INHERITANCE OF ERUCIC ACID IN *BRASSICA CARINATA* A. BRAUN AND DEVELOPMENT OF LOW GLUCOSINOLATE LINES

A Thesis
Submitted to the Faculty of Graduate Studies and Research
in Partial Fulfilment of the Requirements
for the degree of Doctor of Philosophy
in the Department of Crop Science and Plant Ecology
University of Saskatchewan
Saskatoon

by
Getinet Alemaw
Spring, 1996

UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial Fulfilment of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

Getinet Alemaw

Department of Crop Science and Plant Ecology

Spring 1996

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Inheritance of Erucic acid in *Brassica carianata* A. Braun and Development of Low Glucosinolate Lines

Ethiopian mustard (*Brassica carinata* A. Braun) or gomenzer is an oilseed crop that is well adapted to the highlands of Ethiopia. Evaluation of the local germplasm has resulted in the registration of high yielding cultivars, such as Dodolla and S-67. The oil of gomenzer contains about 40% erucic acid and the meal is high in glucosinolates. The objective of this research was to study the inheritance of erucic acid content in gomenzer and to introgress genes for the non2-propenyl glucosinolate trait from *B. napus* and *B. juncea.*

The erucic acid content of F_1 seed from reciprocal crosses between the high erucic acid cultivars Dodolla and S-67 and a low erucic acid line was intermediate between the parents indicating that erucic acid content was controlled by nondominant additive genes. F_1BC seed derived from the backcross to the low erucic acid parent fell into three erucic acid classes with <1%, 6 to 16% and >16% erucic acid at the ratio of 1:2:1 indicating that erucic acid was under the control of two alleles at two loci. F_2 seed segregation data supported this observation. Each allele contributed approximately 10% erucic acid.

The high glucosinolate *B. carinata* line C90-14, low glucosinolate *B. napus* cultivar Westar and *B. juncea* line J90-4253 were chosen as parents for the development of low 2-propenyl glucosinolate *B. carinata*. Interspecific crosses were carried out between *B. carinata* and *B. napus* and *B. carinata* and *B. juncea* and the interspecific F_1 generations backcrossed to *B. carinata* with the objective to transfer genes for low 2-propenyl glucosinolate content from *B. napus* and *B. juncea* into *B. carinata*. F_1 backcross plants from the two interspecific crosses were intercrossed in an attempt to combine the two sources for low 2-propenyl glucosinolate content in one genotype.

F_1 plants of the interspecific backcrosses [(*B. carinata* x *B. napus*) *B. carinata*] contained high concentration of 2-propenyl glucosinolate similar to those of *B. carinata*. Introgression of C genome chromosomes of *B. napus* into *B. carinata* was not effective in redirecting glucosinolate synthesis away from 2-propenyl and into 3-butenyl glucosinolate. This indicated that C genome chromosomes do not contain genetic factors for C3→C4 glucosinolate precursor chain elongation, and that 2-propenyl is solely controlled by genes on B genome chromosomes.

F_2 backcross plants of the interspecific backcross [(*B. carinata* x *B. juncea*) x *B. carinata*] contained much reduced levels of 2-propenyl glucosinolate indicating that genetic factors for C3→C4 glucosinolate precursor chain elongation were introgressed from the B genome of *B. juncea* into the B genome of *B. carinata*. However, a complete diversion of glucosinolate synthesis from 2-propenyl to 3-butenyl was not achieved. Further selections in segregating generations of *B. juncea* derived *B. carinata* populations could yield the desired zero 2-propenyl glucosinolate *B. carinata*.

The double interspecific cross was unsuccessful.

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ABSTRACT

Ethiopian mustard (*Brassica carinata* A. Braun) or gomenzer is an oilseed crop that is well adapted to the highlands of Ethiopia. Evaluation of the local germplasm has resulted in the registration of high yielding cultivars, such as Dodolla and S-67. The oil of gomenzer contains about 40% erucic acid and the meal is high in glucosinolates. The objective of this research was to study the inheritance of erucic acid content in gomenzer and to introgress genes for the non2-propenyl glucosinolate trait from *B. napus* and *B. juncea*.

The erucic acid content of F_1 seed from reciprocal crosses between the high erucic acid cultivars Dodolla and S-67 and zero erucic acid line C90-14 was intermediate between the parents indicating that erucic acid content in *B. carinata* was controlled by two nondominant genes with two alleles acting in an additive manner. Backcross F_1 seed derived from the backcross to the low erucic acid parent fell into three erucic acid classes with <0.5%, 6 to 16% and >16% erucic acid at the ratio of 1:2:1 indicating that erucic acid was under the control of two alleles each of at two loci. F_2 seed segregation data supported this observation. Each allele contributed approximately 10% erucic acid. The high glucosinolate *B. carinata* line C90-14, low glucosinolate *B. napus* cultivar Westar and *B. juncea* line J90-4253 were chosen as parents for the development of non2-propenyl glucosinolate *B.* carinata. The objective was to transfer genes for non2-propenyl glucosinolate content from *B. napus* and *B. juncea* into *B. carinata*. Interspecific crosses were made between *B. carinata* and *B. napus*, *B. carinata* and *B. juncea* and the interspecific F_1 generations were backcrossed to *B. carinata*. Backcross F_1 plants from the two interspecific crosses were intercrossed in an

attempt to combine the two sources for non2-propenyl glucosinolate content in one genotype.

Seed of backcross F_1 plants of the cropss [(*B. carinata* x *B. napus*) x *B. carinata*] contained a high concentration of 2-propenyl glucosinolate similar to those of *B. carinata*. Introgression of C genome chromosomes of *B. napus* into *B. carinata* was not effective in redirecting glucosinolate synthesis away from 2-propenyl and into 3-butenyl glucosinolate. This indicated that C genome chromosomes do not contain genetic factors for C3→C4 glucosinolate precursor chain elongation, and that 2-propenyl glucosinolate synthesis is primarily controlled by genes on B genome chromosomes.

Seed of ackcross F_2 plants of the cross [(*B. carinata* x *B. juncea*) x *B. carinata*] contained much reduced levels of 2-propenyl glucosinolate indicating that genetic factors for C3→C4 glucosinolate precursor chain elongation were introgressed from the B genome of *B. juncea* into the B genome of *B. carinata*. However, a complete diversion of glucosinolate synthesis from 2-propenyl to 3-butenyl was not achieved. Further selections in segregating F_4 and F_5 generations of *B. juncea* derived *B. carinata* populations could yield the desired zero 2-propenyl glucosinolate *B. carinata*.

The double interspecific cross was unsuccessful.

ACKNOWLEDGEMENTS

The author acknowledges the financial assistance of the International Development Research Centre, Ottawa. I am indebted to the Research Center of Agriculture and Agri-Food Canada (AAFC) Saskatoon for laboratory, greenhouse and growth room facilities for the research. The three year study leave provided to me by the Institute of Agricultural Research, Addis Abeba, Ethiopia, is sincerely acknowledged. I thank my supervisor Dr. G. Rakow for his guidance. I thank members of my advisory committee, Dr. R. K. Downey, Dr. F. W. Sosulski, Dr. M. Devine and Dr. G. Rowland for their input..

I thank Dr. J. P. Raney of AAFC, Saskatoon, who assisted with fatty acid and glucosinolate analyses and I particularly want to thank him for his constrictive criticism and advice in many aspects of this research.. Mr. C. J. Powlowski, Todd Olson, Troy Libke and Rod Costain provided technical assistance of the highest quality.

I am also forever grateful for the encouragement I received from my wife Roman Korma and sisters, Mulunesh Alemaw and Denkayehu Alemaw.

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1.0 INTRODUCTION

The agricultural land of Ethiopia can be divided into three distinct ecological agricultural production zones, and different oilseeds are cultivated in each zone. In the warmer lowland zone (750 to 1500 m above sea level), sesame (*Sesamum indicum* L.), groundnut (*Arachis hypogea* L.) and cottonseed (*Gossypium hirsutum* L.) are grown for local consumption and also export. The greatest concentration of oilseed crops is found in the most productive mid altitude (1500 to 2200 m above sea level) agricultural zone. The oilseed crops grown in this zone are noug (*Guizotia abyssinica* L. Cass), safflower (*Carthamus tinctorius* L.), and sunflower (*Helianthus annuus* L.). In the cooler highlands (2200 to 2800 m above sea level) with temperatures of 14 to 18^{0}C and rainfall of 600 to 900 mm during the growing season from June to December, gomenzer (*Brassica carinata* A. Braun) and linseed (*Linum usitatissimum* L.) are grown. Castor bean (*Ricinus communis* L.) has a wider ecological distribution than other oilseeds. Noug (niger seed), linseed and gomenzer account for more than 90% of the total edible oilseed production in Ethiopia.

Research on rapeseed (*Brassica napus* L. and *B. rapa* L.) and gomenzer started in the early 1970's at the Institute of Agricultural Research, Addis Abeba (Anonymous 1995). Introduced spring rapeseed cultivars from Canada and Germany were tested for the first time in 1968, and preliminary yield tests conducted in 1970. Between 1970 and 1975, nine single plant selections of gomenzer and three rapeseed cultivars were yield tested at Alemaya, Ambo, Awassa, Bekoji, Bako, Holetta and Kulumsa, Ethiopia. On average, rapeseed

cultivars were ten days earlier maturing than gomenzer and their seed oil content was 5% higher. In 1976 the *B. carinata* cultivars S-67, S-71 and S-115 and the Awassa population were recommended for production by the Institute. Later, S-67 was approved and registered by the National Variety Release Committee.

Yield and quality of gomenzer has been compared to that of rapeseed and mustard (*B. juncea*) in observation trials across the Ethiopian highlands (Westphal and Marquard 1980). Seed yields were highest for *B. carinata* followed by *B. napus*, while yields of *B. rapa* were lowest. *B. rapa* and *B. napus* had higher oil contents than *B. carinata* and *B. juncea*. Based on these observations, Westphal and Marquard (1980) recommended that research on gomenzer meal and oil quality should be initiated. Belayneh *et al.* (1983) determined the yield of the *B. rapa* cultivar Torch, the *B. napus* cultivar Target and the *B. carinata* cultivar S-67 for two years at two locations in the highlands of Ethiopia. At locations with short seasons and poorly distributed rainfall, yields of the *B. napus* cultivar Target were equal to that of the *B. carinata* cultivar S-67, but at locations with long seasons and well distributed rainfall, *B. carinata* S-67 yielded up to 3.5 t/ha of seed compared to 2.3 t/ha for Target. Since then selection in *B. carinata* for earliness and higher oil content through yellow seed coat colour, has resulted in earlier maturing higher oil content cultivars which are higher yielding than *B. napus* even under less favourable growing conditions.

The optimum sowing date for gomenzer is late May for areas higher than 2500 m in elevation and mid to late June for elevations lower than 2500 m (Belayneh *et al.* 1983). A seeding rate of 10 kg/ha and fertilizer applied at rates of 46 kg/ha N and 69 kg/ha P_2O_5 are recommended. A yield loss of up to 40% could result from weed competition. Therefore,

a premergence application of Alachlor at 1.0 kg/ha a. i. is recommended for weed control. Hand weeding once during the early stages of plant development has been shown to be economic and increase yield.

Seed of gomenzer contains from 37 to 44 % oil depending on the cultivar and environment. Cool temperatures or higher altitudes and well distributed rainfall result in higher oil contents. The high seed yield of gomenzer in combination with its high oil content makes gomenzer a highly desirable oilseed for the Ethiopian highlands. Additional advantages of gomenzer over other oilseed crops for production of oil for local consumption are the ease of oil extraction using screw press and high oil recovery rates during oil extraction for local consumption.

Gomenzer seed oil contains approximately 40% erucic acid (Westphal and Marquard 1973), and the seed has a high glucosinolate content (Gland *et al.* 1981, Getinet 1986, Mnzava and Olsson 1990, Getinet *et al.* 1996). Low erucic acid *B. carinata* was developed in Spain (Alonso *et al.* 1991) and Canada (Getinet *et al.* 1994). Low erucic acid content is a requirement for high quality edible *Brassica* seed oils. To improve the feed value of the meal, low glucosinolate types are required. However, thus far genotypes with low glucosinolate contents have not been developed.

The objective of this study was to investigate the inheritance of erucic acid in B. *carinata* and to develop low glucosinolate forms in this species. The thesis is organized into two parts, part one covers the inheritance of erucic acid in *B. carinata* and part two reports on the development of low glucosinolates lines.

2.0 INHERITANCE OF ERUCIC ACID IN *BRASSICA CARINATA*

2.1 Literature review

2.1.1 The genus *Brassica*

The genus *Brassica* belongs to the family of Cruciferae and tribe Brassiceae (Gomez Campo 1980). The genus includes many economically important crops which provide edible roots, stems, leaves, flowers and seeds. Many of the wild forms within the genus *Brassica* have a potential to be used as condiments and oilseeds. The wild forms are useful sources of desirable agronomic and seed quality traits in breeding programs.

The genus *Brassica* consists of six cultivated crop species namely, *B. nigra* (L.) Koch, *B . oleracea* L., *B. rapa L.* (syn. *B. campestris* L.), *B. napus* L., *B. carinata* A. Braun and *B. juncea* (L.) Czern. The latter four are cultivated as oilseed crops. *B. oleracea* is a vegetable crop and *B. nigra* is a condiment.

2.1.2 Cytology

The *Brassica* species have been a subject of cytological and breeding studies since the early 1920's. The somatic chromosome number of *B. rapa* 2n=20, *B. oleracea* 2n=18, B. *nigra* 2n=16 and *B. juncea* 2n=36 were reported by Karpetchenko (1922). The chromosome number of *B. napus* 2n=38 and *B. carinata* 2n=34 were determined by Morinaga (1934) and Morinaga and Fukushima (1930). Somatic metaphase cells of the F_1

hybrid between *B. rapa* and *B. napus* had mostly 10 bivalents + 9 univalents (Morinaga 1929) and occasionally (0-3) trivalents +(12-7)bivalents +(4-8) univalents (Catcheside 1934). The F_1 between *B. juncea* and *B. nigra* had 8 bivalents + 10 univalents chromosomes in its somatic cells (Morinaga 1934) and that of *B. juncea* and *B. rapa* showed 10 bivalents + 8 univalents. The F_1 hybrid between *B. carinata* and *B. nigra* and *B. carinata* and *B. oleracea* had 8 bivalents + 9 univalents and 9 bivalents + 8 univalents chromosomes respectively. From these results Morinaga (1934) concluded that *B. napus, B. juncea* and *B. carinata* contained two genomes each in their somatic metaphase cells. He named the genome constitution of *B. rapa* aa, *B. nigra* bb, and *B. oleracea* cc, *B. napus* aacc, *B. juncea* aabb and *B. carinata* bbcc. Morinagas's (1934) proposal was verified by U (1935) by resynthesising of *B. napus* from its diploids and reciprocal crosses between *B. napus* x *B. carinata* as well as crosses of amphidiploids with their diploid species. He presented the genome relationships of *Brassica* species in a form of a triangular diagram (Figure 2.1). The results of the basic studies of Morinaga (1928, 1929, 1931, 1933, 1934) and U (1935) were corroborated following resynthesis of *B. napus* (Olsson 1960b, Chen *et al.* 1988), *B. juncea* (Frandsen 1943, Ramanujam and Srinivasachar 1943, Olsson 1960a, Campbell 1993) and *B. carinata* (Frandsen 1947, Mizushima and Katsuo 1953, Sarla and Raut 1988).

Chloroplast and mitochondrial DNA endonuclease restriction analyses were used to identify the maternal and paternal parents of the amphidiploid species. The chloroplast DNA restriction pattern of *B. carinata* and *B. nigra* were identical, and that of *B. juncea* was similar to that of *B. rapa* (Erickson *et al.* 1983, Palmer *et al.* 1988). The chloroplast DNA

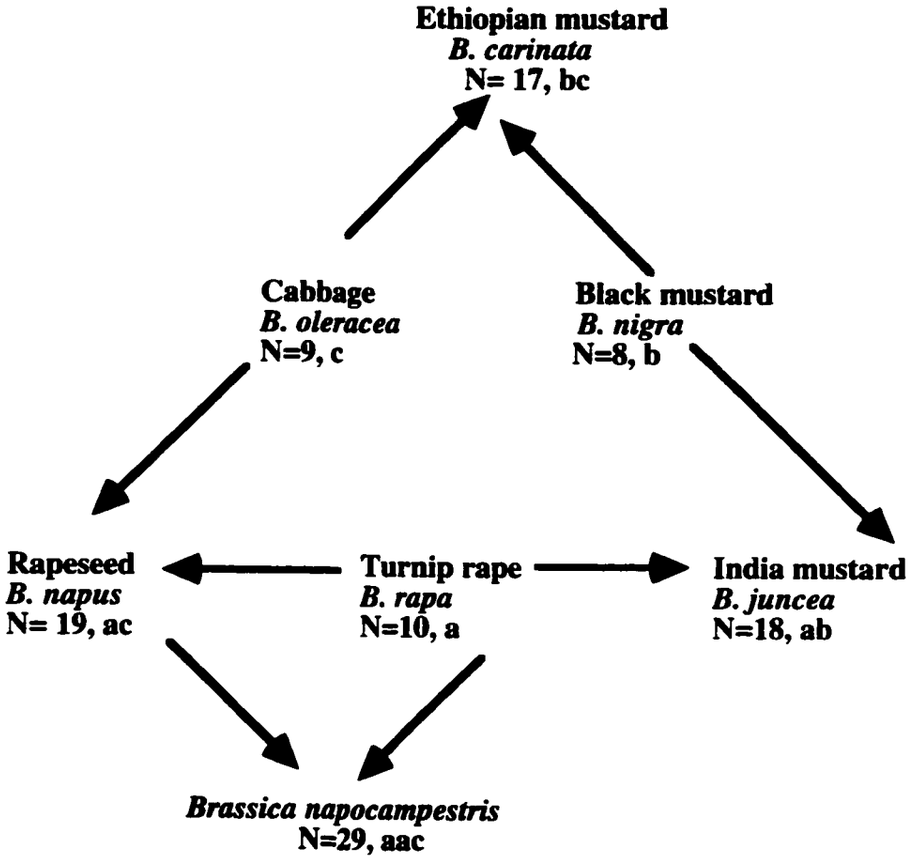

Figure 2.1 Genome relationships of *Brassica* species (U, 1935). Ethiopian mustard issynonymous with gomenzer.

restriction pattern of *B. napus* showed a similarity to *B. oleracea* but it had diverged from its diploid parents more than that of the other two amphidiploid species. Later the evolutionary pathways of the *Brassica* species were divided into two lineages, namely the *nigra* lineage and the *rapa/oleracea* lineage (Warwick and Black 1990). These conclusions based on chloroplast DNA restriction pattern, were further confirmed by mitochondrial DNA restriction analyses. The two species *B. rapa* and *B. oleracea* were more similar to each other in chloroplast DNA restriction pattern than either were to *B. nigra*. *B. nigra* was closer to *Sinapis arvensis* L. The RFLP pattern of the amphidiploids (Lydiate *et al.* 1993, Song *et al.* 1993) was in agreement with the cytological studies of Morinaga (1928, 1929, 1931, 1933 1934) and U (1935). It was suggested that *B. napus* and *B. juncea* had different polyphyletic origins, and parallel variation of different morphotypes of diploid and amphidiploids were observed. The possibility of different interspecific evolutions was suggested by Song *et al.* (1988, 1993).

2.1.3 Oil quality of *Brassica* species

Oils from rapeseed contained 95.8% nonpolar and 4.2% polar lipids (Zadernowski and Sosulski 1978). Of the nonpolar lipids 92% was triacylglyceride. Oil of *Brassica* species contained palmitic, oleic, linoleic, linolenic, eicosenoic and erucic acids (Röbbelen and Thies 1980a). The palmitic acid levels in *B. napus* and *B. rapa* ranged from 4 to 7%, and the total content of other saturated fatty acids such as stearic, arachidic, behenic, and lignoceric was less than 2% (Downey and McGregor 1976, Röbbelen and Thies 1980a). The content of unsaturated fatty acids varied for oleic from 8 to 23%, linoleic from 11 to 16%,

linolenic from 6 to 11%, erucic from 41 to 54% and eicosenoic 6 to 14%. In *B. juncea* a wider variability in erucic and linoleic fatty acids has been observed. Indian *B. juncea* cultivars contained from 42 to 54% erucic acid in their oil whereas European cultivars contained from 18 to 27 % erucic acid in their oil (Kirk and Oram 1981). Linoleic acid varied from 24% in European to 15% in Indian cultivars. Oil from *B. carinata* contained about 40% erucic, 6 to 7% eicosenoic, 11 to 19% linoleic, 7 to 17 % linolenic, 6 to 11% oleic, 2 to 5% palmitic and 0.3 to 1.0% stearic acids (Seegeler 1983). Based on nutritional studies of high erucic acid oils, it is generally accepted that low erucic acid oils are beneficial for human consumption (Vles 1974). Therefore, reduction of erucic acid content through plant breeding has been a major subject of research.

2.1.4 Development of *Brassica* genotypes containing less than 0.2 % erucic acid in their oil

Zero erucic acid (<0.2%) *B. napus* plants were developed through pedigree selection out of the variety Liho (Stefansson *et al.* 1961). Analysis of single plants for erucic acid of the Liho cultivar resulted in plants containing from 6 to 50% erucic acid. Further selection for lower erucic acid content in seed from plants with lowest erucic acid content produced seed free from erucic acid in their oil. Analysis of 28 strains and cultivars of *B. rapa* for fatty acid composition resulted in the identification of the land race Polish containing 18% erucic acid in its oil (Downey 1964). Continued pedigree selection utilizing the half-seed technique (Downey and Harvey 1963) for fatty acid analysis resulted in the identification of plants of *B. rapa* Polish containing oil free from erucic acid.

In *B. juncea,* zero erucic acid mustard (Zem) lines were isolated out of two seed lots of oriental mustard from China containing 11 and 12 % erucic acid, respectively (Kirk and Oram 1981). Half-seed analysis of individual seed for erucic acid resulted in the identification of individual seeds with <0.2% erucic acid in both seed lots. Seed from nine zero erucic acid seedlings out of the original seed lot containing 11% erucic acid was designated as Zem 1 and thirteen zero erucic acid seedlings out of the seed lot containing 12% erucic acid formed Zem 2. Zem 2 had slightly higher linolenic acid content than Zem 1.

