cultivars of *B. rapa*. A 2 brown:1 yellow-brown:1 yellow backcross ratio was observed in four crosses with different brown seeded cultivars and yellow sarson which was consistent with the expectation for a two gene model of dominant inheritance. With this model, brown colour would result from a dominant allele at one of the loci, yellow brown seed from dominance at the other independent locus with recessiveness at the first, and yellow seed colour would be expected when both loci were homozygous recessive. He indicated that some modifier genes were also present which affected pigment production in a genetically yellow seed.

Schwetka (1982) crossed six yellow seeded *B. rapa* strains, two yellow sarson and four European cultivars with one brown seeded cultivar. $F_1$ plants of all six crosses produced brown seeds indicating dominance of brown over yellow. The results of crosses between *B. rapa* yellow sarson and dark seeded types suggested a two gene model of inheritance ($Br_1/br_1$, $Br_3/br_3$) with epistatic effects. Two other yellow seeded strains of the *B. rapa* oleifera group also showed digenic segregation in progeny of crosses with a brown seeded cultivar. One yellow seeded strain exhibited monogenic inheritance with dominance of brown over yellow in a cross with the brown seeded cultivar while $F_2$ segregation data of a cross between another yellow seeded strain and the same brown seeded cultivar suggested control of seed pigmentation by three dominant genes with epistatic effects. This segregation data further suggested that the yellow seed colour of B. *rapa* yellow sarson and that of *B. rapa* oleifera were conditioned by different series of recessive alleles.

Vera *et al.* (1979) studied the inheritance of seed colour in crosses between three brown seeded cultivars and one yellow seeded cultivar of *B. juncea*. They found that seed colour was controlled by two independent loci, $R_1$ and $R_2$, either of which produced brown seed when a single dominant allele was present. Yellow seed resulted when all alleles were recessive. These results were later confirmed in a second study (Vera and Woods, 1982).

Aslamyousuf and Bechyne (1983a) found that seed pigmentation in *B. juncea* was controlled by two independent duplicate genes, each of which separately or both in combination produced brown seeds. The absence of dominant alleles in both loci produced yellow seed. Anand *et al.* (1985) studied seed colour inheritance in crosses between six yellow seeded *B. juncea* cultivars from Poland and four brown seeded Indian types of *B. juncea*. He reported a digenic mode of inheritance with epistatic interaction due to recessive alleles. Dhillon *et al.* (1986) reported that seed colour was controlled by two duplicate genes in crosses between yellow and brown seeded cultivars of *B. juncea*. He also reported that brown seed colour was dominant over yellow.

In *B. carinata*, inheritance of seed colour was investigated by Yousuf (1982) in crosses between brown and yellow seeded lines. He reported that a gene B produced brown seeds with brown being dominant over yellow. Another gene Y produced yellow seeds when the allele at the B locus was recessive, while homozygous recessive alleles at both loci also produced yellow seeds. The interaction between both the genes resulted in the production of yellowish brown seed. Getinet *et al.* (1987) studied the inheritance of the brown seed colour of *B. carinata* S-67 in crosses with two yellow seeded lines. They found that seed colour was controlled by two alleles at one locus, and brown seed colour was incompletely dominant over yellow seed colour.

Inheritance of seed colour in *B. napus* was studied by Shirzadegan and Röbbelen (1986) in crosses between four yellow seeded lines and the black seeded winter rape cultivar Quinta. It was concluded that the black seed colour in winter rape was controlled by three genes which were designated $Bl_1$, $Bl_2$ and $Bl_3$. Yellow seeds were produced when all alleles at the three loci were recessive. Black colour resulted from dominance at the $Bl_1$ locus, while a dominant allele at either $Bl_2$ or $Bl_3$ and recessive alleles at the first locus ($bl_1bl_1$) resulted in brown seeds. Diallel crosses between the four different yellow seeded lines indicated that the loci responsible for the yellow seed character were not identical in

these lines. Hou-Li and Yong-Tong (1987) studied the genetic behaviour of yellow seed colour genes in crosses between yellow seeded and black seeded strains of *B. napus*. They found 1.63% yellow seeded plants in $F_2$ progenies which indicated trigenomic control of inheritance, but backcross data suggested a more complex inheritance.

# 3 MATERIALS AND METHODS

## 3.1. Interspecific Crossing Scheme for the Introgression of Genes for Yellow Seed Colour from the two Mustard Species *B. juncea* and *B. carinata* into rape *B. napus*

The mustard species *B. juncea* and *B. carinata* are related to rape *B. napus* as depicted in the triangle of U (1935) (Figure 2.1). *B. juncea* contains the genomes A and B, and *B. carinata* the genomes B and C while *B. napus* contains the genomes A and C. Yellow seeded forms of *B. juncea* and *B. carinata* may contain genes for yellow seed colour in both of their genomes as is evident from the digenomic inheritance pattern for seed colour in both species. The presence of genes for yellow seed colour in the A genome of *B. juncea* and the C genome of *B. carinata* was the basis for an interspecific crossing scheme designed to introgress genes for yellow seed colour from the A and C genomes of *B. juncea* and *B. carinata*, respectively, into the A and C genomes of *B. napus*. The objective of the crossing scheme was to block seed colour pigmentation in *B. napus* and to develop yellow seeded forms in this species. The interspecific crossing and selection scheme consisted of the following steps (Figure 3.1).

Black seeded fully pigmented cultivars of *B. napus* (AACC) were crossed with yellow seeded forms of *B. juncea* ($A^yA^yBB$) with the objective to transfer genes for yellow seed colour from the A genome of *B. juncea* into the A genome of *B. napus*. The interspecific hybrid plants of this cross were expected to be heterozygous for seed colour in the A genome ($AA^yCB$ ). The same black seeded *B. napus* cultivars (AACC) were also crossed with yellow seeded forms of *B. carinata* ($BBC^yC^y$) with the objective to transfer genes for yellow seed colour from the C genome of *B. carinata* into C genome of *B. napus*. The resulting interspecific hybrid plants were therefore expected to be heterozygous for seed colour in the C genome ($ACC^yB$). The crossing scheme did not require to presence of genes for yellow seed colour in the B genome of both *B. juncea* and *B. carinata*.

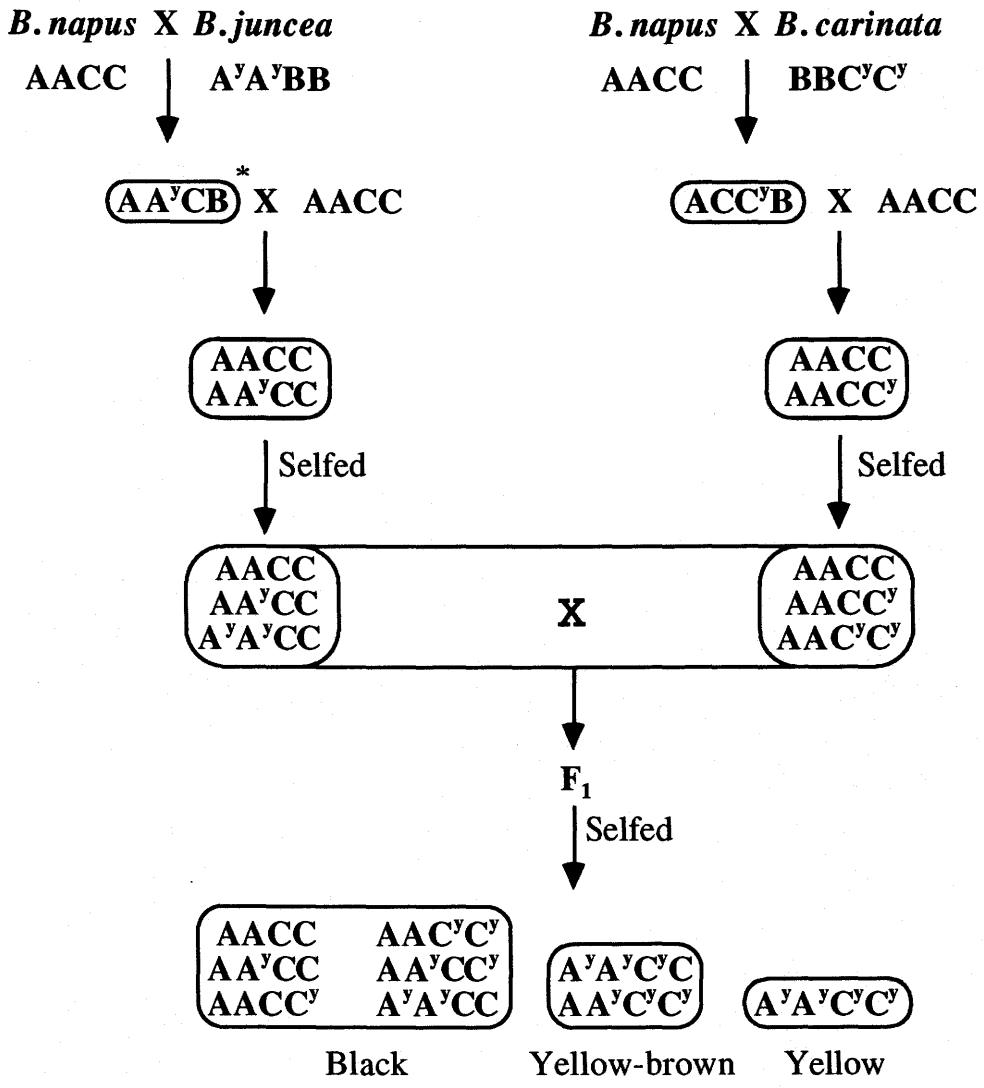


Figure 3.1 Interspecific crossing scheme for the incorporation of genes for yellow seed colour from the A genome of *B. juncea* and C genome of *B. carinata* into the A and C genomes of *B. napus*, respectively.

* Genome composition given in boxes indicate expected genotypes in that generation according to the working hypothesis described in detail in the text

$F_1$ plants of both interspecific crosses were backcrossed to *B. napus* with the objective to shift the genomic composition of the next generation towards the target species *B. napus*, and to eliminate undesirable B genome chromosomes. Backcrossing was also expected to improve fertility of the backcross plants. The backcross generations ($BCF_1$) were expected to contain some true 38 chromosome *B. napus* plants and many aneuploid individuals which would be less fertile than the *B. napus* plants. Plants with *B. napus* like morphology were identified and self pollinated to produce $BCF_2$ seed. The $BCF_2$ generation of the cross (*B. napus* x *B. juncea*) x *B. napus* was expected to contain *B. napus* individuals that were either AACC, $AA^yCC$ or $A^yA^yCC$ while those of the cross (*B. napus* x *B. carinata*) x *B. napus* were expected to be either AACC, $AACC^y$ or $AAC^yC^y$ (Figure 3.1).

Seed colour of all backcross plants from both $BCF_2$ generations was expected to be black, because it was hypothesized that for *B. napus* plants to produce yellow seed, genes for yellow seed colour must be present in both the A and C genomes. Homozygosity for yellow seed colour at either the A or C genome alone would not produce a yellow seed. It was therefore necessary to combine genes for yellow seed colour introgressed into either the A and C genomes of *B. napus* through crosses into one homozygous recessive genotype ($A^yA^yC^yC^y$). Therefore, crosses were made between $BCF_2$ plants of the two interspecific backcross generations. Many plants had to be used from both $BCF_2$ generations on either side of the cross, since plants in which genes for yellow seed colour were introgressed into the A or C genomes, respectively, could not be distinguished from plants that did not carry a yellow seed colour allele. Both types of plants were expected to be black seeded. The cross was expected to produce an $F_1$ generation that contains some double heterozygous individuals for yellow seed colour ($AA^yCC^y$) which would have resulted from crosses between $AA^yCC$ or $A^yA^yCC$ genotypes of (*B. napus* x *B. juncea*) x *B. napus* and $AACC^y$ or $AAC^yC^y$ genotypes of (*B. napus* x *B. carinata*) x *B. napus* crosses.