Development of zero erucic acid *B. carinata* was a recent development. *B. carinata* plants containing oil free from erucic acid were developed through pedigree selection out of the available *B. carinata* germplasm (Alonso *et al.* 1991). The first cycle of selection resulted in the identification of plants containing 19 to 27% of erucic acid. Intercrossing of these plants followed by selection in the F_2 generation for erucic acid content using the half-seed technique resulted in the identification of a single plant containing 13.4% erucic acid in its oil. Analysis of single seeds of this plant for erucic acid resulted in the identification of zero erucic acid *B. carinata.*

Zero erucic acid *B. carinata* was also developed through an interspecific transfer of the zero erucic acid trait from *B. juncea* to *B. carinata* (Getinet *et al.* 1994). High erucic acid *B. carinata* was crossed with zero erucic acid *B. juncea* and the F_1 backcrossed to *B. carinata.* Zero erucic acid seeds were identified among backcross F_2 seed of the interspecific backcross. Zero erucic acid plants, raised from zero erucic acid half-seeds were backcrossed to *B. carinata* four times with selfing and reselection of zero erucic acid plants after each

backcross.

The reduction in erucic and eicosenoic acids resulted in a corresponding increase in oleic and linoleic acids with no apparent effect on total seed oil content (Downey and Craig 1964). The correlation between erucic and oleic acids was strongly negative. This led to the conclusion that fatty acids in *Brassica* seeds were synthesized through a chain elongation process. This was confirmed by feeding radio active labelled ^{14}C acetate to the maturing rapeseed siliquas. The pathway of fatty acid biosynthesis in rapeseed is shown in Figure 2.2.

2.1.5 Inheritance of erucic acid in *Brassica* species

The isolation of zero erucic acid types in *B. napus* and *B. rapa* in the early 1960's stimulated basic genetic research into the inheritance of fatty acid compositions in *Brassica* species. The development of the half-seed analysis technique for the determination of fatty acid composition of individual seed by Downey and Harvey (1963) was a major breakthrough, and this technique was applied worldwide in the genetic analysis of erucic acid and the development zero erucic acid *Brassica* cultivars. The half-seed analysis technique is a nondestructive method which enables the plant breeder to analyse the fatty acid content of a single seed while retaining the inner cotyledon for planting.

The inheritance of erucic acid in *B. napus* was studied in progenies of the cross between the high erucic acid (40%) cultivar Golden and a strain from Liho containing <0.2% erucic acid (Harvey and Downey 1964). The F_1 seed contained 22% erucic acid in its oil. The distribution of erucic acid content in F_2 seeds of reciprocal crosses fell into five classes at the ratio of 1:4:6:4:1 and backcrosses to the zero erucic acid parent segregated into three

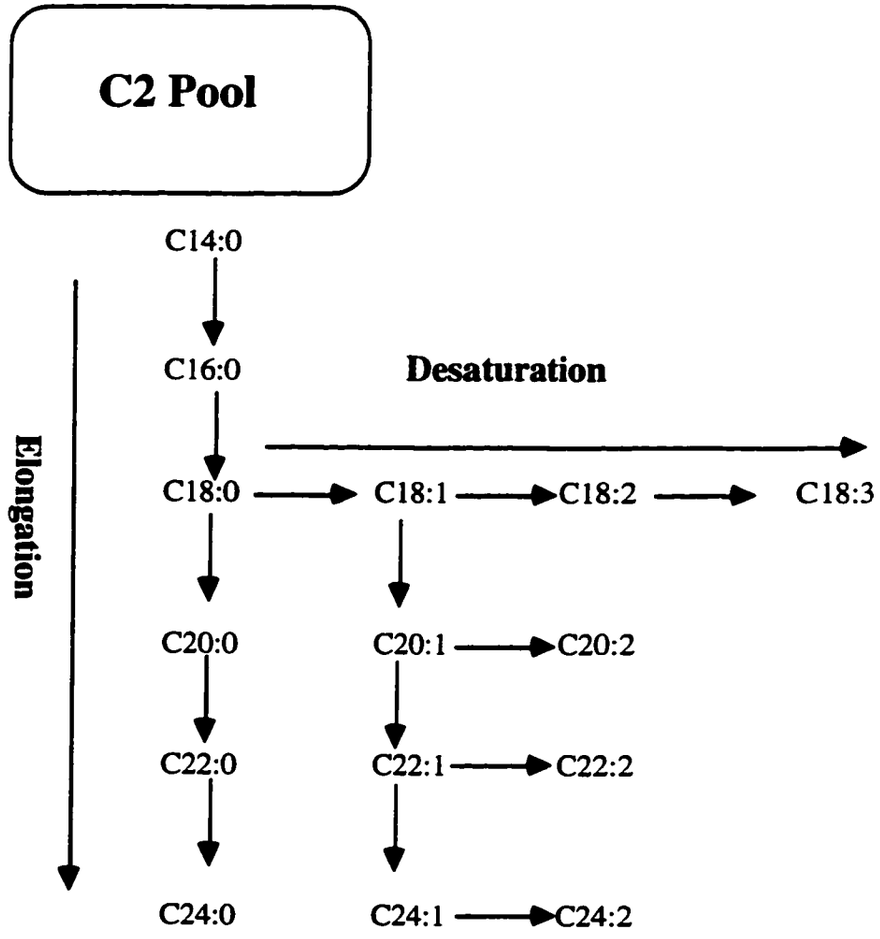

Figure 2.2 Fatty acid biosynthesis pathway in *Brassica* species.
(modified after Downey 1983)

classes at the ratio of 1:2:1 therefore, a two gene model with additive effects was suggested for the genetic control of erucic acid in *B. napus*. Harvey and Downey (1964) concluded that erucic acid in *B. napus* was controlled by two additive genes at two loci. Kondra and Stefansson (1965) analysing segregating generations of crosses between the high erucic acid cultivar Nugget and two sources of zero erucic acid *B. napus* (Liho and Budapest) drew the same conclusions. These authors further concluded that both erucic and eicosenoic acid contents were controlled by the two pair of same genes. Genetic analysis of erucic acid in *B. napus* Bronowski with 7% erucic acid revealed an allele for erucic acid in Bronowski that contributed 3.5% erucic acid (Krzymanski and Downey 1969). Therefore, a new allele (E^d) was suggested. Anand and Downey (1981) crossed *B. napus* Bronowski containing 7% erucic acid with *B. napus* strain SZ66-1626 containing 10% erucic acid. Based on F_1 and backcross data it was concluded that the 3.5% erucic acid allele (E^d) from Bronowski and the 10% erucic acid allele from SZ66-1626 (E^a) were truly allelic. Interspecific crosses between Bronowski and zero erucic acid *B. rapa* were made to determine the genome location of the E^d erucic acid allele in Bronowski. F_1 and backcross F_1 seed derived from backcrosses to the Bronowski parent indicated that the E^d erucic acid allele was located on chromosomes of the C genome of Bronowski.

The inheritance of erucic and oleic fatty acids in *B. napus* were studied using microspore-derived doubled haploid (DH) populations (Chen and Beversdorf 1990). *B. napus* lines containing contrasting erucic and oleic fatty acid contents were crossed and DH plants produced from F_2 gametes of F_1 plants. Selfed seed was produced on DH plants in the field. DH plants derived from crosses between standard zero erucic acid *B. napus* and

the high erucic acid *B. napus* cultivar Reston segregated for erucic acid in a 1:2:1 ratio with erucic acid contents of less than 1%, 20 to 35% and 36 to 45% respectively. This result was in agreement with the result of Harvey and Downey (1964) supporting the two gene model of erucic acid inheritance. In populations cosegregating for oleic and erucic acids plants segregated into three classes with oleic acid contents of 18 to 30%, 30% to 50% and 51 to 69% at a ratio of 1: 2: 1. In populations without cosegregation for erucic acid, three oleic acid classes were observed in the 1:2:1 ratio. The ranges for oleic acid were 58 to 62%, 63 to 67% and 68 to 74%. Chen and Beversdorf (1990) concluded from their segregation data that oleic acid contents were under the genetic control of two additional loci involved in fatty acid desaturation from oleic to linoleic acid. The allele controlling fatty acid chain elongation from oleic to erucic acid had a major impact on oleic acid contents.

The genetic control of erucic acid in *B. rapa* was reported by Dorrell and Downey (1964). These authors crossed a *B. rapa* line free from erucic acid with *B. rapa* yellow sarson containing 58.6% and brown sarson containing 58.9% erucic acid. F_1 seed of both crosses contained 30 to 34% erucic acid. F_2 and backcross plants to the zero erucic acid line segregated into erucic acid classes of <1% erucic acid and greater than 19% erucic acid at a ratio of 1:3 and 1:1 for the F_2 and backcross populations, respectively. Dorrell and Downey (1964) concluded from the data that erucic acid in *B. rapa* was controlled by two alleles with additive effects at one locus.

The inheritance of erucic acid in *B. juncea* was studied by Kirk and Hurlstone (1983). These authors crossed Zem 1 and Zem 2 zero erucic *B. juncea* with Indian *B. juncea* cultivars containing 44 to 51% erucic acid and European *B. juncea* cultivars containing 21.9 to 26.8%

erucic acid in their oil. In addition, crosses between the Canadian condiment cultivar Lethbridge 22A, containing 23% erucic acid, and RH-30-A *B. juncea* from India, containing 57% erucic acid were made. F_1 seeds derived from crosses between Zem lines and European cultivars contained 12% erucic acid while F_1 seeds derived from crosses between Zem lines and Indian cultivars contained 33% erucic acid. The authors classified seed containing less than 5% erucic acid as zero erucic acid types and seed containing more than 5% erucic acid as high erucic acid types. F_2 segregation analysis of the Zem x European and Zem x Indian cultivar crosses indicated the presence of one and two genes for erucic acid synthesis in the European and Indian *B. juncea* cultivars, respectively. The authors did not study erucic acid segregation in backcross populations.

Fernandez-Escobar *et al.* (1988) crossed the high erucic acid *B. carinata* line C-101 with the zero erucic acid *B. napus* cultivar Duplo. The interspecific F_1 plant was selfed and F_2 seed produced. The F_1 was also backcrossed to *B. carinata* and BC_1 seeds produced. F_1 seed contained 24% erucic acid and the backcross seeds segregated into three erucic acid classes with mean erucic acid contents of 20.1%, 33.9% and 47.8%. Based on these data the authors concluded that erucic acid in *B. carinata* was controlled by four alleles at two loci.

2.1.6 Further fatty acid improvement in *Brassica* seed oils

The fatty acid composition of a vegetable oil determines its nutritional as well as industrial value (Downey and McGregor 1976). Canola oils are used as salad oils, for frying and for the production of margarines. Each use requires a specific fatty acid composition. Objectives of fatty acid modification include, increasing oleic acid to improve the heat

stability of cooking oil, increasing the dietary essential linoleic acid and decreasing linolenic acid to reduce taste impairment that results from oxidation of the double bond. High palmitic acid content is essential to reduce the tendency of margarines to form large internal crystals.

Seed mutagenesis was used to reduce linolenic acid content in *B. napus*. Rakow (1973) developed a low linolenic acid mutant that contained 5% linolenic acid in its seed which was half the level of linolenic acid normally found in *B. napus*. This material and further low linolenic acid mutants developed by Röbbelen and Nitsch (1975) were provided to the University of Manitoba, Winnipeg, where Dr. B. R. Stefansson continued selections for the lowest possible levels of linolenic acid in progeny of crosses between different low linolenic acid *B. napus* mutants from Germany. Stellar was the first low linolenic acid (3%) *B. napus* cultivar developed from this work (Scarth *et al.* 1988). Roy and Tarr (1986) developed low linolenic acid *B. napus* from crosses between low linolenic acid *B. napus* mutants and *B. juncea*. The lines that these authors developed had high linoleic (30%) and low linolenic acid (1.5%) contents (Roy and Tarr 1986).

2.2 MATERIALS AND METHODS

2.2.1 Plant material, growing conditions and selfing and crossing techniques

For the erucic acid inheritance study, two high erucic acid cultivars, Dodolla (yellow seeded) and S-67 (brown seeded), and the zero erucic acid line C90-14 (yellow seeded) were used as parents in crosses to produce segregating backcross and F_2 generations. The cultivars Dodolla and S-67 were developed and released by the Institute of Agricultural Research, Holetta Research Centre, in Ethiopia. The zero erucic acid line C90-14 was developed through an interspecific cross between the high erucic acid *B. carinata* cultivar S-67 and zero erucic acid *B. juncea* strain Zem 2330 followed by four backcrosses to *B. carinata* cultivar Dodolla with selection for zero erucic acid after each backcross (Getinet *et al.* 1994).

The half-seed technique for fatty acid analysis (Downey and Harvey 1963) was used to ensure that the plants used for the inheritance study were true breeding for either the high (Dodolla and S-67) or zero erucic acid (C90-14) traits. Plants grown from half-seeds of Dodolla and S-67 were reciprocally crossed with C90-14, and parent plants were also self pollinated (Table 2.1). Five F_1 plants were grown from each cross and self pollinated and backcrossed to both parents to produce F_2 and BCF_1 seed, respectively. Selected backcross and F_2 half-seeds with varying erucic acid contents were grown to verify erucic acid contents of the F_2 and BCF_1 generations. All plants were grown in the growth cabinet except the BCF_2 and F_3 seeds which were produced in the greenhouse.

The Saskatoon Research Centre soilless potting mix was used for growing of plants in pots in the greenhouse and growth cabinet. The mixture was prepared by mixing the following ingredients. One 113 litre bale sphagnum peat moss, 2 x 110 l bags medium grade vermiculite, 4.0 kg fine ground calcium carbonate, 3.0 kg Osmocote 18-6-12 controlled release fertilizer, 655 g 20% superphosphate fines (0-20-0 fertilizer), 33 g fitted trace elements no. 555, 15 g 13% chelated iron, and washed torpedo sand screened to 1 cm maximum particle size. The peat moss mix was shredded to obtain an even texture so that all the ingredients, except the sand and vermiculite were evenly distributed. Finally, four parts of this mix were mixed in a cement mixer with one part of sand. The pot size was 13 cm in diameter and 15 cm deep. Plants were watered every day and fertilized once a week with 20/20/20 fertilizer. Older leaves were removed from plants to avoid insect buildup. An 18 h photoperiod in combination with a $22^0C/18^0C$ day/night temperature was used in the growth chamber. Aphids were controlled by spraying Primor (Primicab) 50% WP at 0.5g/l. Spider mites were controlled by Vendex (Fenbutatin-oxide) 50% WP.

Crossing was done by emasculating immature buds of the female plant followed by pollination with pollen from the male parent on three consecutive days. Pollinated buds were covered with plastic bags for one week to exclude foreign pollen. Selfing was achieved by emasculating buds followed by immediate pollination using pollen from the same plant. Crossed and selfed seed was left in the pod until maturity.

2.2.2 Fatty acid analysis

Fatty acid analysis of parents, F_1, F_2, F_3, BCF_1 and BCF_2 seed generations were conducted on individual seeds according to the standard laboratory technique of the Saskatoon Research Centre (J. P. Raney, unpublished) following a modified version of the technique described by Thies (1971). The average weighed fatty acid content of parents and their reciprocal F_1's was calculated based on the following formula.

$$\% \text{ Fatty acid} = \frac{\text{Area of one fatty acid}}{\text{Total area of all fatty acids}} \times 100$$

This formula reports the fatty acid content of seeds independent of their oil content or seed size.

Table 2.1 Pattern of self and cross pollination and reciprocal crosses made between high erucic acid cultivars (HEC) Dodolla (P1) and S-67(P2) and low erucic acid line C90-14 (P3).

	Male parent			
Female parent	HEC	C90-14	C90-14 x HEC	HEC x C90-14
HEC	Self	Cross	Cross	Cross
C90-14	Cross	Self	Cross	Cross
C90-14 x HEC	Cross	Cross	Self	Not Done
HEC x C90-14	Cross	Cross	Not Done	Self

Single seeds were placed in 0.2 ml vials containing 0.01 ml methylating solution. The seed was then crushed with a glass rod. In case of single cotyledon analysis, single seeds were separated into outer and inner cotyledon and the dried outer cotyledons were placed in vials. The inner cotyledons were placed in germinating trays containing sand, and

seedlings were transferred to pots in the growth cabinet or greenhouse. After crushing of either the whole seed or the dried outer cotyledon, 0.05 ml of methylating solution, 0.08% sodium metal in methanol, was added for transesterification of fatty acids, and left to react for 30 min at room temperature. Then, 0.01 ml of phosphate buffer was added to each vial. Then the vials were put under a stream of air to evaporate excess methanol. Finally 0.05 ml of hexane was added and the vials were put on the autosampler of the gas chromatograph for fatty acid analysis. A Hewlett Packard gas chromatograph model 5890 equipped with a flame ionization detector, an autosampler model 7673 and a capillary column Supelcowax 10 0.05 μm by 32 mm, 15 m and hydrogen carrier gas was used for separation and quantification of fatty acid methyl esters.

2.3 RESULTS

2.3.1 Fatty acid composition of original parent seed

The erucic acid contents of the high erucic acid cultivars Dodolla and S-67 were 39.6 and 40.2%, respectively, and that of the zero erucic acid line C90-14 was 0.2% (Table 2.2). Eicosenoic acid contents of Dodolla and S-67 were 7.9 and 7.5% while that of C90-14 was 0.6%. The oleic, linoleic and linolenic acid contents of the two high erucic acid cultivars were similar and averaged 10.3%, 15.4%, and 21.4%, respectively.

Table 2.2 Mean and standard deviation of fatty acid composition of twelve half-seeds each of high erucic acid cultivars Dodolla (P1) and S-67 (P2), and zero erucic acid line C90-14 (P3) grown in the field at Saskatoon.

	Fatty acids (% of total)							
Parents	Erucic	Eicosenoic	Oleic	Linoleic	Linolenic	Palmitic	Stearic	Others[1]
P1= Dodolla								
Mean	39.6	7.9	10.6	15.2	21.9	2.1	0.6	1.8
±SD	2.1	0.8	1.1	1.4	1.6	0.2	0.1	0.2
P2=S-67								
Mean	40.2	7.5	10.1	15.6	21.0	2.3	0.7	2.6
±SD	1.9	0.7	1.0	1.5	1.8	0.2	0.1	0.1
P3=C90-14								
Mean	0.2	0.6	26.2	36.7	29.1	4.1	1.8	1.8
±SD	0.1	0.1	2.3	2.4	2.9	0.5	0.2	0.1

[1] Others include saturated (myristic, arachidic and lignoceric) and unsaturated (palmitoleic, eicosadenoic, nervonic) fatty acids.

2.3.2 Fatty acid composition of parents and F_1 seed

The fatty acid composition of selfed seed of the high erucic acid parents Dodolla and S-67 and the low erucic acid C90-14, when grown in the growth cabinet (Table 2.3), was somewhat different from that of the original field grown seed (Table 2.2) used as a seed source in this study. The average erucic acid content of Dodolla and S-67 seed produced in the growth cabinet was 35.5% which was 4% lower than that of the original seed. Eicosenoic and oleic acids of Dodolla and S-67 were lower in the growth cabinet while linoleic and linolenic acid contents were higher. The erucic acid and eicosenoic acid contents of selfed seed of the low erucic acid parent C90-14 were low in both the original and growth room produced seed. Oleic acid content of C90-14 was lower under growth room conditions (19.3 versus 26.2%) while linoleic and linolenic acid contents were higher.

The erucic acid content of F_1 seed borne on the high erucic acid parents Dodolla and S-67 was similar and averaged 21.5% (Table 2.3). The erucic acid content of F_1 seed borne on the low erucic acid parent C90-14 was similar to that of F_1 seed born on the high erucic parent in both crosses. Eicosenoic acid content of F_1 seed was somewhat elevated and higher than those of the high erucic acid parents Dodolla and S-67. Oleic acid contents of F_1 seed was similar to that of the high erucic acid parents. In contrast, the linoleic acid content of F_1 seed was about 8% higher than that of Dodolla and S-67; however the linolenic acid content of F_1 seed and high erucic acid parent seed was similar (Table 2.3).

Table 2.3 Average fatty acid composition of parents and F_1 seed from reciprocal crosses between high erucic acid cultivars Dodolla (P1) and S-67 (P2) and zero erucic acid line C90-14 (P3) grown in the growth cabinet.

	Fatty acids (% of total)[1]							
Genotype	Erucic	Eicosenoic	Oleic	Linoleic	Linolenic	Palmitic	Stearic	Others[2]
Parents								
P1	35.7	4.7	7.4	18.2	24.9	4.3	0.5	4.3
P2	35.4	4.5	7.0	19.6	23.1	4.3	0.7	5.4
P3	0.1	0.8	19.3	38.0	34.0	5.6	1.0	1.2
F_1								
[P1 x P3]	22.2	5.5	9.2	25.6	26.9	4.9	1.1	4.6
[P2 x P3]	20.0	6.3	11.1	28.0	22.4	5.8	0.7	5.7
[P3 x P1]	21.4	5.8	9.2	28.3	23.1	5.3	0.5	6.4
[P3 x P2]	22.4	6.9	8.6	25.6	25.6	4.6	1.1	5.2
Mean F_1	21.5	6.1	9.5	26.9	24.4	5.1	1.0	5.5

[1] Single 10 seed bulk analysis

[2]Others include saturated (myristic, arachidic, lignoceric,) and unsaturated (palmitoleic, eicosadenoic, nervonic) fatty acids.

2.3.3 Erucic acid content of F_1 and F_2 seed from backcrosses to the low erucic acid parent C90-14

This study focuses on the inheritance of erucic acid in *B. carinata* seed oil. For this reason, only erucic acid data are presented for the segregating backcross and F_2 generations of two crosses of high erucic acid parents Dodolla and S-67 with the low erucic acid line C90-14.

The erucic acid content of individual backcross (BCF_1) seed derived from the backcross (Dodolla x C90-14) x C90-14 ranged from <0.5 to 30% (Figure 2.3b). The frequency distribution was separated into three classes consisting of seed with 0.5%, 6 to 16% and >16% erucic acid. The 1 : 2: 1 genetic ratio which represents a two gene model with additive effects was tested for the two reciprocal cross combinations (Table 2.4). The observed ratios were not significantly different from the theoretical ratio and also the pooled data fitted the 1:2:1 ratio. The erucic acid content of individual BCF_1 seeds from the (S-67 x C90-14) cross, backcrossed to C90-14, ranged also from 0.5% to 30% erucic acid (Figure 2.4b) and the frequency distribution was separated into three classes of seed with 0.5%, 6 to 16% and >16% erucic acid (Table 2.4). As with the backcross [(Dodolla x C90-14) x C90-14)] the 1:2:1 genetic ratio of two additive genes was tested, and this ratio gave a good fit to observed ratios for the four backcross combinations as well as for the pooled data (Table 2.4). The combined data from both backcrosses also supported the two gene model. The F_2 generation of the backcross to the low erucic acid parent was not produced since the BCF_1 data showed good fit to the 1:2:1 theoretical ratio.