$F_1$ plants were grown and self pollinated with the objective to produce double homozygous recessive $F_2$ seed which, when grown out, would produce a yellow seeded *B. napus* plant. A large $F_2$ population would be required to identify the desired yellow seeded plants of the $A^yA^yC^yC^y$ genotype. It was in this sixth plant generation including the initial interspecific crosses that the working hypothesis would be proven to be feasible.

## 3.2. Parent Materials

Parents used in the interspecific crosses designed to transfer genes for yellow seed colour from the mustard species, *B. juncea* and *B. carinata,* into rape, *B. napus* were five yellow seeded *B. juncea*, two yellow seeded *B. carinata* and four black seeded *B. napus* cultivars/strains (Table 3.1). Seed was obtained from the *Brassica* collection residing at the Agriculture Canada Research Station, Saskatoon.

Table 3.1 Parents used in interspecific crosses

| Species | Seed colour | Cultivar/strain | Origin of cultivar or strain |
|---|---|---|---|
| *B. juncea* | Yellow | Lethbridge 22A | Agri. Canada, Lethbridge |
| | | Domo | Agri. Canada, Saskatoon |
| | | Cutlass | Agri. Canada, Saskatoon |
| | | Zem 83-406A | Agri. Canada, Saskatoon |
| | | ZYR-6 | Agri. Canada, Saskatoon |
| *B. carinata* | Yellow | PGRC/E 21164 | Ethiopian introduction |
| | | Dodolla | Ethiopian introduction |
| *B. napus* | Black | Argentine | Argentinian introduction |
| | | Midas | Agri. Canada, Saskatoon |
| | | Westar | Agri. Canada, Saskatoon |
| | | Profit | Agri. Canada, Saskatoon |

### 3.3. Growing conditions

Interspecific crosses were made using plants grown in the greenhouse and/or growth cabinet. Plants in the growth cabinet were grown under an 18 hours photoperiod and temperature of 18°C (day) and 14°C (night). Plants were grown in a soilless potting mixture in 13 cm diameter plastic pots (one plant per pot). Seeds from the $F_1$, $BCF_1$, and $BCF_2$ generations were first germinated on filter paper in petri dishes at room temperature (22°C) and germinated seeds transplanted into pots. The plants were watered regularly and fertilized weekly with a 20-20-20 NPK nutrient solution beginning one month after planting.

### 3.4. Identification of hybrid plants

The $F_1$ hybrids from the interspecific crosses were expected to have intermediate morphology and have reduced male fertility. Five $F_1$ plants were grown and the interspecific nature of hybrid plants was confirmed by plant morphological characteristics and pollen viability. Only those plants which were morphologically intermediate between the two parents and had poor pollen viability were classified as interspecific hybrids. This was possible since all three species employed in the interspecific crosses, differed from each other in a number of morphological characters such as upper leaf margin, upper leaf clasping, leaf surface and leaf colour. Interspecific hybrid plants were identified at an early stage of plant development.

Pollen viability of hybrid plants was evaluated by staining with aceto-carmine. Fresh anthers were removed from buds, which would be open the next day, using tweezers and a scalpel. Pollen grains were squeezed from the anthers onto a microscopic slide and stained with a drop of 2% aceto-carmine and covered with a coverslip applied without pressing. Pollen grains that did take up the stain were considered to be viable while those that did not were considered non-viable. Pollen viability was calculated as the percentage of stainable pollen grains.

## 3.5. Crossing and Selfing Techniques used in Interspecific Crosses and Backcrosses

Four parents plants from each *B. juncea*, *B. carinata* and *B. napus* variety or strain were grown. *B. juncea* parents were sown seven days later than the other two species to synchronize flowering. At flowering, buds on the female parents of *B. napus* plants which were about to open the following day were selected for crossing. Immature buds and already opened flowers were removed. Selected buds were emasculated with sterile tweezers and covered with a glassine bag to avoid contamination with foreign pollen. The following day, the emasculated buds were hand pollinated with fresh pollen from the designated male parent plant of *B. juncea* or *B. carinata* and a tag attached to the flower stalk which recorded. Pollination was repeated the following day to improve the seed set in these interspecific crosses. Pollinated buds were kept covered with glassine bags for a week to exclude contaminating pollen. At maturity seeds were harvested and stored in a cold room. The number of buds crossed, pods developed and seeds harvested for each cross were recorded.

Interspecific hybrid plants were backcrossed to their respective black seeded *B. napus* parent variety or strain to produce $BCF_1$ seed using the same crossing technique as for the production of $F_1$ seed. All $BCF_1$ seeds from all crosses were grown and the main raceme of each plant was covered with a glassine bag during flowering. All plants were hand pollinated to increase the seed set. $BCF_2$ seed was harvested from these selfed plants.

There were total 49 cultivar/strain cross combinations including 20 *B. napus* x *B. juncea*, and 15 *B. napus* x *B. carinata* interspecific crosses and 8 (*B. napus* x *B. juncea*) x *B. napus* and 6 (*B. napus* x *B. carinata*) x *B. napus* backcrosses (Table 3.2).

Table 3.2 Number of interspecific crosses and backcrosses made

| Cross | Number of crosses |
|---|---|
| *B. napus* x *B. juncea* | 20 |
| $F_1$ (*B. napus* x *B. juncea*) x *B. napus* | 15 |
| *B. napus* x *B. carinata* | 8 |
| $F_1$ (*B. napus* x *B. carinata*) x *B. napus* | 6 |

## 3.6. Intercrossing of $BCF_2$ plants and production of $F_2$ generation

Twenty $BCF_2$ plants of the cross (*B. napus* cv. Westar x *B. juncea* strain ZYR-6) x *B. napus* cv. Westar were crossed with twenty $BCF_2$ plants of the cross (*B. napus* cv. Westar x *B. carinata* strain PGRC/E 21164) x *B. napus* cv. Westar. It was necessary to concentrate the work on one cross, since it would have been physically impossible to evaluate a sufficient numbers of $F_2$ progeny in all crosses to identify the expected yellow seeded plants. It was felt that twenty paired crosses would be required to ensure the inclusion of backcross plants carrying genes for yellow seed colour in either the A or C genomes, a prerequisite for the identification of the double homozygous recessive ($A^yA^yC^yC^y$) yellow seeded plants in the $F_2$ generation. A total of forty paired crosses (including reciprocals) were made and $F_1$ seed harvested for each cross individually.

Four hundred $F_1$ plants (ten plants from each paired cross) were grown in pots in the greenhouse. Twenty one plants died during the seedling stage. Upon self pollination under glassine bags, 334 plants produced sufficient quantities of seed so that these could be evaluated in the field in single rows.

Fifty $F_2$ seeds were counted from each of 334 single $F_1$ plants and sown in three metre long single row plots in the field in the summer of 1991 at Saskatoon. Rows of the parental varieties of strains of *B. napus* cv. Westar, *B. juncea* strain ZYR-6 and *B. carinata* strain PGRC/E 21164 were also grown as checks. At maturity all plants from each row were harvested individually and threshed with a single plant thresher. A total of 4858 single open pollinated plants were harvested from 334 rows. Single plants of the parental varieties or strians were also harvested for comparison of seed colour.

The seed of the individual plants was placed in small plastic trays and visually rated for seed colour. Seed of plants was rated as being either black, yellow-brown or yellow in colour. The number of plants in each colour class was recorded for each of the twenty $F_2$ families.

# 4 RESULTS

## 4.1. Interspecific Crosses

The *B. napus* x *B. juncea* interspecific cross was accomplished with relative ease and an average of 1.3 seeds were obtained from each pollinated bud (Table 4.1). Individual crosses gave varying results ranging from 0 to 7.5 seeds per pollination depending on the genotypes used in the cross. The cross *B. napus* cv. Westar x *B. juncea* cv. Zem 83-406A produced 7.5 seeds per pollination while no seed was obtained from the three crosses of *B. napus* cv. Midas with *B. juncea* cv. Lethbridge 22A, Domo and Cutlass. In crosses where no seeds were obtained pods did develop but contained only seed-like structures devoid of embryos.

In the *B. napus* x *B. carinata* interspecific hybrid, seeds were readily obtained and an average of 9.8 seeds were harvested per pollination (Table 4.2). There were small differences in seed set among crosses using different cultivars/strains of *B. napus* or *B. carinata.* Seed set ranged from 7.6 to 11.7 seeds per pollination.

## 4.2. Identification of hybrid plants

It was found that interspecific $F_1$ plants were phenotypically intermediate when compared with their respective parents. $F_1$ plants were vigorous and flowered earlier compared to their parents. Pollen viability of $F_1$ plants of the *B. napus* x *B. juncea* cross was relatively high and ranged from 1 to 60% viable pollen (Table 4.3). However, most interspecific hybrid plants produced 1 to 25% viable pollen. Seed set on $F_1$ plants under open pollination was extremely poor and no seed was harvested from most of the interspecific hybrid plants. Pollen viability in interspecific $F_1$ plants of the *B. napus* x *B. carinata* cross was lower than in $F_1$ plants of the *B. napus* x *B. juncea* cross. Pollen viability ranged from 0 to 40%. The majority of interspecific $F_1$ plants in the *B. napus* x *B. carinata* cross had 0 to 20% viable pollen. Seed set under open pollination was zero for all crosses.

Table 4.1 Number of buds pollinated, pods formed, seeds obtained and seeds harvested per pollination from interspecific crosses between varieties/strains of *B. napus* and *B. juncea*

| Female *B. napus* | | Male *B. juncea* | Buds pollinated | Pods formed | Seeds harvested | Seeds/ pollination |
|---|---|---|---|---|---|---|
| Argentine | x | Lethbridge 22A | 55 | 50 | 64 | 1.2 |
| | | Domo | 57 | 55 | 62 | 1.1 |
| | | Cutlass | 57 | 55 | 83 | 1.5 |
| | | Zem 83-406A | 57 | 52 | 38 | 0.7 |
| | | ZYR-6 | 71 | 65 | 116 | 1.6 |
| Midas | x | Lethbridge 22A | 40 | 38 | 0 | 0.0 |
| | | Domo | 35 | 34 | 0 | 0.0 |
| | | Cutlass | 25 | 25 | 0 | 0.0 |
| | | Zem 83-406A | 36 | 33 | 9 | 0.2 |
| | | ZYR-6 | 53 | 50 | 80 | 1.5 |
| Westar | x | Lethbridge 22A | 18 | 16 | 76 | 4.2 |
| | | Domo | 19 | 17 | 45 | 2.4 |
| | | Cutlass | 21 | 20 | 10 | 0.5 |
| | | Zem 83-406A | 22 | 20 | 164 | 7.5 |
| | | ZYR-6 | 33 | 30 | 69 | 2.1 |
| Profit | x | Lethbridge 22A | 43 | 40 | 19 | 0.4 |
| | | Domo | 37 | 35 | 15 | 0.4 |
| | | Cutlass | 43 | 41 | 204 | 4.7 |
| | | Zem 83-406A | 45 | 43 | 40 | 0.9 |
| | | ZYR-6 | 76 | 70 | 11 | 0.1 |
| | Total | | 844 | 789 | 1105 | -- |
| | Average | | 42 | 40 | 55 | 1.3 |