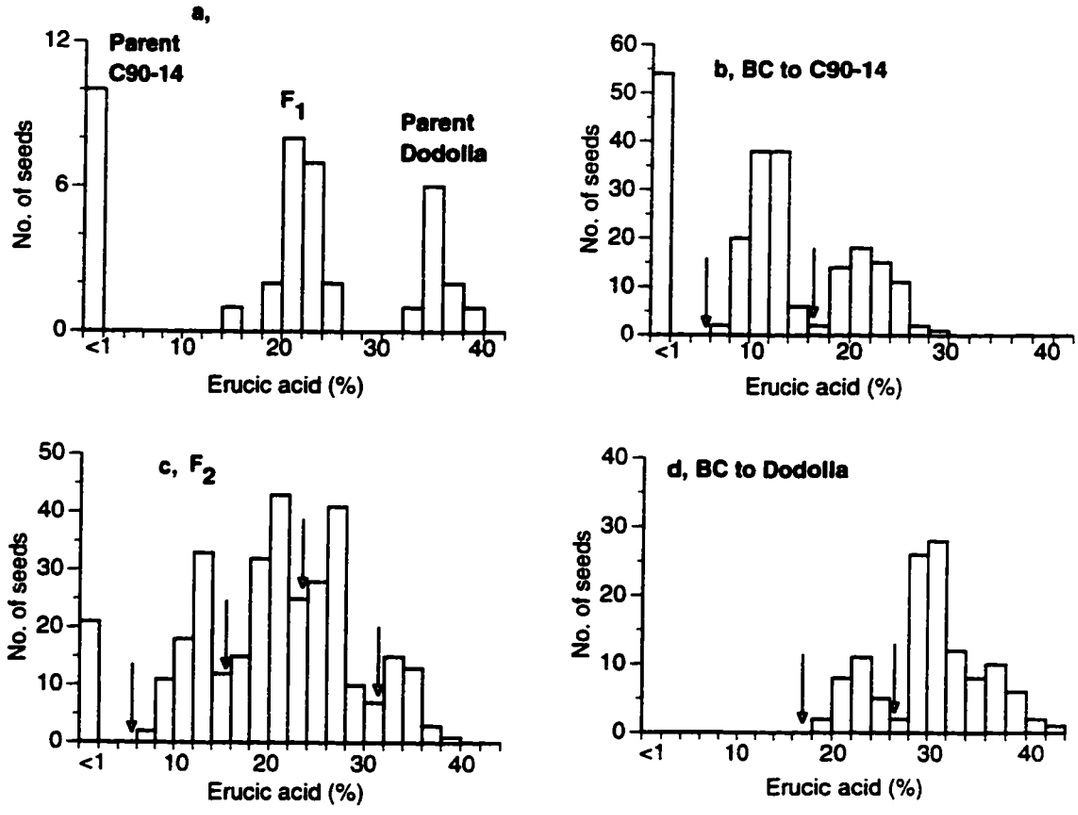

Figure 2.3 Frequency distribution of erucic acid content in individual seeds of the high erucic acid cultivar Dodolla, zero erucic acid line C90-14 and their F_1, BCF_1 and F_2 generations grown in the growth cabinet. Arrows indicate class separations in BCF_1 and F_2 generations.

Table 2.4 Number of single seeds in three erucic acid classes with chi-square values and probabilities for goodness of fit for four backcross populations each from two back crosses to the low erucic acid parent C90-14 grown in the growth cabinet.

Backcross generation	No of seeds with erucic acid content <0.5%	6-16%	>16%	Ratio tested	Chi-quare value	P
[(P3 x P1) x P3]	18	40	22	1:2:1	0.40	0.90-0.80
[(P1 x P3) x P3]	10	20	8	1:2:1	0.31	0.95-0.90
[P3 x (P1 x P3)]	17	27	22	1:2:1	2.94	0.30-0.20
[P3 x (P3 x P1)]	9	19	11	1:2:1	0.23	0.90-0.80
Pooled	54	106	63	1:2:1	1.27	0.70-0.50
Heterogeneity					2.61	0.50-0.30
[(P3 x P2) x P3]	12	25	12	1:2:1	0.02	0.99-0.98
[(P2 x P3) x P3]	13	19	7	1:2:1	1.87	0.50-0.30
[P3 x (P2 x P3)]	7	12	8	1:2:1	0.41	0.90-0.80
[P3 x (P3 x P2)]	8	17	7	1:2:1	0.19	0.95-0.90
Pooled	40	73	34	1:2:1	0.50	0.80-0.70
Heterogeneity					2.00	0.50-0.30
Total	94	180	97	1:2:1	0.37	0.90-0.80
Heterogeneity					1.40	0.50-0.30

P1=Dodolla, P2=S-67, P3=C90-14.

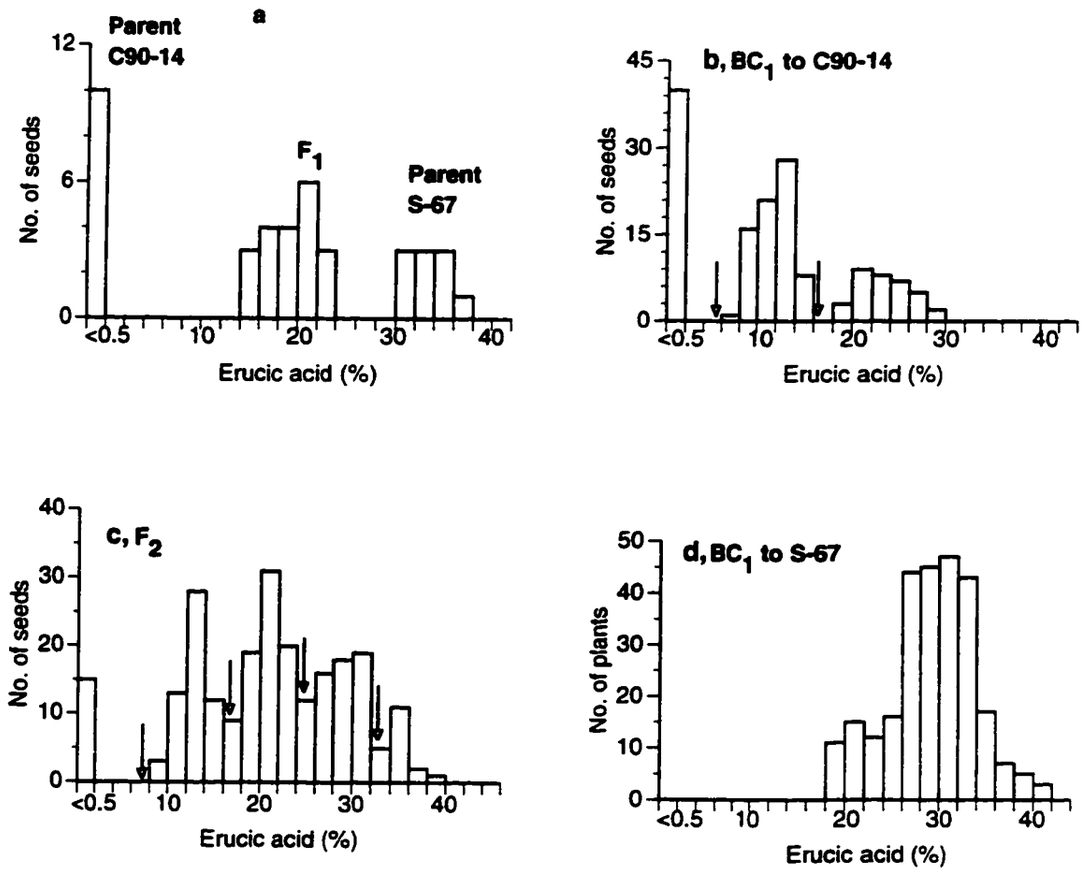

Figure 2. 4 Frequency distribution of erucic acid content in individual seeds of the high erucic acid cultivar S-67, zero erucic acid line C90-14 and their F_1, BCF_1 and F_2 generations grown in the growth cabinet. Arrows indicate class separations in BC F_1 and F_2 generations.

2.3.4 Erucic acid content of F_1 and F_2 seed from backcrosses to the high erucic acid parents Dodolla and S-67

The erucic acid content of individual backcross seeds derived from the backcross [(Dodolla x C90-14) x Dodolla)] ranged from 18 to 44% (Figure 2.3d). The frequency distribution was separated into two classes with the division at 26% erucic acid. The two gene model with additive effects that was used for testing of segregation ratios in backcross generations derived from backcrosses to the low erucic acid parent (see above) was also applied for testing segregation ratios in backcrosses to the high erucic acid parent Dodolla. The 1:2:1 theoretical ratio characterizing BC seed that contained either two (E^aE^a) or three ($E^aE^aE^a$) or four ($E^aE^aE^aE^a$) alleles for erucic acid synthesis, respectively, could be used for testing of segregation ratios, because seeds containing either three ($E^aE^aE^a$) or four ($E^aE^aE^aE^a$) alleles for erucic acid synthesis could not be separated from each other but these two genotypes could be separated from seeds containing two (E^aE^a) alleles for erucic acid synthesis. Therefore, the 1:3 ratio was used for testing of segregation ratios and this ratio was not different from the observed ratios and the pooled data (Table 2.5). The range in erucic acid content observed in BCF_1 seed of the backcross [(S-67 x C90-14) x S-67)] was similar to the ranges in erucic acid for BCF_1 seed of the Dodolla backcross (Figure 2.4d). However, the frequency distribution was continuous and no theoretical ratios could be tested.

Eight BCF_1 seeds of the backcross [(C90-14 x S-67) x S-67)] ranging in erucic acid content from 19.0 to 46.1% based on half-seed analysis were grown for BCF_2 progeny testing

Table 2.5 Number of single seeds in two erucic acid classes with chi-square values and probabilities for goodness of fit for four backcross populations from backcross to the high erucic acid parent Dodolla grown in the growth cabinet.

Backcross generation	No of seeds with erucic acid content		Ratio tested	Chi-square value	P
	18-26 %	>26%			
[P1 x (P3 x P1)]	8	32	1:3	0.52	0.50-0.30
[P1 x (P1 x P3)]	6	33	1:3	1.92	0.20-0.10
[(P3 x P1) x P1]	5	26	1:3	1.30	0.30-0.20
[(P3 x P1) x P1]	9	34	1:3	0.38	0.70-0.50
Pooled	28	125	1:3	3.66	0.10-0.05
Heterogeneity				0.46	0.50-0.30

P1 = Dodolla, P3= C90-14.

to verify their erucic acid genotypes. The BCF_1 half-seed with 19.0% (plant E1) erucic acid based on fatty acid analysis of its outer cotyledon, produced BCF_2 seed that contained from <1 to 40% erucic acid (Figure 2.5, E-1). The frequency distribution was separated into five classes consisting of seed with 0.5%, 8 to 16%, 18 to 26%, 26 to 32% and > 32% erucic acid. The observed ratio was not significantly different from the theoretical ratio, and identified the BCF_1 half-seed plant as a double heterozygote (E^ueE^ue) genotype. The BCF_1 half-seed with 30.4% erucic acid (E-8) produced BCF_2 seed that had erucic acid contents ranging from 16 to 40% erucic acid (Figure 2.5, E-8). The frequency distribution was separated into two classes of seed with 16 to 24% and >24% erucic acid. The observed segregation ratio was tested for goodness of fit to the 1:3 theoretical ratio and was found not to be significantly different from it. It was assumed that BCF_2 seed containing three ($E^uE^uE^a$) or four ($E^uE^uE^uE^a$) alleles for erucic acid synthesis could not be separated from each other but could be separated from seed containing two (E^uE^a) alleles for erucic acid syntheses (see above). Plant E-8 was therefore of the $E^uE^uE^ue$ genotype.

Six additional BCF_1 seeds with 39.2% (E-3), 38.4% (E-6), 46.1% (E-10), 38.5% (F-1), 42.3% (F-8) and 39.9% (F-9) erucic acid were also progeny tested. BCF_2 seed from these plants all contained more than 34% erucic acid, and the frequency distributions were continuous (Figure 2.5). It was concluded that all six plants were of the $E^uE^uE^uE^u$ genotype.

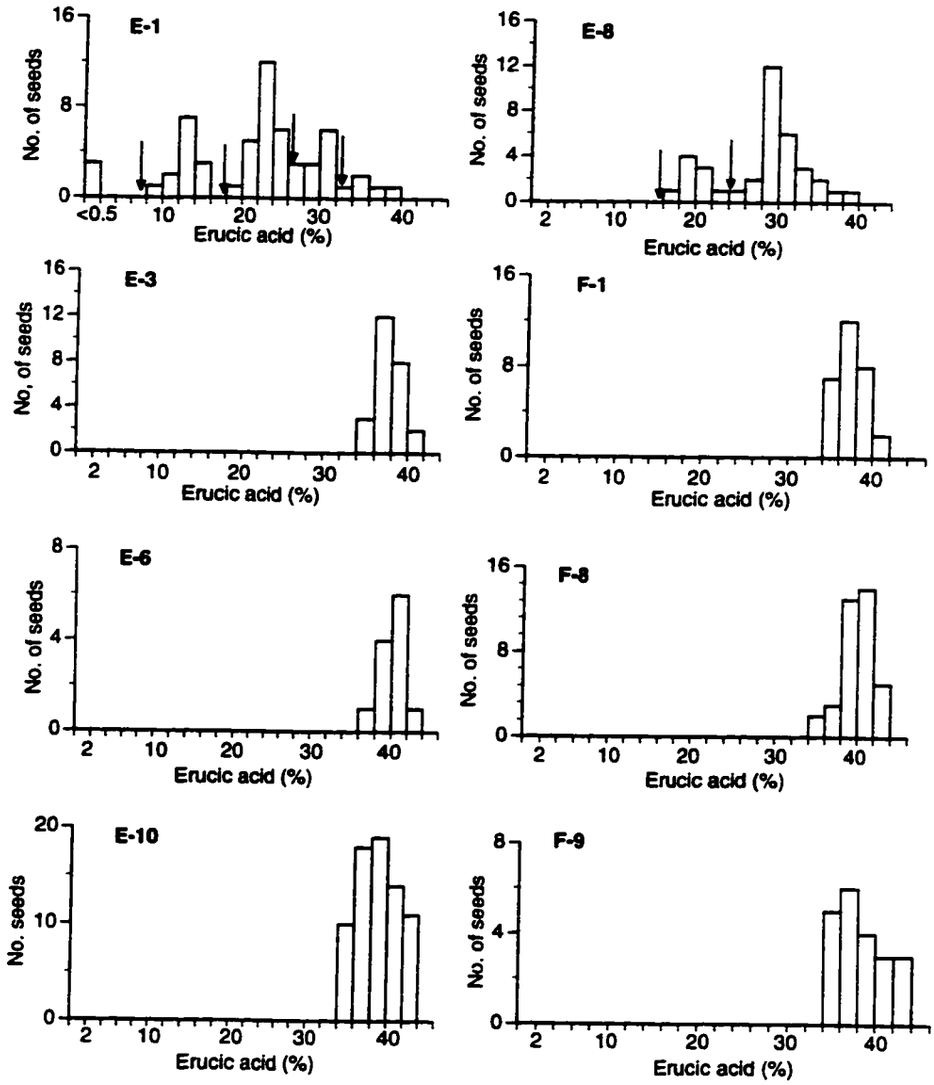

Figure 2.5 Frequency distribution of erucic acid content in individual seeds from eight BCF_2 families of the cross [(C90-14 x S-67) x S-67] generations grown in the greenhouse. Arrows indicate class separations.

2.3.5 Erucic acid content of F_2 and F_3 seed

The erucic acid content of individual F_2 selfed seeds from the two reciprocal crosses of (Dodolla x C90-14) and (S-67 x C90-14) ranged from <0.5 to 40% (Figures 2.3C, 2.4c and 2.6c, Table 2.6). The frequency distributions for both crosses were separated into five classes consisting of seed with erucic acid contents of < 0.5%, 8 to 16%, 16 to 24%, 24 to 32% and >32%. The genetic ratio of 1:4:6:4:1 which represents a two gene model with additive effects for F_2 segregation was tested for goodness of fit to the observed segregation ratios. It was found that the observed ratios were not significantly different from the theoretical ratio (Table 2.6).

Nine F_2 half-seed plants from the cross (C90-14 x S-67) with 0.1% (C-9), 9.8% (D-6), 15.4% (A-10),10.7% (B-3), 11.3% (B-8), 13.2% (C-10), 25.0% (D-7), 27.4% (A-12) and 39.8% (A-11) erucic acid content (Table 2.7) were grown and F_3 seed analysed for erucic acid content. All 36 F_3 seed of the F_2 half-seed plant C-9 contained less than 0.2% erucic acid and were thus true breeding for the zero erucic acid characteristic. In contrast the erucic acid contents of all 36 F_3 seed of the F_2 half-seed plant A-11 were all greater than 32% identifying this plant as a homozygous ($E^aE^aE^aE^a$) high erucic acid plant. The five F_2 half-seed plants that contained from 9.8% to 15.4% erucic acid produced F_3 seed with erucic acid contents ranging from <0.5 to about 30%. The frequency distributions for erucic acid of these five plants separated into three classes (Figure 2.7). The theoretical segregation ratio of 1:2:1 representing a two gene model with additive effects and one ($E)^a$ allele for erucic acid synthesis was tested against the observed ratios and was found to be not significantly different (Table 2.7). All five F_2 plants were thus carrying one (E^a)allele for erucic acid

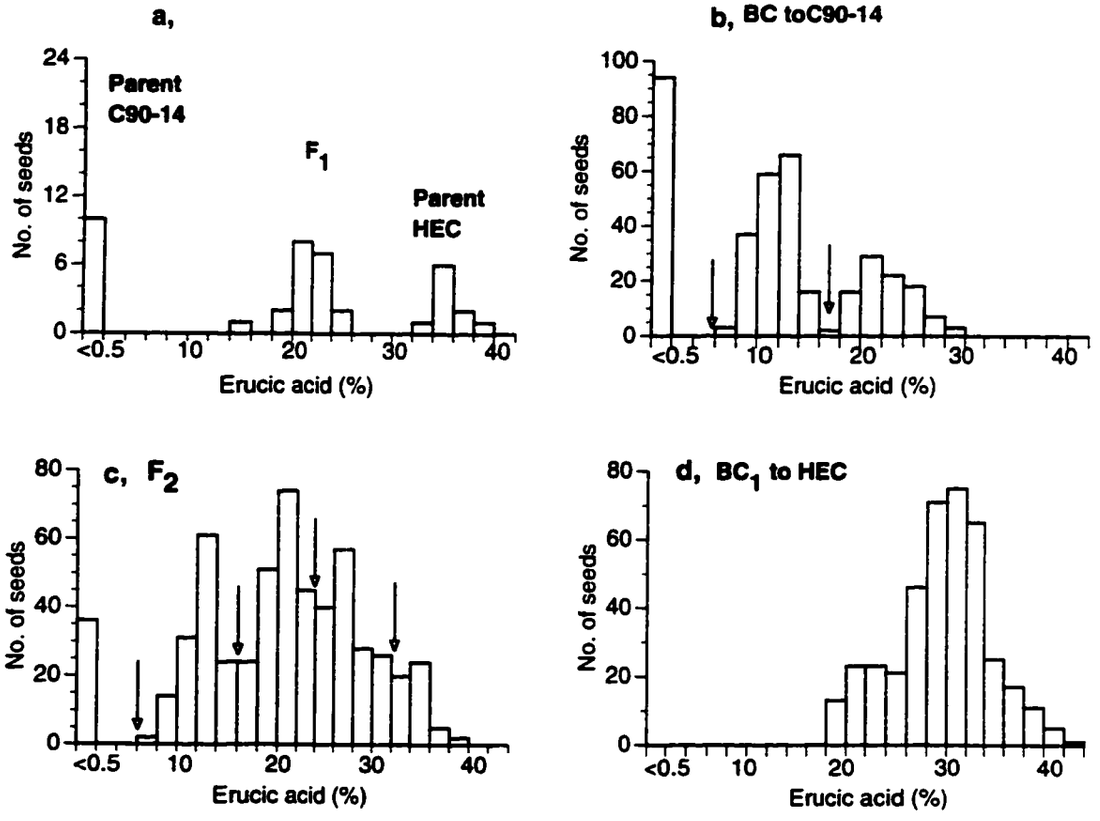

Figure 2.6 Frequency distribution of erucic acid content in individual seeds from high erucic acid cultivars (HEC) Dodolla and S-67, zero erucic acid line C90-14 and their F_1, BCF_1 and F_2 generations grown in the growth cabinet. Arrows indicate class separations in BCF_1 and F_2.

synthesis.

F_2 plants D-7 and A-12 produced F_3 seed that spand the whole range of erucic acid contents (Figure 2.7, D-7, A-12). Their frequency distributions were characteristic of plants that contained two alleles (E^aE^a) for erucic acid synthesis their genotypes was E^aeE^ae (Table 2.8).

Table 2.6 Number of single seeds in five erucic acid classes with chi-square values and probabilities for goodness of fit for four F_2 populations from the cross of the high erucic acid parents Dodolla (P1) and S-67 (P2) and zero erucic acid line C90-14 (P3) grown in the growth cabinet.

	No of seeds with % erucic acid content							
F_2 generation	<0.5	8-16	16-24	24-32	>32	Ratio tested	Chi-square value	P
[P3 x P1]	9	25	37	28	12	1:4:6:4:1	5.09	0.50-0.25
[P1 x P3]	12	51	78	58	20	1:4:6:4:1	3.77	0.50.0.25
Pooled	21	76	115	86	32	1:4:6:4:1	7.55	0.25-0.10
Heterogeneity							1.31	0.90-0.75
[P3 x P2]	7	28	41	29	6	1:4:6:4:1	0.07	1.00-0.99
[P2 x P3]	8	28	38	37	11	1:4:6:4:1	4.41	0.50-0.25
Pooled	15	56	79	66	17	1:4:6:4:1	2.37	0.75-0.50
Heterogeneity							2.11	0.75-0.50
Total	36	132	194	152	48	1:4:6:4:1	7.52	0.25-0.10
Heterogeneity							2.40	0.75-0.50

Table 2.7 Number of single seeds in three erucic acid classes with chi-square values and probabilities for goodness of fit for F_3 seed from six F_2 plants from (C90-14 x S-67) grown in the greenhouse.

F_2 plant no.	Erucic acid	No. of seeds with % erucic acid content			Ratio tested	Chi-square value	P
		<0.5	6-18	>18			
C-9	0.1	36					
D-6	9.8	12	23	14	1:2:1	0.35	0.90-0.80
A-10	15.4	8	14	9	1:2:1	0.35	0.90-0.80
B-3	10.7	10	23	13	1:2:1	0.39	0.90-0.80
B-8	11.3	5	12	5	1:2:1	0.18	0.95-0.90
C-10	13.2	9	19	10	1:2:1	0.05	0.98-0.95

Table 2.8 Number of single seeds in five erucic acid classes with chi-square values and probabilities for goodness of fit for F_3 seed from three F_2 plants from (C90-14 x S-67) grown in the greenhouse.