Table 4.2 Number of buds pollinated, pods formed, seeds obtained and seeds harvested per pollination from interspecific crosses between varieties/strains of *B. napus* and *B. carinata*

| Female *B. napus* | | Male *B. carinata* | Buds pollinated | Pods formed | Seeds harvested | Seeds/ pollination |
|---|---|---|---|---|---|---|
| Argentine | x | PGRC/E 21164 | 50 | 48 | 586 | 11.7 |
| | | Dodola | 17 | 12 | 145 | 8.5 |
| Midas | x | PGRC/E 21164 | 58 | 54 | 621 | 10.7 |
| | | Dodola | 35 | 31 | 310 | 8.9 |
| Westar | x | PGRC/E 21164 | 37 | 35 | 302 | 8.2 |
| | | Dodola | 11 | 8 | 84 | 7.6 |
| Profit | x | PGRC/E 21164 | 69 | 62 | 672 | 9.7 |
| | | Dodola | 39 | 32 | 363 | 9.4 |
| | Total | | 316 | 282 | 3083 | -- |
| | Average | | 40 | 35 | 385 | 9.8 |

Table 4.3 Pollen viability (%) of $F_1$ plants resulting from the interspecific crosses *B. napus* x *B. juncea* and *B. napus* x *B. carinata*

| | Number of $F_1$ plants in pollen viability(%) class | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Interspecific cross | 0-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 | ≧40 |
| *B. napus* x *B. juncea* | 4 | 12 | 15 | 13 | 16 | 6 | 3 | 4 | 2 |
| *B. napus* x *B. carinata* | 2 | 4 | 6 | 10 | 4 | 2 | 1 | 1 | |

## 4.3. Backcrosses

Only those $F_1$ plants that were morphologically intermediate between their parents and also had low pollen viability in comparison to other $F_1$ plants were used as backcross females. On average 1.5 seeds per pollination were harvested from the backcross (*B. napus* x *B. juncea*) x *B. napus* (Table 4.4). One backcross (*B. napus* cv. Profit x *B. juncea* strain ZYR-6) x *B. napus* cv. Profit produced 2.4 seeds per pollination while the backcross (*B. napus* cv. Profit x *B. juncea* cv. Lethbridge 22A) x *B. napus* cv. Profit produced only 0.4 seeds per pollination.

Seed set in (*B. napus* x *B. carinata*) x *B. napus* backcrosses was extremely poor and on an average only one seed was produced per pollination (Table 4.5). No difference in seed set was observed between backcrosses of different cultivars/strains. Overall the seed set ranged from 0.8 to 1.5 seeds per pollination. Although *B. napus* x *B. carinata* interspecific crosses were achieved with relative ease, seed set on the interspecific hybrid plants was severely limited by very poor female fertility of the interspecific hybrid plants.

Table 4.4 Number of buds pollinated, pods formed and seeds harvested per pollination of backcrosses (*B. napus* x *B. juncea*) x *B. napus*

| $F_1$ female backcrossed to its *B. napus* parent | | | Buds pollinated | Pods formed | Seeds harvested | Seeds/ pollination |
|---|---|---|---|---|---|---|
| Argentine | x | Lethbridge 22A | 23 | 18 | 55 | 2.4 |
| | | Domo | 36 | 15 | 38 | 1.1 |
| | | Cutlass | 17 | 6 | 21 | 1.2 |
| | | Zem 83-406A | 10 | 5 | 19 | 1.9 |
| | | ZYR-6 | 14 | 8 | 36 | 2.6 |
| Westar | x | Lethbridge 22A | 16 | 10 | 31 | 1.9 |
| | | Domo | 12 | 8 | 22 | 1.8 |
| | | Cutlass | 20 | 13 | 18 | 0.9 |
| | | Zem 83-406A | 21 | 11 | 8 | 0.4 |
| | | ZYR-6 | 44 | 14 | 33 | 0.8 |
| Profit | x | Lethbridge 22A | 20 | 6 | 8 | 0.4 |
| | | Domo | 31 | 11 | 34 | 1.1 |
| | | Cutlass | 37 | 30 | 81 | 2.2 |
| | | Zem 83-406A | 13 | 12 | 25 | 1.9 |
| | | ZYR-6 | 26 | 10 | 63 | 2.4 |
| | | Total | 340 | 177 | 494 | -- |
| | | Average | 23 | 12 | 33 | 1.5 |

Table 4.5 Number of buds pollinated, pods formed and seeds harvested per pollination of backcrosses (*B. napus* x *B. carinata*) x *B. napus*

| $F_1$ female backcrossed to its *B. napus* parent | | | Buds pollinated | Pods formed | Seeds harvested | Seeds/ pollination |
|---|---|---|---|---|---|---|
| Argentine | x | PGRC/E 21164 | 15 | 10 | 22 | 1.5 |
| | | Dodola | 20 | 18 | 25 | 1.3 |
| Westar | x | PGRC/E 21164 | 26 | 14 | 31 | 1.2 |
| | | Dodola | 31 | 12 | 28 | 0.9 |
| Profit | x | PGRC/E 21164 | 42 | 15 | 32 | 0.8 |
| | | Dodola | 25 | 12 | 21 | 0.8 |
| | Total | | 159 | 81 | 158 | -- |
| | Average | | 27 | 13 | 26 | 1.0 |

## 4.4. Seed Colour of Interspecific $F_1$, $BCF_1$ and $BCF_2$ generations

The seed colour of the interspecific $F_1$, $BCF_1$ and $BCF_2$ generations was black and identical to that of the recurrent black seeded *B. napus* parent. No yellow or partially yellow seeds were observed in any of these generations. However, one plant from the $BCF_2$ generation of the cross (*B. napus* x *B. juncea*) x *B. napus* produced partially yellow seeds. Progeny of the best yellow seeds of this plant segregated black, brown and yellow brown seeded plants and no pure yellow seeded plant was obtained.

## 4.5. $F_2$ Generation

The $BCF_2$ plants of crosses (*B. napus* cv. Westar x *B. juncea* strain ZYR-6) x *B. napus* cv. Westar and (*B. napus* Cv. Westar x *B. carinata* strain PGRC/E 21164) x *B. napus* cv. Westar had *B. napus* like plant morphology and good female and male fertility. They produced good quantities of seed. $F_1$ hybrid plants derived from crosses between the two $BCF_2$ populations produced sufficient quantities of seed so that, on average, more than 200 $F_2$ plants could be grown from each $F_1$ plant (Table 4.6). $F_2$ plants had also *B. napus* like morphology and produced good quantities of seed.

The frequency distribution for black, yellow-brown and yellow seeded plants for each $F_2$ family is presented in Figure 4.1. Eight of the 20 $F_2$ families ($F_2$ family No's 2, 7, 9, 11, 13, 16, 19 and 20) produced only black seeded plants. Of the 249 plants of $F_2$ family No. 4 (Table 4.6), 92% were black seeded and 8% had yellow-brown seed colour; while the 238 plants of $F_2$ family No. 10 segregated 86% black and 14% yellow-brown seeded plants. $F_2$ family No. 1 segregated 80% black and 20% yellow-brown seeded plants; no true yellow seeded plants were found in these three families. Six of the 20 $F_2$ families ($F_2$ families No's 5, 6, 8, 12, 14 and 15) produced black, yellow-brown and yellow seeded plants (Figure 4.2). One $F_2$ family, No. 5, had 75% black, 19% yellow-brown and 6% yellow seeded plants. The five $F_2$ families No. 3, 12, 14, 17 and 18 contained 60 to 66% black seeded plants; three of these five families (No. 3, 17, 18) contained 39,38 and 40% yellow-brown seeded plants but no yellow seeded plants, while two of the five families (No. 12 and 14) contained 30 and 36% yellow-brown and 4% each yellow seeded plants. The remaining three $F_2$ families No. 6, 8 and 15 had 44, 41 and 38% black; 50, 51 and 55% yellow-brown; and 6, 8 and 7% yellow seeded plants, respectively.

Table 4.6 Number and percent of $F_2$ plants in each of 20 $F_2$ families producing black, yellow-brown or yellow seed

| $F_2$ family No. | Total Plants | No. of plants | | | % of plants | | |
|---|---|---|---|---|---|---|---|
| | | Black | Yellow -brown | Yellow | Black | Yellow -brown | Yellow |
| 1 | 158 | 126 | 32 | 0 | 80 | 20 | 0 |
| 2 | 252 | 252 | 0 | 0 | 100 | 0 | 0 |
| 3 | 224 | 137 | 87 | 0 | 61 | 39 | 0 |
| 4 | 249 | 230 | 19 | 0 | 92 | 8 | 0 |
| 5 | 247 | 186 | 46 | 15 | 75 | 19 | 6 |
| 6 | 229 | 100 | 116 | 13 | 44 | 50 | 6 |
| 7 | 271 | 271 | 0 | 0 | 100 | 0 | 0 |
| 8 | 216 | 88 | 110 | 18 | 41 | 51 | 8 |
| 9 | 201 | 201 | 0 | 0 | 100 | 0 | 0 |
| 10 | 238 | 205 | 33 | 0 | 86 | 14 | 0 |
| 11 | 251 | 251 | 0 | 0 | 100 | 0 | 0 |
| 12 | 211 | 139 | 63 | 9 | 66 | 30 | 4 |
| 13 | 249 | 249 | 0 | 0 | 100 | 0 | 0 |
| 14 | 248 | 149 | 89 | 10 | 60 | 36 | 4 |
| 15 | 354 | 132 | 196 | 26 | 38 | 55 | 7 |
| 16 | 251 | 251 | 0 | 0 | 100 | 0 | 0 |
| 17 | 232 | 143 | 89 | 0 | 62 | 38 | 0 |
| 18 | 353 | 211 | 142 | 0 | 60 | 40 | 0 |
| 19 | 219 | 219 | 0 | 0 | 100 | 0 | 0 |
| 20 | 205 | 205 | 0 | 0 | 100 | 0 | 0 |