F_2 plant no.	erucic acid	No of seeds with % erucic acid					Ratio tested	Chi-square value	P
		<0.5	6-18	18-24	24-32	>32			
D-7	25.0	3	13	15	11	5	1:4:6:4:1	2.02	0.80-0.70
A-12	27.4	3	11	14	8	6	1:4:6:4:1	5.21	0.30-0.20
A-11	39.8					36			

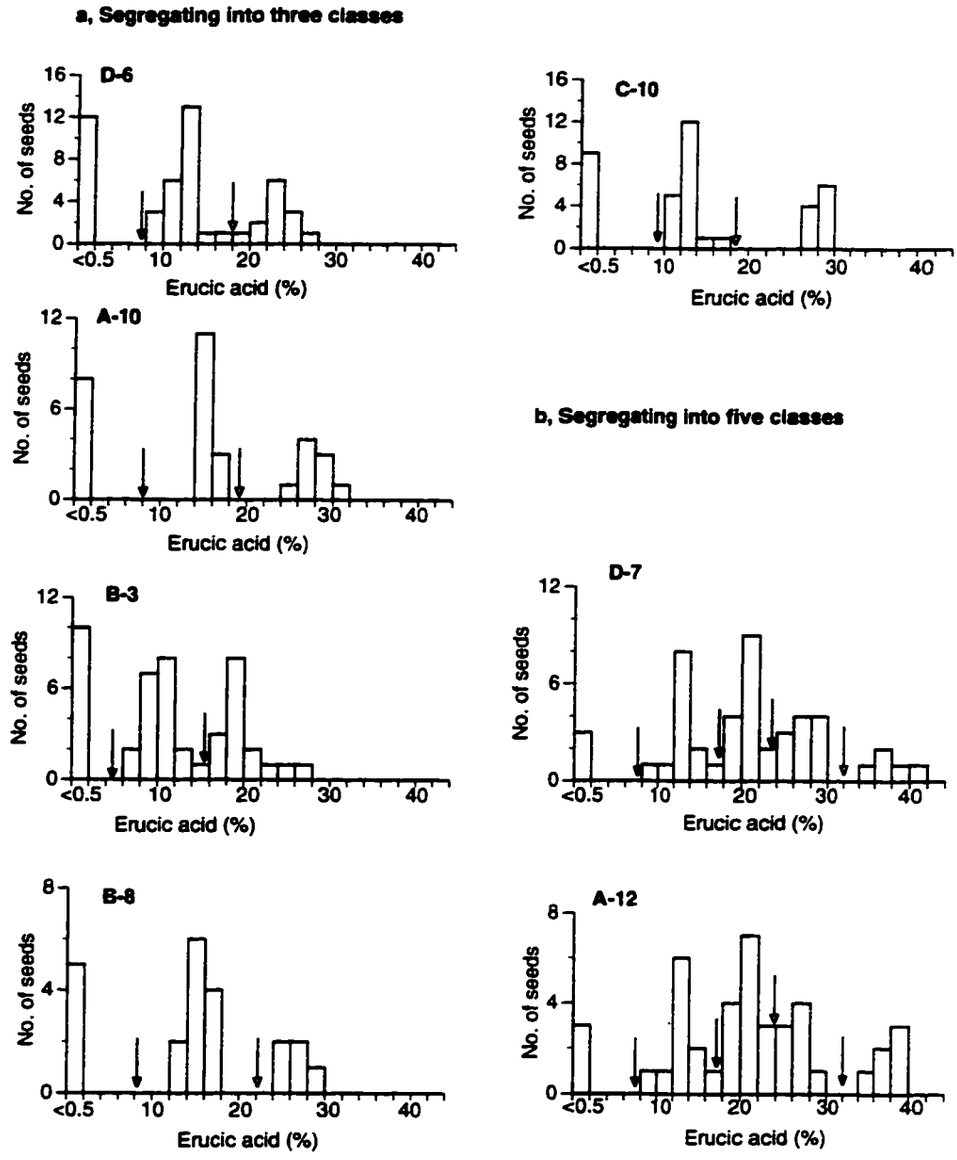

Figure 2.7 Frequency distribution of erucic acid content in individual F_3 seeds from seven F_2 families of the cross [(C90-14 x S-67] grown in the greenhouse. Arrows indicate class separations.

Seven seeds of Dodolla and ten seeds of S-67, the high erucic acid parents used in this study, were analysed for erucic acid content by the half-seed method, and were found to be high in erucic acid (Table 2.9). Selfed seed of these plants were again analysed for erucic acid content. There was a narrow range in erucic acid contents of these seeds but all were high in erucic acid thus confirming that the seed used of the high erucic acid parent plants, Dodolla and S-67, was true breeding for the high erucic acid trait.

Table 2.9. Erucic acid content of seven S_1 Dodolla and ten S-67 high erucic acid parent seed mean, range and standard deviation of S_2 seed for each parent half seed plant produced in the greenhouse.

Variety	S_1 half-seed plant no.	Erucic acid of S_1 half-seed	No. of S_2 seeds	Erucic acid (%) of S_2 seed		
				Range	Mean	±SD
Dodolla	A-3	43.8	40	37.9-40.5	39.0	0.83
	A-7	42.2	34	38.0-44.7	41.4	0.84
	A-1	43.4	18	39.5-44.5	43.2	0.83
	B-4	41.1	44	36.1-42.1	38.2	0.82
	B-1	43.8	37	39.9-43.1	40.8	0.81
	B-5	43.9	31	36.0-43.5	41.8	0.82
	A-12	43.8	29	38.0-45.2	42.0	0.78
	Mean	43.1			42.0	
S-67	C-1	42.6	36	39.3-43.8	41.7	1.30
	C-2	43.4	34	39.3-45.2	43.0	3.40
	C-4	45.4	27	42.6-46.1	44.5	2.10
	C-5	40.6	21	42.1-45.7	44.7	1.20
	C-7	42.4	36	40.2-47.2	45.3	1.60
	B-12	44.2	41	42.9-47.6	44.7	2.60
	E-10	36.9	68	36.7-44.7	39.8	2.30
	B-10	44.3	39	42.2-46.8	45.4	0.83
	C-3	38.0	16	41.0-44.5	42.7	1.20
	C-6	45.6	38	42.2-47.0	45.0	1.30
Mean		42.3			43.6	

2.3.6 Fatty acid composition at different levels of erucic acid heterozygosity

The decrease of erucic acid from about 40% to 0.5% was associated with increases in oleic acid from 7 to 30%, linoleic acid from 14 to 36% and linolenic acid from 17 to 25% (Table 2.10). Palmitic acid was about two percentage points higher in zero erucic acid seed than in high erucic acid seed. On other hand, the eicosenoic acid content, which was 0.5% in zero erucic acid seed reached 10.9% in seed carrying one erucic acid allele and decreased to 5.7% as erucic acid contents increased in seed with four erucic acid alleles.

Table 2.10 Fatty acid composition of seed with 0, 1, 2, 3 and 4 erucic acid alleles based on 20 analyses of single seeds of each genotype.

Genotype	Range in erucic acid		Fatty acid (% of total)					
			Erucic	Eicose.	Oleic	Linoleic	Linolenic	Palmitic
eeee	<0.5	Mean	0.1	0.4	29.8	36.0	25.2	5.1
		±SD	0.1	0.3	2.5	2.6	2.3	0.6
E^{u}eee	8-16	Mean	13.4	10.9	19.1	24.3	22.5	4.5
		±SD	0.2	1.7	3.2	2.9	3.0	0.4
E^{u}eE^{u}e	16-24	Mean	23.8	9.2	14.6	22.3	20.9	4.8
		±SD	1.5	2.1	2.0	3.1	2.8	0.7
E^{u}E^{u}E^{u}e	24-32	Mean	29.7	8.1	11.3	19.6	20.6	4.2
		±SD	2.3	1.7	2.4	2.3	2.7	0.6
E^{u}E^{u}E^{u}E^u	>32	Mean	43.9	5.7	7.0	14.1	17.3	3.2
		±SD	2.8	1.2	1.7	1.5	10.3	0.2

2.4 DISCUSSION: INHERITANCE OF ERUCIC ACID IN *B. CARINATA*

The *B. carinata* cultivars Dodolla and S-67 are the only gomenzer cultivars which are certified for production in Ethiopia. The fatty acid composition of the two cultivars is similar and typical of *B. carinata* accessions (Westphal and Marquard 1980, Seegeler 1983, Getinet 1986) and is representative of the Ethiopian germplasm. The small differences in erucic acid content of 4% between growth cabinet and field grown seed of S-67 and Dodolla observed in this study is an indication that environmental factors have an effect on erucic acid content of *B. carinata* seed. Both cultivars synthesized more C18 fatty acids in the cooler grown cabinet environment compared to the field.

The zero erucic acid line C90-14 was developed by introgression of the zero erucic acid allele from *B. juncea* Zem 2330 into *B. carinata* S-67 and Dodolla (Getinet *et al.* 1994). It contains high levels of oleic, linoleic and linolenic acids and very low levels of erucic and eicosenoic acids compared to oils from the of high erucic acid cultivars Dodolla and S-67. Polyunsaturated fatty acids linoleic and linolenic are higher and oleic acid is lower in zero erucic acid *B. carinata* than in zero erucic acid *B. napus* (Getinet *et al* 1994, Raney *et al.* 1995). This indicates that zero erucic acid *B. carinata* has a higher fatty acid desaturation activity from oleic→linoleic→ linolenic acids than *B. napus*. The higher level of linolenic acid content is undesirable since it makes the oil more susceptible to autoxidation.

No maternal effects were observed for erucic acid content in crossed seed from reciprocal crosses between high and low erucic acid parents. Erucic acid contents of F_1 seed were intermediate between the parents indicating embryonic control of erucic acid synthesis in *B. carinata*. Similar observations with regard to embryonic control of erucic acid synthesis were made in *B. napus* (Harvey and Downey 1964, Kondra and Stefansson 1965), *B. rapa* (Dorrell and Downey 1964) and *B. juncea* (Kirk and Hurlstone 1983). However, in all cases the erucic acid content of F_1 seed was shifted towards that of the high erucic acid parents. The 1:2:1 three class segregation of BCF_1 seeds for erucic acid content derived from the backcrosses to the low erucic acid parent indicated that erucic acid in Dodolla and S-67 was controlled by two alleles at each of two loci acting in an additive manner. The 1:2:1 segregation ratio of progenies of BCF_1 half-seed plants containing from 9% to 15% erucic acid confirmed the above assumption. The BCF_1 to Dodolla segregated into a 1:3 ratio instead of the expected 1:2:1 ratio. This was because genotypes containing 26 to 32% erucic acid and those containing >32% erucic acid were not clearly distinguishable from each other. These two upper classes were also indistinguishable in *B. napus* (Harvey and Downey 1964) and *B. juncea* (Kirk and Hurlstone 1983). Therefore, the 1:3 ratio observed in the BCF_1 to Dodolla was in fact a 1:2:1 ratio. The erucic acid segregation ratio of 1:4:6:4:1 observed in F_2 seed supported the two gene inheritance model.

The allelic series for erucic acid in oilseed *Brassica* has been described by Krzymanski and Downey (1969). The allele responsible for production of 10% erucic acid in *B. napus* var. Golden (Harvey and Downey 1964) and Nugget (Kondra and Stefansson 1965) was designated as E^a. The allele responsible for the production of 3.5% erucic acid

in *B. napus* var. Bronowski was designated as E^{l} (Krzymanski and Downey 1969). The allele contributing 15% erucic acid in *B. rapa* var. Polish and 30% in var. yellow Sarson (Dorrell and Downey 1964) were designated as E^{b} and E^{c} respectively. In *B. juncea*, Kirk and Hurlstone (1983) described two alleles E_1 contributing about 12% erucic acid in European type *B. juncea* and E_2 contributing about 20% erucic acid in Indian type *B. juncea*. It could well be that the E_1 allele described by Kirk and Hurstone (1983) was in fact the same as the E^{b} allele in *B. rapa* Polish and E_2 the same as the E^{c} allele in yellow sarson described by Dorrell and Downey (1964). Yellow and brown sarson types of *B. rapa* trilocularis with 55 to 60% erucic acid were the suspected diploid ancestors for Indian type *B. juncea* germplasm which would explain their high erucic acid contents. The allele E_0 described by Kirk and Hurstone (1983) as producing 0.5% erucic acid should be designated as *e* as is customary for a recessive mutant genotype.

In this study the proposal of Krzymanski and Downey (1969) and Anand and Downey (1981) for naming alleles was followed. There were two identical alleles E^{a} and E^{a} at two loci producing about 10% erucic acid in *B. carinata*. These two alleles were the same alleles described in *B. napus* (Downey and Harvey 1964, Kondra and Stefansson 1965). The genotype of the high erucic acid cultivars Dodolla and S-67 was designated as $E^{a}E^{a}E^{a}E^{a}$ and zero erucic acid line C90-14 as *eeee*, F_1 seed was of the genotype $E^{a}eE^{a}e$. Theoretically, the genotypes $E^{a}E^{a}ee$ and $eeE^{a}E^{a}$ would contain erucic acid levels similar to the F_1 but would not segregate. Such genotypes could be used to develop zero erucic acid lines if crossed with other double heterozygous genotypes such as $E^{a}eE^{a}e$.

Erucic acid expression in the three amphidiploid species, namely *B. napus* (Harvey

and Downey 1964), *B. juncea* (Kirk and Hurlstone 1983) and *B. carinata* (this study) was controlled by two genes at two loci. On the other hand in the diploid *B. rapa*, erucic acid was controlled by a single gene with additive alleles interaction. Furthermore it was suggested that the two genes controlling erucic acid in *B. napus*, *B. juncea* and *B. carinata* lie on chromosomes of the AC, AB and BC genomes of these three species. Therefore the A, B and C genomes of the genus *Brassica* each carry one gene with two alleles for erucic acid synthesis.

3.0 DEVELOPMENT OF LOW GLUCOSINOLATE LINES IN *BRASSICA CARINATA*

3.1 Literature review

3.1.1 Meal quality of *Brassica* species

Rapeseed and mustard meals, remaining after oil extraction from the seed contain about 40% protein on a dry weight basis (Josefsson and Åppelqvist 1968, Bengtsson 1985). The amino acid composition of the protein of *Brassica* species seed meal is well balanced with the exception of methionine and isoleucine which are limiting. The seed meal from *Brassica* species is an excellent feed for animals, but its utilization is limited, particularly for monogastric animals, due to its high glucosinolate contents.

3.1.2 Glucosinolates and their breakdown products

Glucosinolates (Figure 3.1, Table 3.1) are nontoxic nonvolatile hydrophilic compounds which are stable in neutral pH (Belzile *et al.* 1963, Underhill 1980, Larson 1981). Glucosinolates are hydrolysed by the enzyme myrosinase enzyme into isothiocyanates, thiocyanates, nitriles, and elemental sulphur upon crushing of the plant tissue (Benn 1977, Tookey *et al.* 1979, Röbbelen and Thies 1980b). The myrosinase enzyme reaction yields D-glucose and an aglucone moiety which is converted into isothiocyanates at a neutral pH. Nitriles are formed at low pH in the presence of ferrous ions. Isothiocyanates are volatile with a pungent taste and smell and are chemically very reactive.

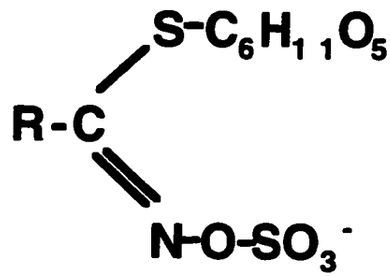

Figure 3.1. General structure of glucosinolates (Ettlinger and Lunden 1956). R= glucosinolate side chain.

Table 3.1 Side chain structure of alkenyl glucosinolates in *Brassica* species (Röbbelen and Thies 1980b).

Trivial name	Side chain structure	Semisystematic name[2]
Sinigrin	$H_2C=CH-CH_2-X$	2-Propenyl (allyl)
Gluconapin	$H_2C=CH-CH_2-CH_2-X$	3-Butenyl
Progoitrin	$H_2C=CH-\underset{\displaystyle OH}{\underset{\vert}{C}}H-CH_2-X$	2-Hydroxy 3-Butenyl
Glucobrassicanapin	$H_2C=CH-CH_2-CH_2-CH_2-X$	4-Pentenyl
Gluconapoleiferin	$H_2C=CH-CH_2-\underset{\displaystyle OH}{\underset{\vert}{C}}H-CH_2-X$	2-Hydroxy 4-Pentenyl

[1] X is common to all glucosinolates.
[2] The suffix glucosinolate is added to each semisystematic name (eg 2-propenyl glucosinolate).

Isothiocyanates with a hydroxy group at the ß position of the side chain cyclize to yield substituted 5- vinyloxazolidine-2-thione. Isothiocyanates derived from indole glucosinolates are unstable in neutral and alkeline pH and give rise to thiocyanate ions (Larson 1981). The formation of thiocyanates requires two enzymes, first myrosinase and then an aglucone rearranging enzyme (Benn 1977). Attempts to isolate the enzymes forming thiocyanate have failed due to their susceptibility to denaturation. The enzyme myrosinase, a thioglucoside glucohydrolase, was found in many isozyme forms with no specificity for a specific glucosinolate (Chew 1988).

3.1.3 Seed glucosinolate composition of *Brassica* species

Several authors have reported on the glucosinolate composition of the seed meal of *Brassica* species. Gland *et al.* (1981) analysed 1000 seed meal samples of various *Brassica* species. *B. oleracea* had the greatest variation in content and pattern of glucosinolates compared to the other five *Brassica* species. Different taxa of *B. oleracea* contained 2-propenyl, 3-butenyl, 4-pentenyl, 2-hydroxy 3-butenyl and 2-hydroxy 4-pentenyl glucosinolates in varying concentrations and compositions.

B. rapa has been found to contain 3-butenyl, 2-hydroxy 3-butenyl, 4-pentenyl, 2-hydroxy 4-pentenyl and indole glucosinolates (Daxenbichler *et al.* 1979, Gland *et al.* 1981). The proportion of each glucosinolate as a percent of the total amount was different for individual genotypes. However, 3-butenyl glucosinolate was the main glucosinolate in *B. rapa* seed. Seed meal of *B. nigra* (Gland *et al* 1981), and *B. carinata* (Mnzava and Olsson 1990, Getinet *et al.* 1996) were found to contain mainly 2-propenyl glucosinolate with trace

amounts of 3-butenyl, 2-hydroxy 3-butenyl, 4-pentenyl and 2-hydroxy 4-pentenyl glucosinolates. Kirk and Oram (1976) reported that *B. juncea* from the Indian subcontinent contained 2-propenyl and 3-butenyl glucosinolates while European genotypes of *B. juncea* contained 2-propenyl glucosinolate only. The pattern of glucosinolates in *B. napus* seed was more uniform in that two thirds was 2-hydroxy 3-butenyl and one third 3-butenyl glucosinolates (Josefsson and Åppelqvist 1968, Gland *et al.* 1981, Sang 1988).

3.1.4 Glucosinolate content of vegetative tissue and developing seed

The root, stem, leaf, flower heads (in vegetables) and pod tissues of *Brassica* species also contain glucosinolates (Daxenbichler *et al.* 1979, 1981, Sang *et al.* 1984, Mithen *et al.* 1987, Glover *et al.* 1988, McGregor 1988, DeMarch *et al.* 1989, Bilsborrow *et al.* 1993a, 1993b). As *Brassica* seeds germinate and the seedling grows, the glucosinolate content in cotyledons decreases and the amount in the developing young leaf increases acropitally. In *B. napus*, *B. juncea*, *B. carinata* and *B. rapa* the young leaves contain the highest concentration of glucosinolates, and as the leaves age the glucosinolate contents decrease (McGregor 1988). It appears that different organs contain various proportions of alkenyl and indole glucosinolates at different growth stages. Young pod tissue contains high levels of glucosinolates but the glucosinolate content in the pod tissue decreases as the seed in the pod develops and the plant matures (McGregor and Love 1987, McGregor 1988, DeMarch *et al.* 1989).

McGregor and Love (1987) found that the amount of total glucosinolates in seed continued to increase as the seed developed in the pod, and the seed was the most important

sink for glucosinolates. The level of 2-propenyl and 3-butenyl glucosinolates in leaves of *B. juncea* was correlated with the amount in the seed. In *B. napus* and *B. rapa* the amount of 3-butenyl and 4-pentenyl glucosinolates and their hydroxy products in leaf tissue were not correlated with the amount in the seed. High and low glucosinolate *B. napus* cultivars had similar glucosinolate composition in the leaf (DeMarch *et al.* 1989, Milford *et al.* 1989a and b). Pods contained also a similar distribution but a higher amount of glucosinolates than the leaf. High and low glucosinolate cultivars contained similar indole glucosinolate contents but showed major difference in levels of alkenyl glucosinolates (Milford *et al.* 1989a and b).

3.1.5 Glucosinolate biosynthesis

Glucosinolates are synthesized from amino acids through side chain elongation and modification (Underhill 1980). Glucosinolates and amino acids are similar in structure with the loss of the carboxyl carbon of amino acids. First the methylthioalkyls (eg. 3-methylthiopropyl) are formed and then the removal of the H_3CS terminal produces the alkenyl glucosinolates (eg. 2-propenyl glucosinolate). The hydroxylation of 3-butenyl and 4-pentenyl glucosinolates results in 2-hydroxy 3-butenyl and 2-hydroxy 4-pentenyl glucosinolates (Figure 3.2).