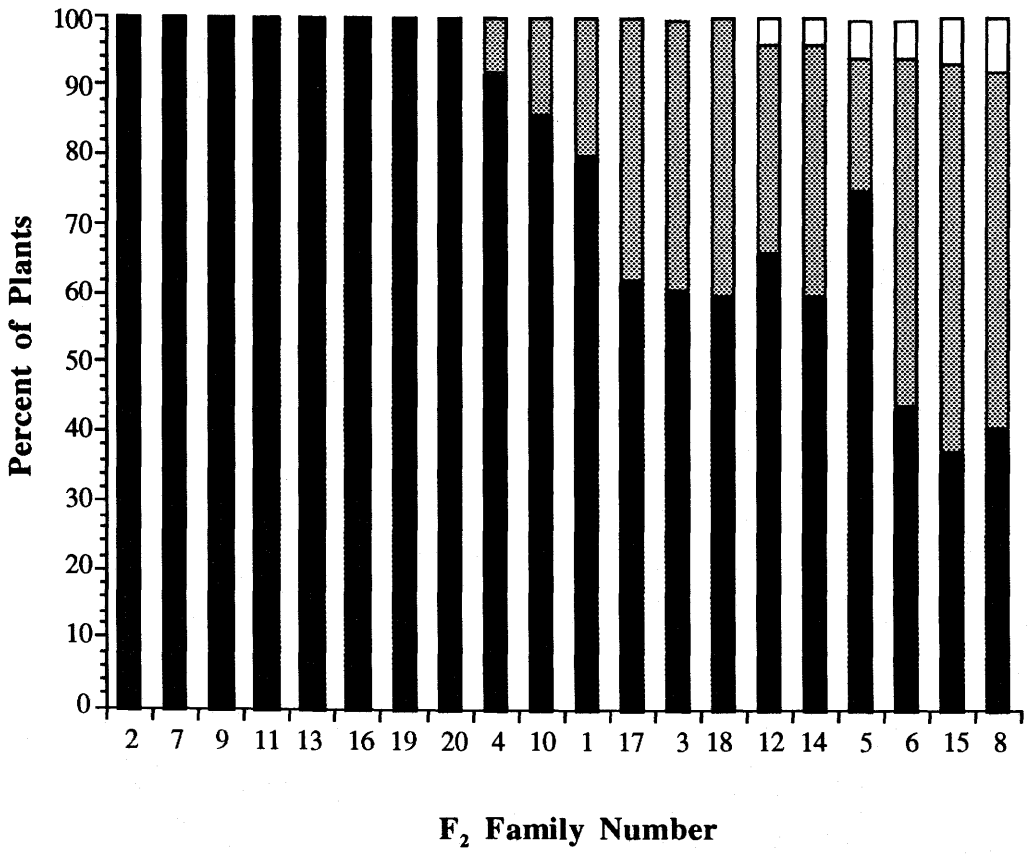


Figure 4.1 Percent black ■ yellow-brown ▨ and yellow □ seeded plants observed in 20 $F_2$ families, derived from the intercrossing of $BCF_2$ plants of the interspecific backcrosses (*B. napus* cv. Westar x *B. juncea* strain ZYR-6) x *B. napus* cv. Westar and (*B. napus* cv. Westar x *B. carinata* strain PGRC/E 21164) x *B. napus* cv. Westar

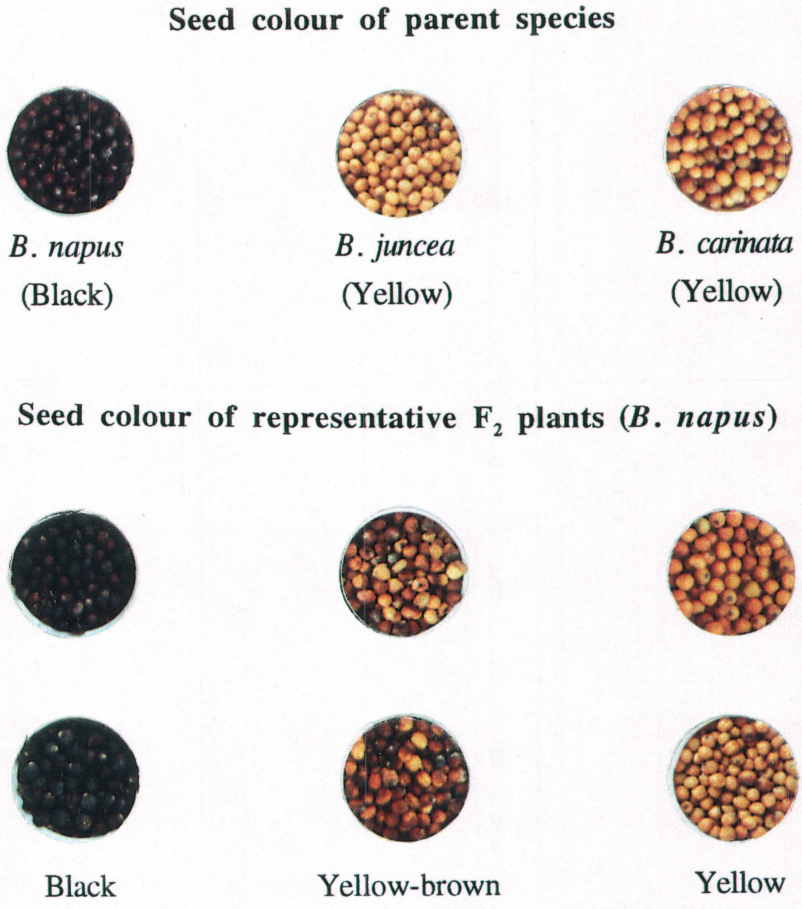


Figure 4.2 Seed coat colour of parent plants of *B. napus* (black), *B. juncea* (yellow) and *B. carinata* (yellow) and representative $F_2$ plants (black, yellow-brown and yellow) derived from intercrossing $BCF_2$ plants of the backcrosses (*B. napus* cv. Westar x *B. juncea* strain ZYR-6) x *B. napus* cv. Westar and (*B. napus* cv. Westar x *B. carinata* strain PGRC/E 21164) x *B. napus* cv. Westar

# 5 DISCUSSION

## 5.1 Interspecific Crosses

The interspecific crosses between *B. napus* x *B. juncea* and *B. napus* x *B. carinata* as well as the backcrosses of the interspecific $F_1$'s with *B. napus* were achieved with relative ease. It was not necessary to apply embryo rescue techniques. The crossing technique of delayed, repeated bud pollination is believed to have significantly improved survival of hybrid embryos. Furthermore, proper care of parent plants (cleaning debris to control the spread of disease, control of insects etc.) and optimum growing conditions for plants (temperature, light, water, timely application of fertilizer etc.) are additional factors that might have contributed to the high success rate recorded for these two interspecific crosses.The number of viable hybrid seeds obtained in this study are far greater than those obtained by other workers (Roy, 1977; Aslamyousuf and Bechyne, 1985; Wahiduzzaman, 1987).

## 5.2 Segregation for Seed Colour in $F_2$ Generation

The fact that yellow and yellow-brown seeded *B. napus* plants segregated in the $F_2$ generation as a result of intercrossing of $BCF_2$ plants of crosses (*B. napus* x *B. juncea*) x *B. napus* and (*B. napus* x *B. carinata*) x *B. napus*, indicates that the crossing scheme proposed for the development of yellow seeded *B. napus* was effective. Genes for yellow seed colour present in the A genome of *B. juncea* and the C genome of *B. carinata* were successfully introgressed into the A and C genomes of *B. napus* through interspecific crosses. The *B. napus* (AACC) x *B. juncea* ($A^yA^yBB$) interspecific cross produced an $F_1$ plant with the genomic constitution $AA^yBC$ in which half of the 10 A genome chromosomes were derived from the *B. napus* parent and the other 10 A genome chromosomes were derived from *B. juncea* parent. Genes for yellow seed colour, present in the A genome of *B. juncea* (Vera *et al.* 1979) were thus transferred into the interspecific hybrid. Similarly, the *B. napus* (AACC) x *B. carinata* ($BBC^yC^y$) interspecific cross resulted in an $F_1$ plant

with the genomic constitution ABCC$^{y}$ in which half of the 9 C genome chromosomes were derived from the *B. napus* parent and the other 9 C genome chromosomes from the *B. carinata* parent. The presence of genes for yellow seed colour in the C genome of *B. carinata*, an assumption of this study, would therefore allow its transfer into the resulting interspecific hybrid.

Backcrossing of the two interspecific $F_1$'s to *B. napus* produced morphologically *B. napus* like plants. The $BCF_1$ plants had sufficient female and male fertility which allowed the production of selfed $BCF_2$ seed. The fact that $BCF_2$ seed of both the backcrosses, (*B. napus* x *B. juncea*) x *B. napus* and (*B. napus* x *B. carinata*) x *B. napus* were black seeded indicated that the presence of genes for yellow seed colour from mustard in either A or C genome of *B. napus* alone does not result in a reduction of seed coat pigmentation or a non-pigmented (yellow) seeded plant (Figure 3.1).

It was therefore necessary to intercross $BCF_2$ *B. napus* like plants of the (*B. napus* x *B. juncea*) x *B. napus* cross, carrying genes for yellow seed colour in their A genome with $BCF_2$ *B. napus* like plants of the (*B. napus* x *B. carinata*) x *B. napus* cross, carrying genes for yellow seed colour in their C genome. It was hypothesised that $F_2$ plants of this cross should segregate yellow seeded *B. napus* plants. The yellow seeded plants would be recombinant genotypes which should contain genes for yellow seed colour in both (A and C) genomes.

Seed colour segregation was studied in the $F_2$ generation derived from intercrossing of $BCF_2$ plants of (*B. napus* cv. Westar x *B. juncea* strain ZYR-6) x *B. napus* cv. Westar and $BCF_2$ plants of *B. napus* cv. Westar x *B. carinata* strain PGRC/E 21164) x *B. napus* cv. Westar. This was done because it was not physically possible to evaluate all possible crosses with accuracy and to test sufficient number of plants to identify the desired genotypes that carried genes for yellow seed colour at their both A and C genomes. The use of only one yellow seeded *B. juncea* and *B. carinata* parent is tenable since inheritance studies in

*B. juncea* have indicated that yellow seed colour is always controlled by two independent gene pairs (Vera et al. 1979; Aslamyousuf and Bechyne, 1983a; Anand *et al.* 1985; Dhillon *et al.* 1986). In the same way, it is suspected that yellow seeded genotypes of *B. carinata* also have identical genes for yellow seed colour at their C genome. Therefore, it is expected that all other crosses will produce similar results to those obtained using *B. napus* cv. Westar x *B. juncea* strain ZYR-6 and *B. napus* cv. Westar x *B. carinata* strain PGRC/E 21164 crosses.

Six of the 20 $F_2$ families segregated from 4% to 8% yellow seeded plants which closely approximated a 1:15 two gene segregation ratio (Table 4.6). Therefore, these yellow seeded plants might have contained two genes, both in the homozygous condition ($A^yA^yC^yC^y$) for yellow seed colour, one gene each in their A and C genomes, derived from *B. juncea* and *B. carinata*, respectively. It is also concluded that genes for yellow seed colour from the A genome of *B. juncea* and from the C genome of *B. carinata* are complementary and both genes must be present in *B. napus* for the expression of the yellow seed colour. The 91 yellow seeded plants obtained from these six $F_2$ families are expected to carry identical seed colour genes . This conclusion is based on the fact that all plants were derived from one cross in which seed colour genes were introgressed from the A genome of *B. juncea* strain ZYR-6 and from the C genome of *B. carinata* strain PGRC/E 21164 into the A and C genomes of *B. napus*, respectively. The six $F_2$ families that segregated yellow seeded plants, segregated also partially yellow (yellow-brown) and black seeded plants (Table 4.6). The proportion of yellow-brown plants varied from 19% to 55%.

An additional six $F_2$ families segregated yellow-brown and black seeded plants but no yellow seeded plants. It is speculated that the yellow-brown seed colour in these plants resulted from partial dominance of the yellow seed colour genes from the C genome of *B. carinata* (Getinet *et al.* 1987). They observed that $F_1$ plants of the cross *B. carinata*

(brown) x *B. carinata* (yellow) produced partially yellow seeded $F_1$ plants which indicated incomplete dominance of brown over yellow. This observation may explain the yellow-brown seed colour of the intercross $F_2$ plants in that the genotypic constitutions $AA^yC^yC^y$ and $A^yA^yC^yC$ likely result in yellow-brown seeded phenotypes. It should be possible to isolate true breeding yellow seeded *B. napus* plants ($A^yA^yC^yC^y$) from yellow-brown seeded plants. This study remains to be conducted.

Eight $F_2$ families contained only black seeded plants (Table 4.6). It was expected that a number of $F_2$ families would not segregate any yellow seeded plants. This can be explained by the fact that neither parent, $BCF_2$ plants of (*B. napus* x *B. juncea*) x *B. napus* or $BCF_2$ plants of (*B. napus* x *B. carinata*) x *B. napus*, contained genes for yellow seed colour in their A and C genomes, respectively; or that only one parent contained genes for yellow seed colour. In both cases no yellow seeded plants would be expected in the $F_2$ generations of crosses between such forms.

The yellow seeded *B. napus* plants developed in this study provide a new source of yellow seededness in *B. napus* which can be utilized for the breeding of yellow seeded *B. napus* cultivars. The inheritance of the yellow seed characteristic and effect of yellow seed colour on oil, protein and fibre contents remain to be investigated and are an essential prerequisite for its efficient utilization in breeding programs.

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