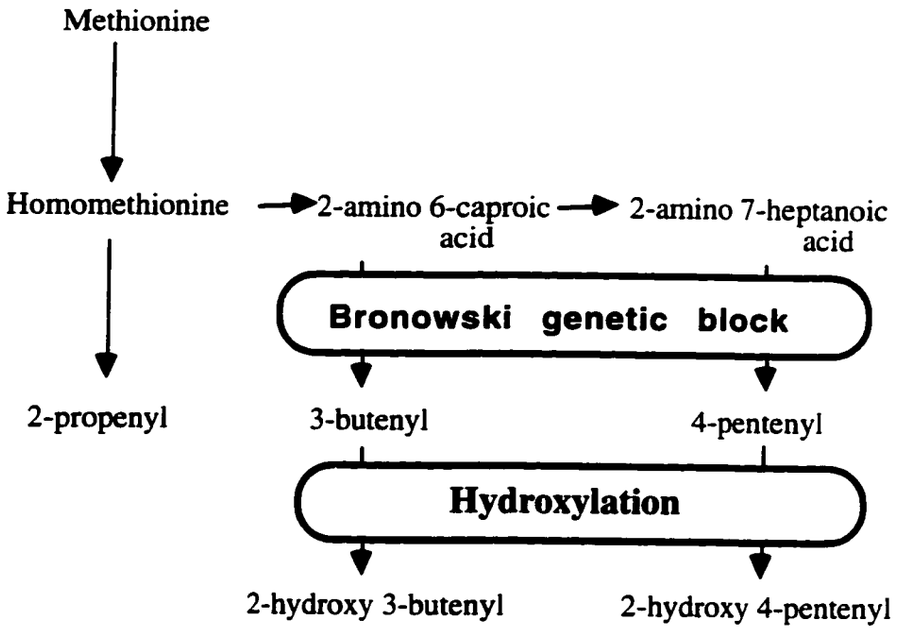

Figure 3.2 Biosynthetic pathway of alkenyl glucosinolates in *Brassica* species (Underhill *et al.* 1973)

The synthesis of glucosinolates was studied by feeding radio active labelled tracer elements to plants that normally synthesize glucosinolates in their tissue. Radio labelled ^{14}C methionine when fed to horseradish plants was correspondingly incorporated into carbon 2, 3 and 4 of 2-propenyl glucosinolate (Chisholm and Wetter 1964). Feeding of double radio labelled ^{14}C and ^{15}N methionine and homomethionine to horseradish showed that the amino acids and 2-propenyl glucosinolate in horseradish had similar $^{14}C/^{15}N$ ratios (Matsuo and Yamazaki 1966). Lee and Serif (1970) fed labelled ^{14}C 2-amino 6-caproic acid at C2 and ^{15}N at the amino group to the leaves of rutabaga. The labelled ^{14}C at the C2 position was incorporated selectively into the C1 position and ^{15}N at the 2-hydroxy 3-butenyl nitrogen position. Lee and Serif (1970) concluded that 2-amino 6-caproic acid was a direct precursor of 2-hydroxy 3-butenyl glucosinolate. The biosynthesis of benzyl glucosinolate in *Tropaeolum majus* L. was reported by Underhill (1965). The radio active labelled compounds phenylalanine 2-^{14}C and phenylalanine-3-^{14}C were recovered at high rates in benzyl glucosinolate. He concluded that benzyl glucosinolate was derived from the C6 to C2 carbon of phenylalanine. Double labelling of $^{14}C/^{15}N$ of phenylalanine and feeding to the leaves of *T. majus* demonstrated that benzyl glucosinolate had the same C/N ratio as phenylalanine. They concluded that the carbon skeleton and the amino nitrogen, except the carboxyl group was incorporated into benzyl glucosinolate. Similarly, the biosynthesis of the 2-hydroxy 2-phenylethyl glucosinolate in *Reseda luteola* L. from phenylalanine (Underhill and Kirkland 1972) and 3-indolylmethyl glucosinolate in *B. oleracea* from tryptophan (Kutacek *et al.* 1962) was demonstrated by feeding radio labelled elements to plant tissue.

Conclusions derived from studies involving amino acids established that labelled carbon and nitrogen were incorporated intact. This established the idea that the intermediates between the amino acids and glucosinolates are nitrogenous. The first compound identified as being intermediate was aldoxime. Underhill (1967) demonstrated that feeding labelled 1-^{14}C phenylacetaldhyde oxime to *T. majus*. resulted in incorporation of the tracer elements in the aglucone moiety of benzyl glucosinolate. Underhill (1967) concluded that the biosynthetic pathway of benzyl and 2-phenylethyl glucosinolates was amino acid→ aldehyde oxime → mustard oil glucoside → glucosinolate.

Several glucosinolates are formed from a single amino acid through side chain elongation and modification (Underhill 1980). Incorporation of radio labelled DL-methionine-2 ^{14}C and DL-homomethionine-2^{14} C into *Cheiranthus kewensis =Erysimum cheiranthoides* L (wallflower) showed that 2-propenyl glucosinolate was synthesized from methionine → homomethionine → 3-methylthiopropyl → 3-methylsulfinylpropyl glucosinolates (Chisholm 1972). When the same compounds were fed to *Armoracia lapathifolia* Gilib. (Chisholm and Matsuo 1972), the biosynthetic pathway followed 4-methylthiobutyrothiohydroximate→desulfo-3-methylthiopropyl → 3-methylthiopropyl → 3-methysulfinylpropyl → 2-propenyl glucosinolates. Josefsson (1971a) fed labelled ^{35}S and ^{14}C to plants of the *B. napus* cultivar Regina II and Bronowski and concluded that desulfo 3-butenyl glucosinolate was the precursor of both 3-butenyl and 2-hydroxy 3-butenyl glucosinolates. Feeding radio labelled synthetic D-4-pentenethiohydroximic acid to *B. napus* seedlings of cv. Bienvenu resulted in the incorporation of radio activity into 3-butenyl glucosinolate and 2-hydroxy 3-butenyl glucosinolate (Rossiter *et al.* 1990). In all cases of

B. napus, *Isatis tinctoria* L. and *Reseda luteola* L. hydroxylated glucosinolates were formed at the end of the pathway from their deoxy analogs.

3.1.6 Inheritance of glucosinolates in *B. napus*

The inheritance of 3-butenyl, 4-pentenyl and 2-hydroxy 3-butenyl glucosinolates was studied in *B. napus* cv Bronowski and a selection from the cultivar Target (Kondra and Stefansson 1970). Selfed seed and crossed seed borne on the same plant had similar 3-butenyl isothiocyanate, 4-pentenyl isothiocyanate and 5-vinyloxazolidine-2-thione contents indicating maternal control of glucosinolate contents. The 3-butenyl isothiocyanate and 5-vinyloxazolidine-2-thione contents of F_2 seed born on F_1 plants was greater than the mid parent value and that of 4-pentenyl isothiocyanate was greater than the higher parent. Therefore partial dominance for 3-butenyl isothiocyanate and 5-vinyloxazolidine-2-thione, and over dominance for 4-pentenyl isothiocyanate was reported. In the BC_1 two classes at a ratio of 1:7 for 3-butenyl isothiocyanate, and 1:15 for 5-vinyloxazolidine-2-thione were obtained. For 4-pentenyl isothiocyanate two ratios, 1:15 and 1:31 fitted equally. Therefore, Kondra and Stefansson (1970) concluded that three loci controlled the content of 3-butenyl, four loci determined the 2-hydroxy 3-butenyl level and three to four loci controlled presence or absence of 4- pentenyl isothiocyanates in seed of *B. napus*.

The inheritance of total glucosinolates was studied in crosses involving six *B. napus* cultivars (Mou and Liu 1988). Three of the cultivars, namely Huayou no. 16, Huayou no. 13 and 71-79 contained high glucosinolates in their seed and the other three namely Wesroona, Andor and Marnoo were low glucosinolate types. The reciprocal F_1 generations

between high and low glucosinolate cultivars contained a higher total glucosinolate level than the mid parent value but lower than the high parent. The glucosinolate content of the F_2 populations was continuous and low glucosinolate types were recovered at a the ratio of 1: 64 in two crosses and 1:16 in one cross. Based on F_2 and BC_1 generation segregations. Mou and Liu (1988) concluded that, in the crosses Hua you no. 16 and Marnoo, and Huayou no. 13 and Wesroona three loci controlled the total glucosinolate content of the seed and in the 71-79 and Andor cross two loci were involved. Siebel and Pauls (1989) studied the glucosinolate segregation in double-haploid (DH) populations derived from the F_1 generation of crosses between high x low and low x low glucosinolate strains. Analysis of seed meal from field grown DH plants of both populations revealed continuous distributions for 3-butenyl, 2-hydroxy 3-bytenyl and 4-pentenyl glucosinolates with no class separations. The distributions were skewed towards high glucosinolate levels.

Rücker and Röbbelen (1994) studied the genetics of total and individual glucosinolate contents in three sets of DH populations. In the first experiment, eight DH lines containing 7 to 115 μ moles g^{-1} seed were crossed in a complete diallel. In crosses involving lines containing < 20 μ moles g^{-1} seed, the F_1 plant did not exceed the mid parent value in glucosinolate content and most of the variation was due to general combining ability. The broad sense heritability (h^2_b) was 0.69 and the narrow sense was (h^2_n) was 0.66. The F_1 plants exceeded the mid parent value in glucosinolate content in crosses involving high x low glucosinolate parents and their reciprocals. The number of genes controlling total alkenyl glucosinolate content in *B. napus* was estimated from crosses between high (115 and 122 μ moles g^{-1} seed) x low glucosinolate (8 μ moles g^{1} seed) materials. Total glucosinolate

content of 120 μ moles g^{-1} seed was controlled by four to five independent genes. At least one gene was assumed to control glucosinolate contents below 20 μ moles. In the second experiment four resynthesised *B. napus* parents containing contrasting levels of 3-butenyl and 2-hydroxy 3-butenyl and 4-pentenyl glucosinolates were crossed in three combinations. Each cross involved contrasting levels for each of the glucosinolates. Based on F_2 class segregation, Rücker and Röbbelen (1994) concluded that hydroxylation of 3-butenyl and 4-pentenyl glucosinolates was controlled by two loci. Two loci controlled the ratio of 3-butenyl to 4-pentenyl glucosinolates, but this was in contrast to the result reported by Magrath *et al.* (1993) and Parkin *et al.* (1994).

B. napus lines containing 2-propenyl and methylthioalkyl glucosinolates were developed through interspecific crosses between *B. oleracea* and *B. rapa* (Kräling *et al.* 1990, Magrath and Mithen 1993). Such resynthesised *B. napus* lines were used extensively in genetic studies to elucidate the genetic control of individual steps in the glucosinolate biosynthetic pathway in *B. napus*. Synthetic *B. napus* was developed from the cross *B. atlantica* (CC) (Coss) Schultz (syn. *B. insularis* Moris)containing very high levels of 2-propenyl and *B. rapa* (AA) containing 3-butenyl glucosinolate (Magrath and Mithen 1993). *B. napus* derived from this cross containing 2-propenyl, and 3-butenyl glucosinolates was crossed with the *B. napus* canola quality winter rape cv. Cobra containing 3-butenyl and 4-pentenyl glucosinolates. Glucosinolate content of F_1 seed from the reciprocal crosses were similar and identical to their maternal parents. Glucosinolate hydroxylation in F_1 cotyledons was under embryonic control. Therefore, Magrath and Mithen (1993) suggested that fully formed glucosinolates rather than intermediates were transported from the pod tissue to the

developing seed and that hydroxylation occurred in the seed.

Magrath *et al.* (1993) studied the leaf alkenyl glucosinolate content of *B. napus*. In the first experiment, synthetic *B. napus* was developed from crosses between *B. atlantica* (wild form of *B. oleracea*) containing 2-propenyl glucosinolate and *B. rapa* containing 3-butenyl glucosinolate. Both the diploid parents and the synthetic *B. napus* line contained no hydroxylated glucosinolates in their leaf tissue. The synthetic *B. napus* line containing 2-propenyl and 3-butenyl glucosinolates was crossed with winter *B. napus* cv Cobra. In the second experiment a synthetic *B. napus* was developed from a cross between *B. oleracea (B. macrocorapa* Guss. Caruel. containing exclusively 2-propenyl glucosinolate and *B. rapa* ssp. *oleifera* containing a low level of glucosinolates. The synthetic line contained 2-propenyl and methylalkyl homologues of 2-propenyl, 3-butenyl and 4-pentenyl glucosinolates. This synthetic *B. napus* line was crossed with low glucosinolate spring type *B. napus* cv. Westar. This combination provided several unique opportunities to study the genetics of removal of the H_3CS terminal, elongation and hydroxylation steps in the biosynthesis of glucosinolates. The F_1 and F_2 generations along with diploid and synthetic and natural amphidiploid parents were grown in the greenhouse and the field in Cambridge, England. The authors concluded that two sets of genes controlled the side chain elongation and modification in leaf and seed of *B. napus*. Among loci that controlled side chain elongation, one each controlled the presence and absence of propyl (2-propenyl and 3-methylthiopropyl) and pentyl (4-pentenyl, 2-hydroxy 4-pentenyl and 5- methylthiopentyl) glucosinolates. Two loci controlled the presence or absence of butyl (3-butenyl, 2-hydroxy 3-butenyl, 4-methylthiobutyl) glucosinolates. Four loci were described controlling side chain modification, two loci

controlled the H_3CS terminal glucosinolate chain removal, and two loci regulated the hydroxyaltion of 3-butenyl and 4-pentenyl glucosinolates. The interaction of these loci resulted in an array of glucosinolate pattern in *B. napus*. As the *B. rapa* parent used for the interspecific cross lacked 2-propenyl glucosinolate, Magrath *et al.* (1993) suggested that the 2-propenyl glucosinolate was controlled by genes located on C genome chromosomes of *B. oleracea*. They also suggested that the genes controlling the biosynthesis of 3-butenyl and 4-pentenyl and their hydroxy analogues were located on chromosomes of both the A and C genomes.

The genetic control of side chain elongation in *B. napus* was studied by Magrath *et al.* (1994). The *B. napus* cultivar Cobra, containing butenyls and pentenyls only, was crossed with a synthetic *B. napus* line containing 2-propenyl and 3-butenyl glucosinolates. The F_1 contained alkenyl and hydroxy alkenyl glucosinolate with trace amount of 3-methylthiopropyl. Glucosinolate content of the DH plants derived from the F_1 segregated in a 1 :1 ratio for the presence and absence of 2-propenyl glucosinolate and presence and absence of 4-pentenyl glucosinolate confirming the reports made by Magrath *et al.* (1993) as well as Rücker and Röbbelen (1994). One of these loci was designated as *Gsl-elog-pro* and controlled the presence or absence of 2-propenyl glucosinolate. Alleles at two other loci *Gsl-elog-A* and *Gsl-elong-C* regulated the production of butyl and pentyl glucosinolates. These two alleles were mapped on a pair of homologous linkage groups each on the A and C genomes. Further evidence for the genetic control of hydroxylation in *B. napus* was presented by Parkin *et al.* (1994). Microspore derived double haploid populations from F_1 plants of the cross *B. napus* cv. Cobra, containing 60% 2-hydroxy 3-butenyl, and a synthetic

line containing <1% 2-hydroxy 3-butenyl were developed. First, the hydroxylation capacity in seed and leaf of the doubled haploid populations segregated into two major classes in the ratio of 1:1, second, there was segregation within the major classes. The two major and minor loci were designated as *Gsl-oh-C* and *Gsl-oh-A* respectively. The loci *Gsl-oh-A* and *Gsl-oh-C* are analogous to the A and C genome in *B. napus*. These two loci were mapped on linkage groups 3 and 13 of the A and C genomes respectively. It was also noted that *Gsl-oh-C* had a much greater effect on glucosinolate hydroxylation than *Gsl-oh-A*. The differential efficiency of hydroxylating loci in *B. napus* was reported earlier by Magrath *et al.*(1993).

Uzunova *et al.* (1995) crossed DH lines of the *B. napus* winter rape cultivar Hamburger Raps (high glucosinolate content) with DH lines the *B. napus* winter rape cultivar Samourai low in glucosinolate conent. The F_1 derived DH populations were then used for mapping. The glucosinolate content of 151 DH lines was normally distributed. Nineteen linkage groups were constructed using 204 randem fragment length polymorphism (RFLP) genomic, 2 RAPD and one phenotypic markers. Glucosinolate contents were linked to four linkage groups which were identified using interval mapping. The additive effect of the quantitative trait loci's (QTL's) varied from 3.4 to 7.8 μ moles glucosinolate g^{-1} seed for a total of 38 μ moles glucosinolate g^{-1} of seed. This accounted for 74% of the difference in glucosinolate content between the two cultivars and for 61.7% of the variance in glucosinolate content in the DH populations. The QTL's controlling total alkenyl glucosinolates in the seed of *B. napus* were also mapped in DH populations derived from F_1 plants of the cross between the low glucosinolate summer rape cv. Stellar and high glucosinolate winter rape cultivar cv. Major (Toroser *et al.* 1995). Two loci, *GSL-1* and

GSL-2 with major influence on total alkenyl glucosinolate content in the seed were mapped on linkage groups *LG-20* and *LG-1*, respectively. Three other loci, namely *GSL-3, GSL-4* and *GSL-5* with smaller effects were also identified. These loci accounted for 71% of the variation in glucosinolate content between Stellar and Major. The two loci identified by Uzunova *et al.* (1995) as *GSL-1* on linkage group 16 and *GSL-3* on linkage group 18 were the same loci identified as *GSL-1* and *GSL-2* by Toroser *et al.* (1995).

3.1.7 Variation of glucosinolate contents in *B. juncea*

Isolation of *B. juncea* plants containing a low level of glucosinolates was reported by Cohen *et al.* (1983), however, the low glucosinolate trait was not confirmed in the progeny. Love *et al.* (1990a) developed a low glucosinolate *B. juncea* through interspecific crossing of *B. juncea* containing 3-butenyl glucosinolate only and canola quality *B. rapa*. The F_1 was backcrossed to the *B. juncea* parent and the glucosinolate content of 257 BC F_2 plants ranged from 57 to 204 μ moles g^{-1} of oil-free meal Selfing of a plant containing 57.0 μ moles g^{-1} of meal resulted in progeny that ranged glucosinolate content from 0.7 to 46.3 μ moles g^{-1} of meal. In the BCF_2 generation the glucosinolate content of individual plants ranged 0.8 to 2.9 μ moles g^{-1} of meal.

The inheritance of 2-propenyl and 3-butenyl glucosinolates in *B. juncea* was studied in progeny of crosses between line ZEM 84-2293 and cv. Domo containing 2-propenyl glucosinolate and line no. 60143 containing 3-butenyl glucosinolate only (Love *et al.* 1990b). The F_1 and selfed seed on the maternal parent plants contained a similar glucosinolate pattern and content. F_2 seed borne on the F plants contained both

glucosinolates. However a small but significant cytoplasmic effect of line 60143 favouring higher levels of both, 3-butenyl and 2-propenyl, was observed. The seed meal of backcross plants derived from the backcross to the 3-butenyl type parent (60143) contained either 3-butenyl only or a mixture of 3-butenyl and 2-propenyl glucosinolates while seed meal of backcross plants derived from the backcross to the 2-propenyl parents contained either 2-propenyl only or a mixture of 3-butenyl and 2-propenyl glucosinolates. Seed meal of F_2 plants contained either 2-propenyl, 3-butenyl or both glucosinolates. Love *et al.* (1990b) concluded that in this cross 2-propenyl and 3-butenyl glucosinolate synthesis was controlled by more than one locus with multiple alleles having additive effects.

3.1.8 Genetic block of glucosinolate biosynthesis in *B. napus* cultivar Bronowski

The *B. napus* cultivar Bronowski is the only known germplasm source with low glucosinolate level in its seed meal (Josefsson and Åppelqvist 1968). It has been intensively used to develop low glucosinolate *B. napus* and *B. rapa* cultivars all over the world. The differences in chemical composition and glucosinolate metabolic pathways of *B. napus* cv. Bronowski and the high glucosinolate Swedesh *B. napus* cultivar Regina II were studied by Josefsson (1971a, 1971b, 1973). The total sulfur, protein and amino acid composition in vegetative tissues of the two cultivars was similar. Josefsson (1971b) fed radio labelled desulfoglucosinolate to Bronowski and Regina II. In Regina II the desulfoglucosinolate was a good precursor for 3-butenyl and 2-hydroxy 3-butenyl glucosinolates. Josefsson (1971b) concluded that one of the metabolic blocks must be at the hydroxylation step. Josefsson (1973) fed radio labelled synthetic 5-methylthiopentyl oxime 1-^{14}C to Regina II and

Bronowski. The tracer elements were a poor precursor of 3-butenyl glucosinolate in Bronowski. Therefore, Josefsson (1973) concluded that there were two metabolic blocks, one before the formation of 3-butenyl glucosinolate and a second one at the hydroxylation point. The second suggestion was questioned by Finlayson *et al.* (1973).

3.1.9 Interspecific crossing among *Brassica* species

Interspecific crosses have been utilized in the *Brassica* species to transfer disease resistance (Roy 1978, 1994), herbicide resistance (Ayotte *et al.* 1985, 1986), oil quality (Roy and Tarr 1985, 1986, Getinet *et al.* 1994) meal quality (Love *et al.* 1990a) and seed color (Rashid *et al.* 1994) from one species of *Brassica* to another. Club root (*Plasmodiophora brassicae* Wor.) race 2 resistance was transferred from *B. napus* (rutabaga) to *B. oleracea* (cabbage) through interspecific crossing (Chiang *et al.* 1977). Hybrid seed set was improved when vigorous young plants, grown at a moderate temperature of 18^0C were used. It was also noted that cultivars within species differed in their ability to outcross with other species. Hybrid seed set was obtained from the cross *B. napus* x *B. oleracea* but not from the reciprocal. All F_1 plants were resistant to the disease. Similarly resistance to blackleg (*Leptosphaeria maculans*) in *B. juncea* was transferred to *B. napus* through an interspecific cross (Roy 1978). The F_1 plants were resistant to blackleg and a *B. napus* like plant with complete seedling and adult plant resistance was isolated in the F_2 generation. The gene(s) conferring resistance to blackleg in *B. juncea* were designated as JR and the introgressed resistance gene(s) in B. *napus* as $ONAP^{JR}$. A *B. napus* type plant resistant to the blackleg fungus and with complete absence of leaf lesions was isolated (Roy 1984). Atrizine

resistance from the *B. napus* to *B. oleracea* was introgressed (Ayotte *et al.* 1985). Ovary development was only possible from *B. napus* x *B. oleracea* cross and the ovules were collapsing after the 12th day of pollination and on the 18th day ovules were discoloured and shrivelled. However, F_1 plants were raised using embryo culture. Most $_1$F plants had a somatic chromosome number of 2n= 28 with the occasional plant having 37 chromosomes. At meiosis the most frequent chromosome association was 9 bivalents + 10 univalents at 40%, 10 univalents +8 bivalents at 11.2% and 8 bivalents at 6.0% of cells. Multivalents, mostly trivalents were observed in 32.8% of the cells. The backcross to *B. napus* gave 2.2 seeds per pollination and an atrizine resistant plant was isolated in the BC_3 generation.

Wojciechowski (1985) crossed the *B. rapa* subspecies *chinensis*; *pekinensis* Laur. and *oleifera* Mtzg var. sarson with *B. oleracea* var. *acephala* and *gemnifera* DC. When *B. oleracea*was used as female, 1.1% of pollinated buds produced seed. In the cross *B. oleracea* var. *gemnifera* x *B. rapa* var. sarson 3.4% of pollinated buds produced seed. When *B. rapa* was the maternal parent, 317 seeds was obtained from 2375 pollinated buds. Campbell (1993) crossed four cultivars of *B. rapa* with four *B. nigra* lines. When *B. rapa* was used as a female an average of 4.4% of the pollinated buds produced and half of these seeds were hybrids. The *B. rapa* cultivar Tobin showed the best result but no hybrid seed was obtained from the *B. rapa* cultivar Echo. When *B. nigra* was used as a female parent, an average 3.7% of pollinated bud produced seeds. However hybrid plants were obtained only from two *B. nigra* female genotypes. The failure of the endosperm to develop normally is the major reason for embryo abortion and rescue of the embryo on culture media increases the chances of a successful cross (Chaing *et al.*1977, Sacristan and Gerdemann 1986, Chen *et al.* 1988).

Sacristan and Gerdemann (1986) used embryo culture to obtain hybrids from reciprocal crosses of *B. napus* x *B. juncea* and B. *napus* x *B. carinata*. Chen *et al.* (1988) crossed *B. rapa* x *B. oleracea* ssp. *alboglabra* to produce a synthetic yellow seeded *B. napus*. The ratio of number of seeds harvested to number of flowers pollinated was 0.013% for seed set *in vivo* while ratio of embryos harvested to flower buds pollinated was 0.55% using embryo rescue. Ovule culture also improved the success of the hybrid seed set (Sacristan and Gerdemann 1986, Sarla and Raut 1988).

In summary interspecific crosses between amphidiploids can be achieved without difficulty (Bing 1991, Getinet *et al* 1994, Rashid *et al.* 1994,). Crosses between amphidiploids and their ancestors were also be achieved. However, crossing involving *B. oleracea* (Ayotte *et al.* 1985) followed by *B. nigra* were difficult. *B. rapa* was the most versatile among diploids even when crossed with *B. carinata* (Yousuf 1981).

Compared to sexual hybridization, protoplast fusion results in the combination of both nuclear and cytoplasmic genomes. Since the first report of resynthesis of *B. napus* using protoplast fusion (Schenck *et al.* 1982), *B. carinata* (Narasimhulu *et al.* 1992, Jordan and Salazar 1993) and B. *juncea* (Campbell 1993) have also been resynthesised from their diploid ancestors by protoplast fusion..

3.2 MATERIALS AND METHODS

3.2.1 Working hypothesis

B. carinata seed contained 2-propenyl glucosinolate only , and the levels observed in different genotypes ranged from 70 μ moles to 160 μ moles g^{-1} oil free meal (Getinet *et al.* 1996). This characteristic, namely the presence of only one glucosinolate, is unique for *Brassica* species carrying B genome chromosomes, such as *B. carinata* (BBCC), *B. juncea*, (AABB) and *B. nigra* (BB) (Love *et al.* 1990a). No low 2-propenyl glucosinolate mutant types have been identified despite intensive screening of germplasm of the mustard species. The observed variations in 2-propenyl glucosinolate levels were environmental variations and had genetic basis. *B. napus* (AACC) and *B. rapa* (AA), on the other hand, do not accumulate 2-propenyl glucosinolate in their seed but instead synthesize 3-butenyl, 4-pentenyl, 2-hydroxy 3-butenyl and 2-hydroxy 4-pentenyl glucosinolates. Low glucosinolate *B. napus* and *B. rapa* has been successfully developed through cross breeding with the low glucosinolate *B. napus* mutant cultivar Bronowski (Josefsson and Åppleqvist 1968).

Early attempts to introduce genes for low glucosinolate content from the Bronowski mutant into *B. juncea* were unsuccessful, and it was therefore concluded that the synthesis of 2-propenyl glucosinolate was not affected by Bronowski mutant genes. Love *et al.* (1990a) speculated that low glucosinolate *B. juncea* could be developed from crosses between *B. juncea* free of 2-propenyl glucosinolate but containing 3-butenyl glucosinolate

and low glucosinolate *B. rapa* containing low glucosinolate mutant alleles from Bronowski. The Bronowski genes, when introduced into *B. juncea* through an interspecific cross, should restrict the accumulation of 3-butenyl glucosinolate in *B. juncea*. This hypothesis was proven correct and low glucosinolate *B. juncea* was developed (Love *et al.* 1990a). Based on these findings, an interspecific crossing and selection scheme was conceived that would allow the development of low glucosinolate *B. carinata*. The assumption was that 2-propenyl glucosinolate was synthesized by genes located on both the B and C genomes of *B. carinata* and chromosomes carrying these genes had to be substituted with B and C genome chromosomes of *B. juncea* and *B. napus*, respectively, that did not synthesize 2-propenyl glucosinolate. The two interspecific crosses: 1) *B. carinata* (BBCC) x *B. napus* (AACC) (Figure 3.3) and 2) B. *carinata* (BBCC) x *B. juncea* (AABB) (Figure 3.4) were designed to produce two types of *B. carinata* in which 2-propenyl glucosinolate was synthesized by genes in the 1) B genome only (*B. napus* derived) and 2) C genome only (*B. juncea* derived). Intercrossing of these two *B. carinata* genotypes (Figure 3.5) would combine traits of the two genotypes in one *B. carinata* plant. It was expected that double recessive low 2-propenyl glucosinolate plants would segregate in the F_2 generation of this double interspecific cross.

It was also expected that the zero 2-propenyl glucosinolate F_2 plants would contain 3-butenyl, 4-pentenyl, 2-hydroxy 3-butenyl and 2-hydroxy 4-pentenyl glucosinolates but that the total concentration of these two glucosinolates would not exceed levels in the Bronowski mutant since the *B. napus* and *B. juncea* parents used for the interspecific cross contained

"Bronowski genes".

3.2.2 Parents of the interspecific cross

1. *Brassica carinata*: The *B. carinata* parent C90-14 is a 2-propenyl containing (150 μ moles g^{-1} of meal) genotype with zero erucic acid derived from an interspecific cross with low erucic acid *B. juncea* (Getinet *et al.* 1994).
2. *Brassica napus*: The *B. napus* parent cv. Westar (Klassen *et al.* 1987) is a zero erucic zero 2-propenyl glucosinolate cultivar that carried "Bronowski alleles".
3. *Brassica juncea*: The *B. juncea* parent J90-4253 (Love *et al.* 1991) is also a zero erucic acid genotype that contained no 2-propenyl glucosinolate in its seed and that also contained"Bronowski alleles".

Seed of the *B. carinata*, *B. napus* and *B. juncea* parents was analysed for erucic acid content by the single cotyledon analysis technique (Downey and Harvey 1963), and only zero erucic acid half seeds were grown and used for the interspecific cross to ensure that the resulting low (zero 2-propenyl) glucosinolate *B. carinata* would be of the zero erucic acid type.

3.2.3 Crossing and selfing techniques

Immature buds of the female parent were emasculated and pollinated for three consecutive days with fresh pollen from the male parents. For selfing, immature buds were emasculated and pollinated with fresh pollen from the same plant for two consecutive days. Cross and self pollinated buds were covered with plastic bags for one week to prevent

contamination with foreign pollen. Developing crossed and selfed pods were left in the pod on the plant until maturity: no embryo rescue techniques were used.

Growing conditions for plants in the growth cabinet and greenhouse were the same as described in section 2.2.1.

3.2.4 [(*B. carinata* x *B. napus*) x *B. carinata*] cross

The *B. carinata* strain C90-14 was crossed as a female with *B. napus* cv. Westar as a male (Figure 3.3). At maturity interspecific F_1 seeds were harvested from C90-14, and the seed germinated in a petri dish on filter paper and germinating seedlings transferred to pots in the growth chamber. Leaf glucosinolate content and pattern of F_1 plants was determined from the youngest leaf at the four leaf stage of plant growth. F_1 plants were backcrossed to *B. carinata* C90-14 to improve fertility. A total of 93 BCF_1 seeds were harvested at maturity, germinated in petri dishes on filter paper and transferred to pots in the greenhouse. Fifty fertile BCF_1 plants were selfed using bud pollination and at maturity, 537 BCF_2 seeds were harvested, germinated in petri dishes on filter paper and transferred to pots in the greenhouse. A total of 223 BCF_2 plants were selfed using bud pollination. The selfed buds were covered with plastic bag for one week to prevent foreign pollen. At maturity both selfed and open pollinated seeds were harvested separately from 144 plants and selfed seed was kept in reserve. Since glucosinolates are maternally inherited (Kondra and Stefansson 1970, Mithen *et al.* 1993, Love *et al.* 1990b) both selfed and out crossed seed was used for glucosinolate analysis. Forty BCF_3 plants from eight BCF_2 selected families were grown for progeny testing.

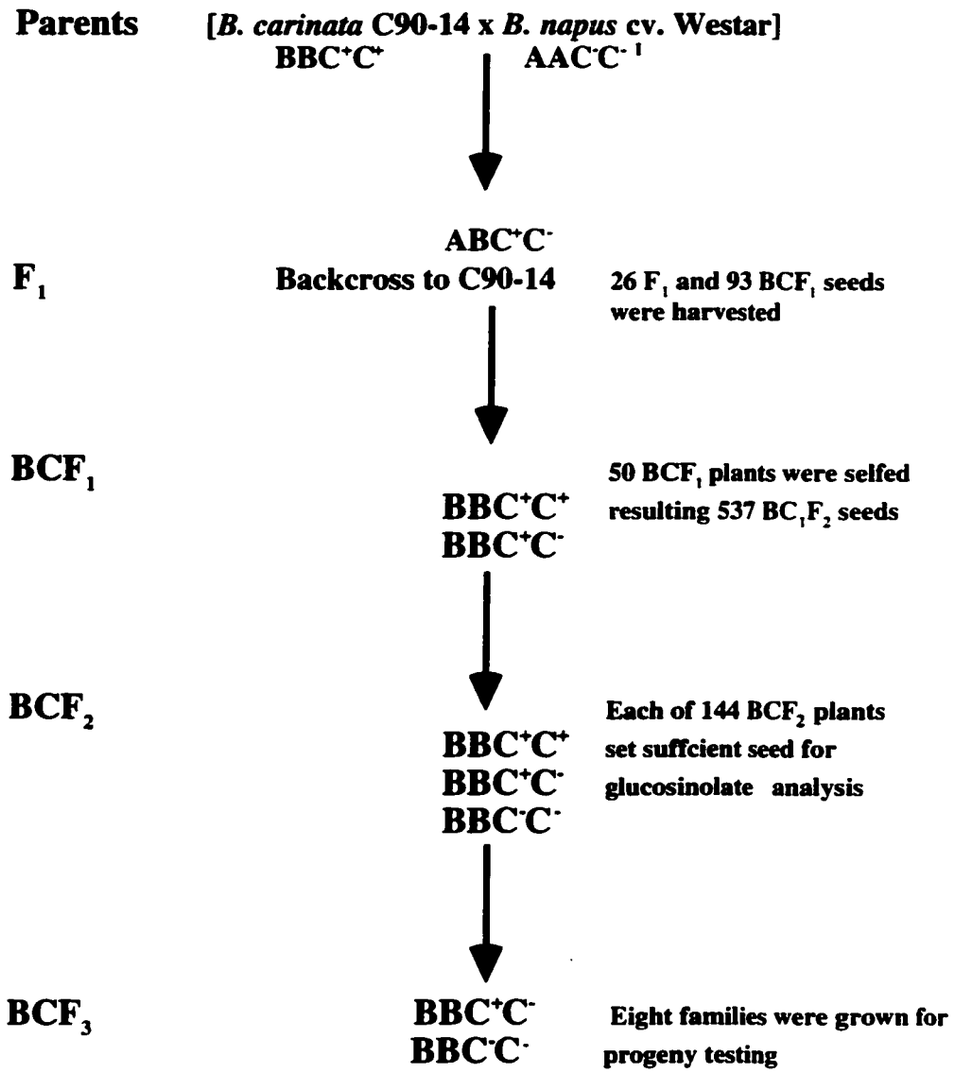

Figure 3.3 Crossing scheme to substitute C genome chromosomes of *B. carinata* (BBCC) synthesizing 2-propenyl glucosinolate with C genome chromosomes of *B. napus* (AACC) that do not synthesize 2-propenyl glucosinolate.
[1] C^+. symbolizes presence of genes synthesizing for 2-propenyl glucosinolate synthesis and C^- symbolizes the absence of genes for 2-propenyl glucosinolate synthesis.

3.2.5 [(*B. carinata* x *B. juncea*) x *B. carinata*] cross

The *B. carinata* strain C90-14 was crossed as a female with *B. juncea* line J90-4253 (Figure 3.4). Eleven F_1 seeds were harvested, germinated in petri dishs on filter paper and transferred to pots in the growth cabinet. Leaf glucosinolate content of F_1 plants were determined from the youngest leaf at the four leaf stage of growth. The F_1 plants were backcrossed to C90-14 to improve fertility. A total of 348 BCF_2 seeds were harvested from two F_1 plants, germinated in petri dishs on filter paper and seedings transferred to pots in the greenhouse. A total of 78 BCF_1 plants were selfed using bud pollination. The selfed buds were covered with plastic bags for one week to exclude foreign pollen. Only 32 BCF_2 seeds germinated in petri dishs on filter paper. After germination seedlings were transferred to pots in the greenhouse. Plants were selfed using bud pollination. The selfed buds were covered with plastic bags to exclude foreign pollen. At maturity only two BCF_2 plants set enough seed for glucosinolate analysis. Four BCF_2 families were grown in the greenhouse for progeny testing.

3.2.6 Double interspecific cross

Two BCF_1 plants with reduced levels of 2-propenyl glucosinolate in the leaf tissue (Plants no. 25 and 30, Table 3.8) *B. juncea* cross and two BCF_1 plants from the *B. napus* cross (Plant no. 39 and 64 Table 3.5) with reduced levels of 2-propenyl glucosinolate in the leaf tissue were selected and propagated from cuttings. The two groups were reciprocally crossed and eight seeds from the cross [(C90-14 x J90-4253) x C90-14] x [(C90-14 x Westar x) C90-14] were harvested (Figure 3.5). No seed was obtained in the reciprocal cross. Five

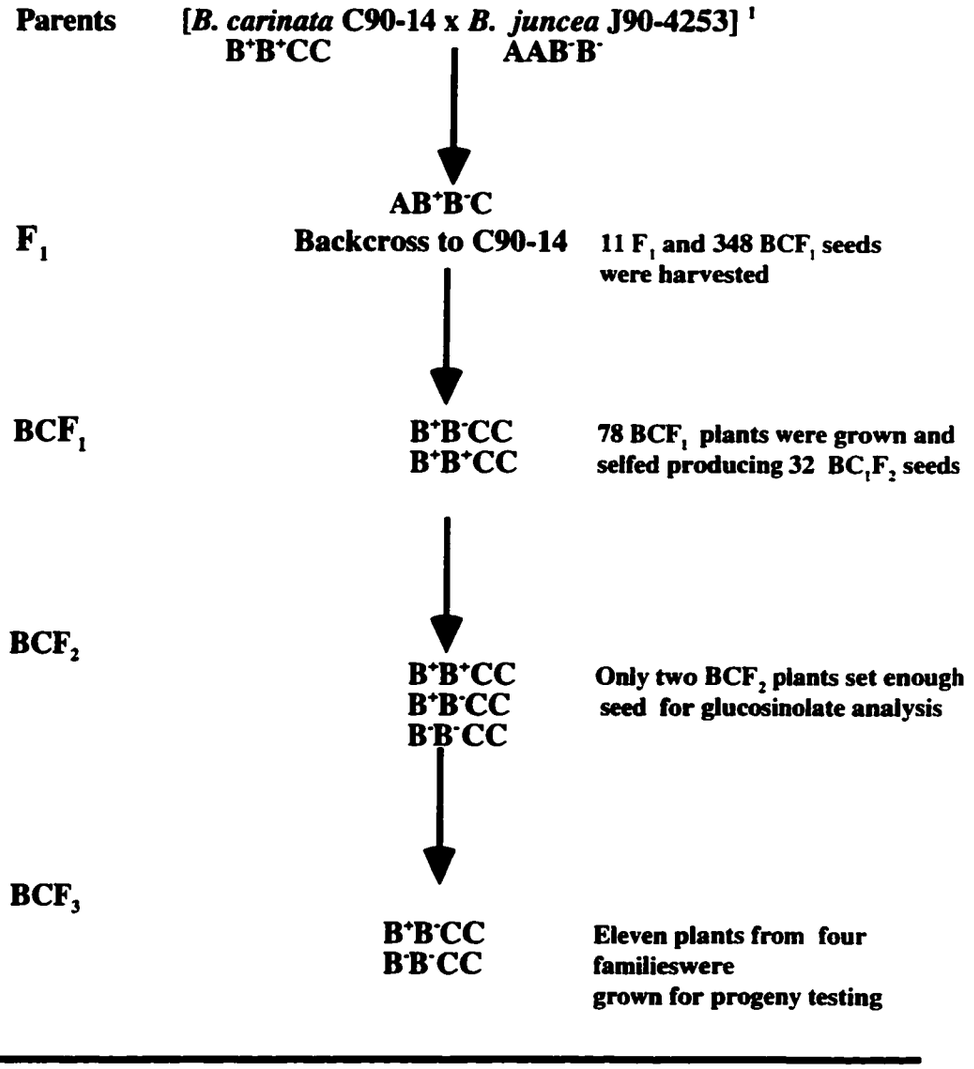

Figure 3.4 Crossing scheme to substitute B genome chromosomes of *B. carinata* (BBCC) synthesizing 2-propenyl glucosinolate with B genome chromosomes of *B. juncea* (AABB) that do not synthesise 2-propenyl glucosinolate.
[1]B^+ symbolizes presence of genes synthesizing for 2-propenyl glucosinolate synthesis and B^- symbolizes the absence of genes for 2-propenyl glucosinolate synthesis.

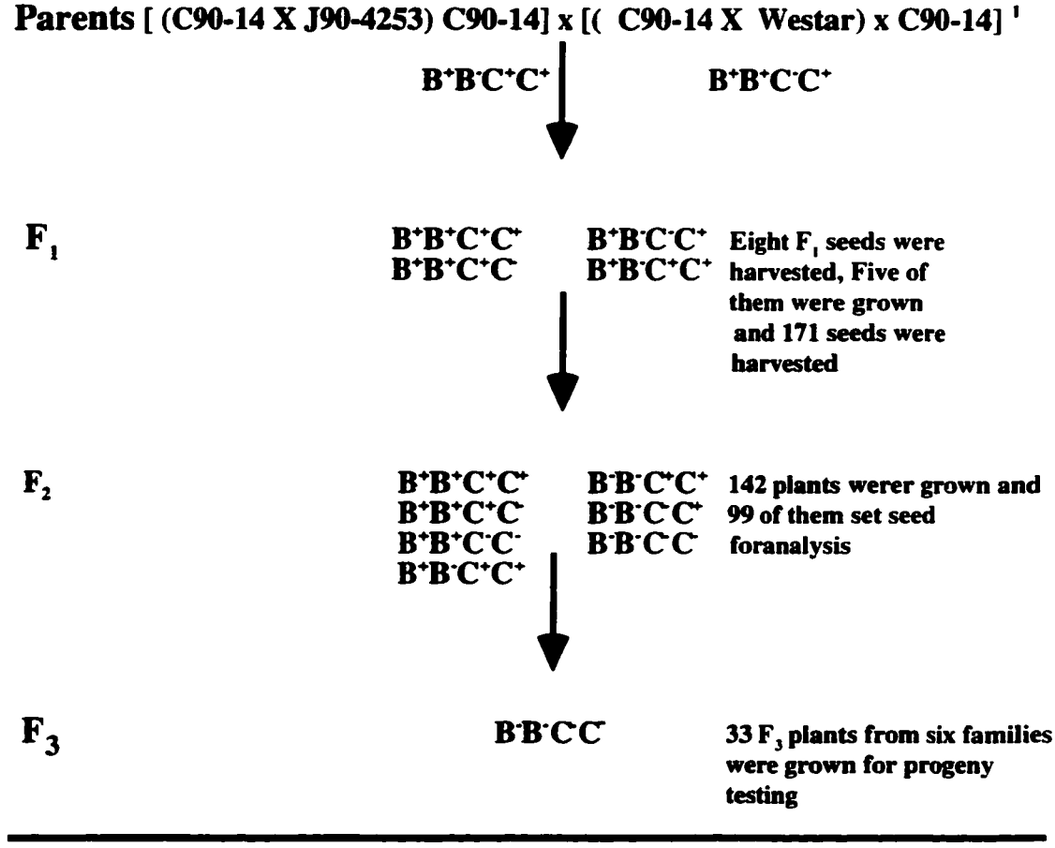

Figure 3.5 Crossing scheme to combine non2-propenyl glucosinolate characteristics of *B. napus* and *B. juncea* derived *B. carinata* genotypes in one B. *carinata* plant.

of the eight hybrid seeds were germinated in a petri dishes on filter paper and transferred to pots in the growth cabinet. During flowering the plants were selfed using bud pollination. A total of 171 F_2 seeds were harvested from three fertile plants. Seeds were germinated in petri dishes and transferred to pots in the greenhouse to raise F_2 plants. At maturity, 99 plants set sufficient seed for glucosinolate analysis. Since the amount of seed was insufficientt to prepare seed meal, glucosinolate content and pattern was determined on a whole seed basis. Five F_2 families that had the lowest glucosinolate content were progeny tested in the greenhouse.

3.2.7 Glucosinolate analysis

Glucosinolate composition of leaf, seed and seed meal was determined according to the laboratory method of Agriculture and Agri-food Canada, Saskatoon Research Center (J. P. Raney personal communication), a modified version of the method described by Thies (1974, 1976, 1977, 1979). Glucosinolate composition of leaves were determined from F_1 plants grown in the growth room and BCF_1 plants grown in the greenhouse. Glucosinolate content of whole seed and seed meal was determined from plants grown in the greenhouse. Seed meal and leaf tissue was prepared in 100 ml tubes in racks of 60. The Canola Normalization Standard (CNS) sample was included every 30 samples as a reference. The Canola Normalization Standard is a reference material distributed by the Canola Council of Canada and contains a known content and composition of glucosinolates (about 27 μ moles/g of total alkenyl glucosinolates defatted dry meal). In the case of leaf analysis, the rack was placed in a boiling water bath and hot water was added to each tube to inactivate the

myrosinase enzyme. In the case of whole seed analysis, moisture-free seed was weighed in plastic tubes and crushed using the Raney crusher (Raney *et al.* 1987) with 70% methanol in water to extract the glucosinolates from the seed. After 30 min. the supernatant is recovered from the tubes.

Once the tubes are prepared in racks of 60, 2 ml methanol, 1 ml of benzyl glucosinolate internal standard and 0.1 ml of 0.6 M lead barium acetate is added to precipitate proteins. The tubes are then vortexed and, left to react for an hour before centrifugation at 4000 RPM for 30 min. After centrifugation the samples are allowed to stand for approximately 10 minutes, 1.5 ml of the supernatant is transferred to the preprepared DEAE sephadex column to capture the glucosinolates. The columns are then washed (in sequence) with 1.8 ml of methanol, 1.8 ml of water and 1 ml of 0. 02 pyridine acetate. Then 0.05 ml of purified sulfatase are added to desulphatize the glucosinolates and the column incubated over night for complete enzymatic removal of the sulfur from all the glucosinolates. The next morning the glucosinolates are eluted in 1.5 ml auto sampler vials. The vials are then placed in a 60^0C drying block under stream of air until the sample is completely dry. The vials are cooled and derivatized. A premix of 40 ml of acetone, 20 ml of BSA (N.O-bis[trimethylsilyl]trifluoraacetamide), and 2 ml part of TMCS (trimethylchlorosilane) and 1 ml of 1-methyl imidazol are added to each vial for derivatization (the desulfo glucosinolates are transmethylsilylated). The vials are then capped, shaken and and placed in the correct sequence on to the autosampler of the gas chromathograph. Regeneration of the Sephadex DEAE columns was accomplished by washing with 1 ml of 1 M sodium acetate, 0.5 M sodium hydroxide and 0.5 M hydrochloric

acid followed by washing twice with 1.8 ml distilled water. A Hewlett Packard gas chromatograph model 5890 equipped with a flame ionization detector, an autosampler model 7673 and a capillary column was used. Each injection was temperature programmed with an initial temperature of 230 ^{0}C and final temperature of 281^{0}C. The carrier gas was helium with a pressure of 5 psi. The glucosinolate content of the BCF_1, BCF_2, and BCF_3 and intercrossed F_2 and F_3 plants were transformed into quotient values using the following formulas:

$$Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

This transformation allows each plant to be characterized as to its ability to elongate (Q1) and hydroxylate (Q2) the glucosinolates.

3.3 RESULTS

3.3. 1 Seed meal glucosinolate content of parents: *B. carinata* C90-14, *B. napus* Westar and *B. juncea* J90-4253

Seed meal of the B. *carinata* parent C90-14 contained a high level of 2-propenyl glucosinolate (159.7 moles g^{-1} of meal) which accounted for 96% of its total alkenyl glucosinolate content (Table 3.2). In contrast seed meal of *B. napus* Westar was free of 2-propenyl glucosinolate, but contained low concentrations of 3-butenyl (3.5 μ moles) and 2-hydroxy 3-butenyl (6.9 μ moles) glucosinolates as well as trace amounts (<0.5 μ moles) of 4-pentenyl glucosinolates and 2-hydroxy 4-pentenyl glucosinolates for a total of 11.0 μ moles g^{-1} meal of total of alkenyl glucosinolates. *B. juncea* seed meal was also low in glucosinolate content with 2-propenyl (1.7 μ moles) and 3-butenyl (1.3 μ moles) being the two principal alkenyl glucosinolates.

Quotients were calculated for two steps in the alkenyl glucosinolate biosynthetic pathway. Quotient 1 (Q1) was low for *B. carinata* indicating the inability of this species to form the longer chain glucosinolates 3-butenyl and 4-pentenyl by chain elongation. *B. napus* and *B. juncea* had Q1 quotients of 1.00 and 0.66, respectively indicating strong glucosinolate precursor chain elongation capacity. Quotient 2 (Q2) which is a measure of 3-butenyl glucosinolate hydroxylation was high for *B. carinata* (0.83) and *B. napus* (0.66) and low for *B. juncea* (0.18).

Table 3.2 Seed meal glucosinolate composition of *B. carinata*, *B. napus* and *B. juncea* parents.

Species and genotype	Glucosinolate μ moles g^{-1} meal[1]							
	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
B. carinata								
C90-14	159.7	1.4	5.6	0.0	0.4	167.1	0.05	0.83
B. napus								
Westar	0.0	3.5	6.9	0.4	0.2	11.0	1.00	0.66
B. juncea								
J90-4253	1.7	1.3	0.3	0.0	0.0	6.8	0.66	0.18

[1] Single analysis based on 100 mg meal sample extracted from 200 mg of seed.

Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl, Hpen= 2-hydroxy 4-pentenyl glucosinolates.

$$^{2}Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

3.3.2 Interspecific crosses between *B. carinata* x *B. napus* and *B. carinata* x *B. juncea* and backcrosses of the two F_1 interspecific crosses to *B. carinata*

In the *B. carinata* x *B. napus* cross, 297 pollinations were made resulting in 286 pods and a total of 26 seeds with an average yield of 0.08 seeds per pollinated bud (Table 3.3). The backcross [(*B. carinata* x *B. napus*) x *B. carinata*] yielded on average 1.36 seeds per pollinated bud. The cross between *B. carinata* x *B. juncea* was less successful. Eleven seeds were harvested from 375 pollinated buds, an average of 0.02 seeds per pollination. The backcross [*(B. carinata* x *B. juncea*) x *B. carinata*] yielded an average of 2.74 seeds per pollinated bud.

Table 3.3 Number of buds pollinated, pods formed, seeds harvested and seeds per pollination of F_1 and BCF_1 generations.

Cross and backcross	Number of			
	Buds pollinated	Pods formed	Seeds harvested	Seeds/ pollinated bud
B. carinata x *B. napus*				
(C90-14 x Westar)	297	286	26	0.08
(C90-14 x Westar)x C90-14	68	56	93	1.36
B. carinata x *B. juncea*				
(C90-14 x J90-4253)	375	367	11	0.02
(C90-14 x J90-4253)x C90-14)	127	124	348	2.74

3.3.3 Leaf glucosinolate composition of parents and F_1 plants

Leaf glucosinolate content was determined for the parents *B. carinata*, *B. napus* and *B. juncea* and F_1 plants of the two interspecific crosses. The *B. carinata* parent contained 6.7 μ moles g^{-1} fresh leaf tissue of 2-propenyl glucosinolate and trace amounts of other alkenyl glucosinolates (Table 3.4). The *B. napus* and the *B. juncea* parents, as well as the F_1 plants of the two interspecific crosses had low 2-propenyl glucosinolate contents in their leaf tissue, and the concentration of other alkenyl glucosinolates were also low. However, the level of 2-propenyl and 2-hydroxy 3-butenyl in the *B. carinata* x *B. napus* cross was higher than in the *B. carinata* x *B. juncea* cross.

Table 3.4 Leaf glucosinolate composition of parents and F_1 plants grown in the growth cabinet.

Genotype	Glucosinolates μ moles g^{-1} fresh leaf weight				
	Pro[3]	But	Hbut	Pent	Total Alk
B. carinata[1]	6.7	0.0	0.1	0.0	6.8
B. napus[1]	0.2	0.1	0.3	0.2	0.8
B. juncea[1]	0.0	0.1	0.1	0.1	0.9
B. carinata x *B. napus*[2]	0.7	0.1	0.9	0.1	1.8
B. carinata x *B. juncea*[2]	0.1	0.1	0.3	0.2	0.7

[1] single analysis.
[2] average of two analyses.
[3]Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl,glucosinolates.

3.3.4 [(*B. carinata* x *B. napus)* x *B. carinata*] cross

3.3.4.1 Leaf glucosinolate composition and glucosinolate quotients of parents and of backcross F_1 plants of the backcross [(*B. carinata* x *B. napus*) x *B. carinata*]

Brassica carinata leaf tissue contained 6.1 μ moles g^{-1} fresh leaf weight of 2-propenyl glucosinolate which represented 95% of its total alkenyl glucosinolate content (Table 3.5). In contrast, *B. napus* leaf tissue was low in 2-propenyl glucosinolate (0.2 μ moles g^{-1} fresh leaf weight).

Leaf tissues from 50 F_1 plants of the backcross [(*B. carinata* x *B. napus*) x *B. carinata*] contained high level of 2-propenyl glucosinolates. F_1 plants 4, 30, 35 and 40 had 2-propenyl glucosinolate contents as high as or higher than the *B. carinata* parent. The remaining F_1 plants had 2-propenyl glucosinolate contents that were intermediate between *B. carinata* and *B. napus*. All F_1 plants of the backcross contained, in their leaf tissues, the longer chain glucosinolates 3-butenyl, 2-hydroxy 3-butenyl and 4-pentenyl glucosinolates in concentrations similar to those observed in the *B. napus* parent.

The quotient Q1, which characterizes the ability of plants to synthesize the longer glucosinolates, was low for *B. carinata* and high for *B. napus*. Q1 values for F_1 plants of the backcross were intermediate between the parents with values ranging from 0.12 for plant no. 50 to 0.64 for plant no. 41.

Table 3.5 Leaf glucosinolate composition and quotients for parents and F_1 plants of the backcross [(*B. carinata* line C90-14 x *B. napus* cv. Westar) x *B. carinata* line C90-14] grown in the greenhouse.

	Glucosinolates μ moles g^{-1} fresh leaf weight						
Plant	Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
Parents							
B. carinata							
C90-14	6.1	0.2	0.1	0.0	6.4	0.05	0.33
B. napus							
Westar	0.2	0.1	0.8	0.3	0.3	0.86	0.89
Backcross F_1							
1	4.0	0.3	0.5	0.0	4.8	0.17	0.62
2	2.2	0.4	1.4	0.1	4.1	0.46	0.78
3	2.3	0.5	1.6	0.1	4.5	0.49	0.76
4	6.8	0.6	1.1	4.9	8.5	0.20	0.65
5	2.8	0.3	1.1	0.7	4.9	0.43	0.79
6	4.5	0.7	1.5	0.0	6.7	0.33	0.68
7	2.7	0.5	0.7	0.3	4.2	0.36	0.58
8	3.9	0.3	0.9	0.5	5.6	0.30	0.75
9	2.8	0.2	1.1	0.9	5.0	0.44	0.85
10	3.7	0.4	0.6	0.0	4.7	0.21	0.60
11	4.0	0.4	2.5	0.2	7.1	0.44	0.86

[1]Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl glucosinolate.

$$^{2}Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.5 Continued.

Plant	Glucosinolates μ moles g^{-1} fresh leaf weight						
	Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
12	2.1	0.8	1.2	0.2	4.3	0.51	0.60
13	2.1	0.6	1.5	0.2	4.4	0.52	0.71
14	1.8	0.6	0.9	0.1	3.4	0.47	0.60
15	4.1	0.6	2.8	0.2	7.7	0.47	0.82
16	3.7	0.7	1.2	0.1	5.7	0.35	0.63
17	4.4	0.8	2.2	0.4	7.8	0.44	0.73
18	2.1	0.3	0.6	0.0	3.0	0.30	0.67
19	1.7	0.2	1.1	0.1	3.1	0.45	0.85
20	3.9	0.7	2.3	0.3	7.2	0.46	0.77
21	2.2	0.3	0.3	0.0	2.8	0.21	0.50
22	4.0	1.5	3.1	0.6	9.2	0.57	0.67
23	4.0	1.1	3.8	0.5	9.4	0.57	0.78
24	2.5	0.7	0.9	0.1	4.2	0.40	0.56
25	1.3	0.2	0.2	0.0	1.7	0.23	0.50
26	3.4	1.3	1.7	0.4	6.8	0.50	0.57
27	2.7	1.1	1.0	0.2	5.1	0.45	0.48
28	0.6	0.3	0.4	0.0	1.3	0.54	0.57
29	2.8	0.8	0.9	0.1	4.6	0.39	0.53
30	8.2	1.2	1.8	0.0	11.2	0.27	0.60
31	3.0	0.5	1.3	0.1	4.9	0.39	0.72
32	4.7	0.5	0.5	0.0	5.7	0.18	0.50
33	3.6	0.3	0.6	0.0	4.5	0.20	0.67
34	5.6	0.8	1.5	0.6	8.5	0.28	0.65
35	9.0	0.9	1.3	0.0	11.2	0.20	0.59
36	3.1	0.7	1.9	0.2	5.9	0.47	0.73
37	1.6	0.9	0.3	1.4	4.2	0.62	0.60
38	4.1	0.5	0.3	0.9	5.8	0.29	0.64
39	2.8	1.3	2.3	0.3	6.7	0.58	0.64
40	8.1	1.3	0.9	0.3	10.6	0.24	0.41
41	3.2	1.7	3.4	0.0	9.2	0.64	0.35
42	3.9	1.3	2.9	0.8	8.9	0.56	0.69
43	4.7	0.3	0.6	0.5	6.1	0.23	0.67
44	4.4	0.6	1.2	0.0	6.2	0.29	0.67
45	4.7	0.4	0.3	0.2	5.6	0.16	0.43

Table 3.5 Continued.

	Glucosinolates μ moles g^{-1} fresh leaf weight						
Plant	Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
46	1.8	0.3	1.9	0.0	4.0	0.55	0.86
47	3.6	0.1	0.5	0.2	4.4	0.18	0.83
48	2.1	0.3	0.7	0.0	3.1	0.32	0.70
49	2.6	0.3	0.7	0.0	3.1	0.32	0.70
50	2.9	0.1	0.3	0.0	3.3	0.12	0.75
Mean	3.7	0.6	1.3	0.1	5.7	0.37	0.70

3.3.4.2 Seed meal glucosinolate composition and glucosinolate quotients of F_2 plants of the backcross [(*B. carinata* x *B. napus*) x *B. carinata*]

Levels of 2-propenyl glucosinolate content in the seed meal of F_2 plants from the backcross [(*B. carinata* x *B. napus*) x *B. carinata*] ranged from 78.1 μ moles in plant no.-67 to 193.1 μ moles g^{-1} meal in plant no. 98 with an average content of 2-propenyl glucosinolate of 126.6 μ moles g^{-1}, somewhat lower than that of the *B. carinata* parent, while *B. napus* Westar seed meal contained only trace amount of 2-propenyl glucosinolate (Table 3.6 and Figure 3.6a). The average 3-butenyl glucosinolate content of backcross F_2 plants was 5.9 μ moles ranging from less than 0.7 to 19.0 μ moles with plant no. 139 containing 32.4 μ moles of 3-butenyl glucosinolate in its seed meal (Figure 3.6b). *B. carinata* seed meal had only low levels of 3-butenyl glucosinolate, while *B. napus* seed meal contained 2.3 μ moles. Backcross F_2 plants had 2-hydroxy 3-butenyl glucosinolate contents from less than 1 to 40.7 μ moles g^{-1} meal, with an average of 14.5 μ moles (Figure 3.6c). Parent levels for 2-hydroxy 3-butenyl glucosinolates were low but both parents exhibited some glucosinolate hydroxylation capacity. The total alkenyl glucosinolate content of backcross F_2 plants, the sum of 2-propenyl+ 3-butenyl + 2-hydroxy 3-butenyl + 4-pentenyl +2-hydroxy 4-pentenyl glucosinolate (Table 3.6), varied greatly from 117.6 to 206.6 μ moles g^{-1} of meal (Figure 3.6d). The average total alkenyl glucosinolate content for backcross F_2 plants was 146.6 μ moles and was somewhat higher than that of the *B. carinata* parent. The total alkenyl glucosinolate content of the *B. napus* parent was low.

Table 3.6 Meal glucosinolate composition and quotients for parents and F_2 plants of the backcross [(*B. carinata* line C90-14 x *B. napus* cv. Westar) x *B. carinata* line C90-14] grown in the greenhouse, arranged in order of the magnitude of Q1.

	Glucosinolates μ moles g^{-1} meal[1]							
Genotype	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
Parents								
Westar	0.2	2.3	4.1	0.3	0.0	6.9	0.96	0.64
C90-14	133.2	0.8	2.9	0.0	0.0	136.9	0.02	0.79
Backcross F_2								
32	151.2	0.7	3.2	0.0	0.7	155.9	0.03	0.82
55	160.8	0.9	3.5	0.0	0.0	165.3	0.03	0.79
43	172.7	0.9	5.8	0.0	0.3	179.6	0.04	0.87
56	149.9	1.2	4.9	0.0	0.0	156.1	0.04	0.80
64	136.9	1.3	3.2	0.0	1.2	142.7	0.04	0.71
68	151.1	1.6	3.5	0.0	1.6	158.1	0.04	0.69
74	137.0	1.4	3.0	0.0	1.4	142.8	0.04	0.68
77	139.3	1.3	3.4	0.0	1.6	145.6	0.04	0.72
126	153.7	1.1	4.0	0.0	1.8	160.6	0.04	0.79
1	116.6	2.7	2.8	0.0	1.0	123.0	0.05	0.51
3	135.9	1.6	3.8	0.0	1.3	142.6	0.05	0.71
4	117.7	1.5	2.8	0.0	1.4	123.4	0.05	0.66
5	131.3	1.6	3.6	0.0	1.1	137.6	0.05	0.69
8	119.7	1.7	3.7	0.0	1.0	126.2	0.05	0.69
11	138.3	1.4	3.7	0.0	2.0	145.4	0.05	0.73
21	113.7	1.2	2.9	0.0	1.4	119.2	0.05	0.71
62	148.7	2.2	4.1	0.0	2.2	157.0	0.05	0.66
63	133.1	1.8	3.2	0.0	1.2	139.3	0.05	0.64
73	137.0	1.5	3.3	0.0	1.6	143.4	0.05	0.69
75	151.6	1.3	3.5	0.0	2.0	158.5	0.05	0.73

[1] Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl, Hpen= 2-hydroxy 4-pentenyl glucosinolates.

$$^{2}\mathbf{Q1} = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}\mathbf{Q2} = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.6 Continued.

Genotype	Glucosinolates μ moles g^{-1} meal							
	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
76	144.9	1.3	4.0	0.0	2.3	152.6	0.05	0.75
78	159.2	3.4	5.4	0.0	0.0	168.0	0.05	0.61
87	112.0	1.5	3.7	0.0	0.7	117.9	0.05	0.71
92	148.9	1.8	4.2	0.0	1.6	156.5	0.05	0.70
93	136.2	1.5	4.1	0.0	1.7	143.5	0.05	0.73
94	124.5	1.6	3.9	0.0	1.1	131.1	0.05	0.71
102	156.4	3.5	3.6	0.0	1.3	164.9	0.05	0.50
104	179.7	3.2	4.4	0.1	2.2	189.5	0.05	0.58
128	150.6	1.0	3.9	0.0	2.1	157.5	0.05	0.79
136	125.7	1.8	3.3	0.0	1.8	132.5	0.05	0.65
138	121.7	1.8	3.4	0.0	1.0	127.8	0.05	0.65
137	122.1	2.3	4.2	0.0	0.1	128.8	0.05	0.65
25	152.7	1.6	5.4	0.0	2.3	161.9	0.06	0.77
138	137.6	1.7	4.2	0.0	1.9	145.4	0.06	0.72
15	117.7	1.2	3.9	0.0	1.6	124.4	0.06	0.70
27	144.1	1.1	5.2	0.0	2.8	153.2	0.06	0.83
37	162.2	1.8	7.4	0.0	1.4	172.8	0.06	0.80
80	133.3	2.1	4.7	0.0	1.4	141.5	0.06	0.69
82	97.3	1.5	3.4	0.0	1.1	103.3	0.06	0.70
83	123.9	2.1	3.8	0.0	1.3	131.2	0.06	0.64
135	140.4	2.2	4.9	0.0	1.5	149.0	0.06	0.69
19	116.2	1.8	4.4	0.0	1.6	124.1	0.07	0.71
23	142.8	2.1	7.1	0.0	1.4	153.4	0.07	0.77
49	146.7	4.8	6.5	0.0	0.1	158.1	0.07	0.58
57	154.2	2.9	7.5	0.0	1.6	166.2	0.07	0.72
60	129.8	2.0	6.4	0.0	1.6	139.8	0.07	0.76
61	132.0	3.6	4.8	0.0	1.6	142.0	0.07	0.57
79	136.2	2.5	6.0	0.0	1.2	145.9	0.07	0.71
88	105.4	1.4	5.9	0.0	0.9	113.6	0.07	0.80
98	193.1	2.0	8.8	0.0	2.7	206.6	0.07	0.82
134	144.4	2.0	8.1	0.0	0.1	154.6	0.07	0.80
66	162.5	2.7	10.5	0.0	0.1	175.8	0.08	0.80
90	103.2	2.1	5.5	0.0	1.0	111.8	0.08	0.72
106	164.5	3.4	11.2	0.0	0.3	179.4	0.08	0.77
6	131.6	4.1	8.6	0.0	0.1	144.5	0.09	0.68

Table 3.6 Continued.

Genotype	Glucosinolates μ moles g^{-1} meal							
	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
120	138.8	3.7	11.1	0.0	0.1	153.7	0.10	0.75
2	107.7	2.6	10.3	0.0	0.1	120.7	0.11	0.80
29	144.4	5.2	11.6	0.0	0.8	161.9	0.11	0.69
122	153.8	7.3	12.5	0.0	0.1	173.6	0.11	0.63
132	123.4	6.3	8.1	0.0	0.0	137.9	0.11	0.56
143	126.3	3.8	10.8	0.1	0.1	141.2	0.11	0.74
24	177.8	3.8	17.7	0.0	1.7	201.0	0.12	0.82
40	162.8	3.4	17.0	0.0	2.1	185.3	0.12	0.83
46	100.9	4.9	9.2	0.0	0.1	115.1	0.12	0.65
52	133.6	2.6	14.8	0.0	0.1	151.1	0.12	0.85
91	149.2	4.8	15.8	0.0	0.2	170.0	0.12	0.77
109	140.5	2.7	14.3	0.0	1.3	158.8	0.12	0.84
118	121.6	3.5	12.2	0.0	0.1	137.4	0.12	0.78
34	137.5	4.0	15.9	0.2	1.6	159.2	0.14	0.80
131	139.3	4.1	14.1	0.1	0.1	157.6	0.12	0.78
22	110.4	4.8	13.8	0.0	0.1	129.1	0.14	0.74
65	139.7	4.9	15.3	0.0	1.8	161.8	0.14	0.76
81	125.8	4.7	16.1	0.1	0.2	146.8	0.14	0.78
85	115.0	8.9	10.2	0.0	0.1	134.2	0.14	0.53
89	111.9	7.0	9.6	0.0	1.0	129.5	0.14	0.58
96	119.0	5.1	13.6	0.0	0.1	137.7	0.14	0.73
30	110.0	9.3	9.4	0.0	0.1	128.8	0.15	0.50
101	138.9	12.0	12.3	0.0	0.1	163.0	0.15	0.51
110	118.0	4.4	13.2	0.0	2.8	138.4	0.15	0.75
115	108.3	5.5	13.1	0.1	0.2	127.3	0.15	0.70
127	134.0	8.7	15.4	0.0	0.0	158.0	0.15	0.64
130	123.9	3.0	17.7	0.1	0.2	144.9	0.15	0.85
18	108.0	4.3	16.9	0.0	0.1	129.3	0.16	0.80
26	139.3	4.4	20.0	0.0	2.4	166.1	0.16	0.82
51	111.1	5.1	16.3	0.0	0.1	132.7	0.16	0.76
119	127.4	3.7	18.0	0.5	2.2	151.7	0.16	0.83
144	145.3	4.3	22.6	0.0	0.1	172.3	0.16	0.84

Table 3.6 Continued.

Genotype	Glucosinolates μ moles g^{-1} meal							
	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
16	123.7	9.0	14.0	0.0	1.3	148.0	0.17	0.61
39	115.4	5.9	18.0	0.0	0.1	139.6	0.17	0.75
41	134.3	4.7	20.7	0.4	1.7	161.9	0.17	0.81
54	122.3	9.8	15.0	0.0	0.0	147.2	0.17	0.61
71	133.0	8.0	18.4	0.0	0.7	160.0	0.17	0.70
112	114.4	5.1	17.8	0.3	0.1	137.6	0.17	0.78
31	131.3	3.7	23.4	0.4	1.7	160.6	0.18	0.86
48	114.7	4.9	20.3	0.0	0.1	140.0	0.18	0.81
53	115.6	10.0	14.5	0.0	0.1	140.2	0.18	0.59
103	114.6	6.5	18.0	0.0	0.1	139.2	0.18	0.73
72	112.5	3.9	21.2	0.0	0.8	138.4	0.19	0.84
116	128.7	6.8	21.2	0.0	1.5	158.2	0.19	0.76
141	138.9	11.0	20.0	0.0	0.1	165.5	0.19	0.65
33	102.0	6.7	18.1	0.0	0.3	127.2	0.20	0.72
133	132.8	5.5	25.4	0.4	2.1	166.2	0.20	0.82
14	133.8	14.0	18.9	0.0	1.7	168.5	0.21	0.57
28	120.4	8.9	22.6	0.0	1.3	153.2	0.21	0.72
35	118.3	5.3	24.4	0.0	1.0	149.0	0.21	0.82
99	118.2	5.0	24.7	0.3	2.1	150.3	0.21	0.83
105	121.9	9.0	24.1	0.0	0.1	155.1	0.21	0.73
140	141.7	12.0	24.8	0.0	0.1	178.3	0.21	0.68
36	125.4	10.0	24.3	0.3	0.1	160.5	0.22	0.70
84	135.6	15.0	23.7	0.0	0.2	174.6	0.22	0.61
86	116.5	12.0	21.0	0.0	0.1	149.8	0.22	0.63
100	120.5	8.2	25.2	0.0	0.1	153.9	0.22	0.75
123	119.8	6.4	26.2	0.4	1.2	154.1	0.22	0.80
139	116.4	32.0	0.4	0.0	0.1	149.4	0.22	0.01
13	110.4	8.3	23.8	0.0	0.1	142.5	0.23	0.74
42	131.7	5.1	31.1	1.1	1.7	170.7	0.23	0.86
45	105.8	8.3	22.3	0.0	0.6	137.0	0.23	0.73
69	105.4	8.0	22.8	0.0	0.2	136.4	0.23	0.74
111	112.0	3.7	27.0	0.4	1.3	144.4	0.23	0.88
121	119.3	5.9	27.7	1.2	1.5	155.5	0.23	0.82
59	119.3	9.6	26.6	0.0	0.6	156.0	0.24	0.74
108	104.7	10.0	22.3	0.4	0.3	137.7	0.24	0.69

Table 3.6 Continued.

	Glucosinolates μ moles g^{-1} meal							
Genotype	Pro	But	Hbut	Pen	Hpen	Total	Q1[2]	Q2[3]
58	119.2	14.0	25.5	0.1	1.1	159.8	0.25	0.65
95	86.9	9.2	18.1	0.0	0.8	115.0	0.25	0.66
117	109.1	5.7	30.0	0.0	0.3	145.1	0.25	0.84
142	104.8	0.7	26.3	0.0	0.9	139.0	0.25	0.79
17	82.2	15.7	10.9	0.0	1.7	110.5	0.26	0.41
70	107.7	4.8	30.6	0.0	2.1	145.3	0.26	0.86
125	108.7	3.4	30.8	1.3	2.5	146.7	0.26	0.90
12	95.9	13.0	21.5	0.0	1.1	161.6	0.27	0.62
47	111.8	7.6	32.1	0.7	1.1	153.1	0.27	0.81
67	78.1	9.7	19.6	0.0	0.2	107.6	0.27	0.67
107	100.5	7.3	29.0	0.0	0.2	136.8	0.27	0.80
124	115.1	11.0	30.7	0.0	1.6	158.7	0.27	0.73
129	94.7	19.0	14.4	0.4	0.2	128.9	0.27	0.43
20	94.8	18.0	19.0	0.3	0.2	132.4	0.29	0.51
38	107.6	8.0	36.4	1.1	1.6	154.7	0.30	0.82
113	111.8	10.0	37.4	0.0	0.2	159.5	0.30	0.79
10	93.5	11.0	31.8	0.0	0.1	136.2	0.31	0.75
44	112.8	7.0	40.4	1.3	1.0	162.6	0.31	0.85
9	96.7	10.0	35.4	0.0	0.0	142.6	0.32	0.77
97	93.0	17.0	25.8	0.0	0.1	135.6	0.32	0.61
114	98.0	8.5	38.7	0.4	0.2	145.8	0.33	0.82
50	92.2	4.8	40.7	1.4	1.3	140.3	0.34	0.90
Mean	126.6	5.9	14.5	0.1	0.2	146.6	0.14	0.72

Quotient values for C3 ➔ C4 glucosinolate precursor elongation (Q1) of backcross F_2 plants ranged from 0.03 to 0.34 (Figure 3.6e). The frequency distribution of Q1 values was separated into four classes. (Figure 3.6e). The Q1 value for *B. carinata* was very low while that for *B. napus* was very high. The Q2 values of backcross F_2 plants, an indication of 3-butenyl glucosinolate hydroxylation capacity, ranged from 0.41 to 0.90. The backcross

Alkenyl glucosinolates

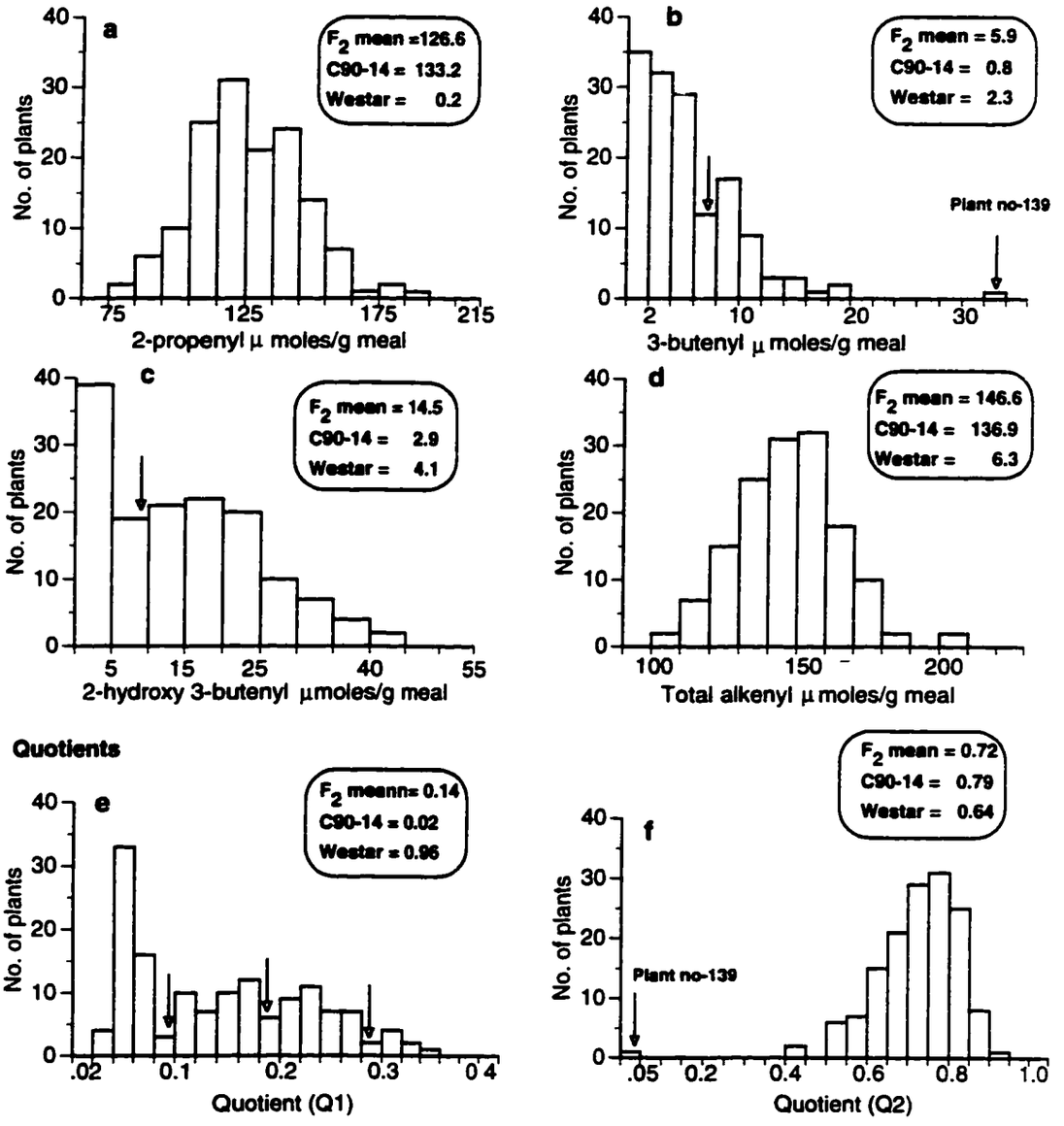

Figure 3.6 Frequency distribution of alkenyl glucosinolate contents and quotients in seed meal of BCF_2 plants from the cross [(*B. carinata* x *B. napus*) x *B. carinata*] grown in the greenhouse. Arrows indicate class separation.

F_2 plant, no.139, had a Q2 value of 0.01 (Table 3.6 and Figure 3.6f) which was well outside the range of Q2 values observed among the F_2 backcross plants (Figure 3.6f). The *B. carinata* and *B. napus* parents had Q2 values of 0.79 and 0.64, respectively.

3.3.4.3 Seed meal glucosinolate composition and glucosinolate quotients of F_3 plants of the backcross [(*B. carinata* x *B. napus*) *B. carinata*]

The alkenyl glucosinolate content of eight selected backcross F_2 plants of the back cross [(*B. carinata* x *B. napus*) *B. carinata*] was verified in F_3 progeny. Backcross F_2 plants no. 4 and 55 had glucosinolate compositions similar to that of the *B. carinata* parent with 2-propenyl glucosinolates as the predominant glucosinolate (Table 3.7). The glucosinolate composition of seven F_3 plants from the F_2 plants 4 and 55, was similar to the glucosinolate composition of the original F_2 plants. Q1 values for the 14 F_3 plants were low which is typical of *B. carinata* glucosinolate profiles.

Backcross F_2 plants 31 and 41, containing approximately 5 µmoles of 3-butenyl glucosinolate, produced F_3 progeny with similar 3-butenyl glucosinolate contents. Both plants had a very strong 3-butenyl glucosinolate hydroxylation F_2 and F_3. Glucosinolate precursor chain elongation capacity was low for plants no. 31 and 41.

Table 3.7 Meal glucosinolate composition of parents and F_3 plants grown from eight F_2 plants of the backcross [(*B. carinata* line C90-14 x *B. napus* cv. Westar) x *B. carinata* line C90-14] grown in the greenhouse.

		Glucosinolates μ moles g^{-1} meal[1]						
Family	Plant no.	Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
Parents								
B. napus								
Westar		2.7	2.3	2.7	0.6	8.3	0.67	0.54
B. carinata								
C90-14		143.0	1.1	4.2	0.0	148.3	0.04	0.80
Backcross								
F_2	4	117.7	1.5	2.8	0.0	122.0	0.04	0.65
F_3	4-1	153.1	3.0	3.3	0.0	159.4	0.04	0.52
	4-2	150.3	1.7	3.3	0.0	155.3	0.04	0.66
	4-3	144.6	1.4	2.5	0.0	148.5	0.03	0.64
	4-4	135.9	1.3	3.1	0.0	140.3	0.03	0.70
	4-5	127.7	1.8	3.4	0.0	132.9	0.04	0.65
	4-6	155.7	1.2	3.1	0.0	160.0	0.03	0.72
	4-7	155.1	1.9	3.6	0.0	160.6	0.03	0.65
F_2	55	160.8	0.9	3.5	0.0	165.2	0.03	0.79
F_3	55-1	152.7	0.8	4.2	0.0	157.7	0.03	0.84
	55-2	162.9	1.1	5.1	0.0	169.1	0.04	0.82
	55-3	134.3	5.6	15.1	0.0	155.0	0.13	0.73
	55-4	185.8	1.5	4.8	0.0	192.1	0.03	0.76
	55-5	133.6	0.8	3.9	0.0	138.3	0.03	0.83
	55-6	157.8	0.7	4.9	0.0	163.4	0.03	0.87
	155-7	141.5	0.7	3.9	0.0	146.1	0.03	0.84

[1]Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl glucosinolates.

$$^{2}\mathbf{Q1} = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}\mathbf{Q2} = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.7 Continued.

Gen.	Plant no.	Glucosinolates μ moles g^{-1} meal					Q1[2]	Q2[3]
		Pro	But	Hbut	Pen	Total		
F_2	31	131.3	3.7	23.4	0.4	158.8	0.17	0.86
F_3	31-1	107.5	8.1	24.6	0.0	140.2	0.17	0.86
	31-2	109.0	8.6	23.9	0.0	141.5	0.23	0.74
F_2	41	134.3	4.7	20.7	0.4	160.1	0.16	0.81
F_3	41-1	103.7	5.0	37.4	1.2	147.3	0.30	0.88
	41-2	117.9	8.0	23.6	0.0	149.5	0.21	0.75
	41-3	112.7	5.0	36.9	1.6	156.2	0.28	0.88
	41-4	72.1	3.2	23.9	1.0	100.5	0.28	0.88
F_2	10	93.5	10.1	31.8	0.0	135.4	0.31	0.76
F_3	10-1	84.8	5.8	30.9	0.0	121.5	0.30	0.84
	10-2	71.8	14.7	26.5	0.0	113.0	0.36	0.64
	10-3	114.6	6.5	31.8	0.0	152.9	0.25	0.87
	10-4	100.4	18.6	17.2	0.0	136.2	0.26	0.48
	10-5	86.1	11.8	35.2	0.0	133.1	0.35	0.75
	10-6	101.4	12.2	34.5	0.0	148.1	0.32	0.74
	10-7	81.3	7.9	24.1	0.0	133.3	0.28	0.75
F_2	36	125.4	10.4	24.3	0.3	160.4	0.22	0.70
F_3	36-1	133.2	11.0	23.8	0.7	168.7	0.21	0.68
	36-2	114.7	13.7	28.3	0.4	157.1	0.27	0.67
	36-3	107.3	15.5	18.1	0.2	141.1	0.24	0.54
	36-4	102.5	6.7	0.3	20.5	130.0	0.21	0.75
F_2	20	94.8	18.1	19.0	0.3	132.2	0.28	0.51
F_3	20-1	100.9	17.5	17.1	0.0	135.5	0.26	0.49
	20-2	71.9	18.6	16.6	0.0	107.1	0.32	0.47
	20-3	113.1	16.2	20.0	0.1	149.4	0.24	0.55
F_2	139	116.4	32.4	0.5	0.0	149.3	0.22	0.02
F_3	139-1	98.8	12.6	0.2	0.0	111.6	0.11	0.02
	139-2	101.5	5.9	0.2	0.0	107.6	0.06	0.03
	139-3	89.6	47.9	1.4	0.0	138.9	0.35	0.03
	139-4	98.0	20.6	0.5	0.0	119.1	0.18	0.02
	139-5	157.6	11.9	0.1	0.0	169.6	0.07	0.01
	139-6	132.3	5.1	0.1	0.0	137.5	0.04	0.02

Backcross F_2 plants 10 and 36 had a stronger chain elongation capacity than F_2 plants no. 31 and 41, resulting in approximately 10 μ moles of 3- butenyl glucosinolate. The F_3 generation of these two F_2 plants had 3-butenyl glucosinolate contents varying from 5 μ moles to 18 μ moles (Table 3.7). The 3-butenyl hydroxylation in these plants was very strong. Q1 values were indicative of strong chain elongation capacities.

The backcross F_2 plant no. 20 had a lower 3-butenyl glucosinolate hydroxylation capacity than F_2 plants no.4 , 55, 31, 41, 10 and 36, but its actual 3-butenyl glucosinolate content was high (Table 3.7). F_3 progeny of F_2 plant no. 20 had a similar composition.

The backcross F_2 plant 139 contained 3-butenyl glucosinolate with only a trace amount of 2-hydroxy 3-butenyl glucosinolate. It was the only F_2 plant identified that was incapable of 3-butenyl glucosinolate hydroxyalation. F_3 progenies of F_2 plant 139 segregated for 3-butenyl glucosinolate content, however all six F_3 plants had very low hydroxylation capacities similar to that of the original F_2 plant. This was also evident from the Q2 values which characterize 3-butenyl glucosinolate hydroxylation (Table 3.7).

3.3.5 [(*B. carinata* x *B. juncea*) x *B. carinata*] cross

3.3.5.1 Leaf glucosinolate composition and glucosinolate quotients of parents and F_1 plants of the backcross [(*B. carinata* x *B. juncea*) x *B. carinata*]

The 2-propenyl glucosinolate content in leaf tissue was high in *B. carinata* (6.0 μ moles) and low in *B. juncea* (0.2 μ moles) (Table 3.8). Seventy eight F_1 plants of the backcross [(*B. carinata* x *B. juncea*) x *B. carinata*] had 2-propenyl glucosinolate contents

ranging from 0.2 to 1.5 μ moles g^{-1} leaf tissue which was somewhat higher than the

Table 3.8 Leaf glucosinolate composition and quotients for parents and F_1 plants of the backcross [(*B. carinata* line C90-14 x *B. juncea* line J90-4253) x *B. carinata* line C90-14] grown in the greenhouse.

	Glucosinolates μ moles g^{-1} fresh leaf weight						
Plant	Pro[1]	But	Hbut	Pen	Total	Q1[2]	Q2[3]
Parents							
B. carinata							
C90-14	6.0	0.0	0.1	0.0	6.1	0.01	1.00
B. juncea							
J90-4253	0.2	2.8	0.0	1.9	4.9	0.96	0.00
Backcross F_1							
1	0.5	0.4	0.6	0.2	1.7	0.71	0.60
2	1.1	1.2	1.3	0.4	4.0	0.73	0.52
3	0.9	0.9	1.2	0.4	3.4	0.74	0.57
4	0.9	0.7	0.9	0.3	2.8	0.68	0.56
5	0.5	0.5	0.5	0.6	2.1	0.76	0.50
6	0.7	0.5	0.3	0.8	2.3	0.70	0.62
7	0.9	0.8	0.9	0.4	3.0	0.70	0.53
8	0.4	0.3	0.5	0.2	1.4	0.71	0.63
9	0.5	0.6	0.7	0.4	2.2	0.77	0.54
10	1.0	0.7	1.6	0.6	3.9	0.74	0.70
11	0.5	0.3	0.6	0.2	1.6	0.69	0.67
12	0.4	0.4	0.5	0.2	1.5	0.73	0.56

[1] Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl glucosinolate.

$$^{2}\mathbf{Q1} = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}\mathbf{Q2} = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.8 Continued.

Plant	Glucosinolates μ moles g^{-1} fresh leaf weight					$Q1^2$	$Q2^3$
	Pro	But	Hbut	Pen	Total		
13	0.4	0.6	0.6	0.4	2.0	0.80	0.50
14	0.4	0.4	0.2	0.4	1.4	0.71	0.50
15	1.2	0.9	0.3	1.1	3.5	0.66	0.55
16	0.6	0.9	0.5	1.1	3.5	0.81	0.55
17	0.4	0.6	0.6	0.3	1.9	0.79	0.50
18	0.4	0.4	0.5	0.2	1.5	0.73	0.56
19	0.3	0.5	0.4	0.3	1.5	0.80	0.44
20	0.3	0.2	0.3	0.2	1.0	0.70	0.60
21	0.3	0.3	0.6	0.3	1.5	0.80	0.67
22	0.5	0.3	0.5	0.3	1.6	0.69	0.63
23	0.5	0.4	0.5	0.2	1.6	0.69	0.56
24	0.7	0.3	1.4	0.4	2.8	0.75	0.82
25	0.8	0.7	0.7	0.2	2.4	0.57	0.50
26	1.0	1.0	1.0	0.4	3.4	0.71	0.50
27	0.3	0.2	0.2	0.1	0.8	0.63	0.50
28	0.4	0.2	0.4	0.1	1.1	0.64	0.67
29	0.3	0.5	0.4	0.3	1.5	0.80	0.44
30	0.4	0.6	0.7	0.5	2.2	0.82	0.54
31	0.7	0.6	0.6	0.3	2.2	0.69	0.50
32	0.5	0.4	0.3	0.4	1.6	0.69	0.43
33	0.6	0.5	0.9	0.5	2.5	0.76	0.64
34	0.9	0.5	0.9	0.7	3.0	0.70	0.64
35	1.1	1.4	1.5	0.7	4.7	0.77	0.52
36	0.7	0.8	0.9	0.5	2.9	0.76	0.53
37	0.3	0.3	0.3	0.2	1.1	0.73	0.50
38	0.5	0.7	0.8	0.5	2.5	0.80	0.53
39	0.1	0.1	0.2	0.1	0.5	0.80	0.67
40	1.0	0.6	1.5	0.4	3.5	0.71	0.71
41	0.3	0.4	0.5	0.3	1.5	0.80	0.56
42	1.5	0.9	2.0	0.6	5.0	0.70	0.69
43	0.5	0.5	0.8	0.3	2.1	0.76	0.62
44	0.3	0.3	0.4	0.2	1.2	0.75	0.57
45	0.2	0.1	0.2	0.1	0.6	0.67	0.67
46	0.5	0.6	0.7	0.3	2.1	0.76	0.54

Table 3.8 Continued.

Plant	Glucosinolates μ moles g^{-1} fresh leaf weight						
	Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
47	0.6	0.6	0.6	0.3	2.1	0.71	0.50
48	0.5	0.5	0.7	0.3	2.0	0.75	0.58
49	0.4	0.4	0.6	0.3	1.7	0.76	0.60
50	0.3	0.3	0.5	0.2	1.3	0.77	0.63
51	0.4	0.4	0.5	0.2	1.5	0.73	0.56
52	0.5	0.6	0.9	0.5	2.5	0.80	0.60
53	0.3	0.3	0.4	0.2	1.2	0.75	0.57
54	1.2	0.7	1.1	0.3	3.3	0.64	0.61
55	1.0	1.4	1.8	0.7	4.9	0.80	0.56
56	0.6	0.5	0.6	0.3	2.0	0.70	0.67
57	0.6	0.5	0.8	0.3	2.2	0.73	0.61
58	0.3	0.3	0.4	0.2	1.2	0.75	0.57
59	0.3	0.5	0.6	0.4	1.8	0.83	0.54
60	0.2	0.3	0.4	0.3	1.2	0.83	0.57
61	0.3	0.3	0.5	0.2	1.3	0.77	0.63
62	1.2	1.0	1.4	0.3	3.9	0.69	0.58
63	0.5	0.1	1.0	0.1	1.7	0.71	0.91
64	0.7	0.3	0.7	0.2	1.9	0.63	0.70
65	0.7	0.4	0.9	0.2	2.2	0.68	0.69
66	0.3	0.3	0.4	0.1	1.1	0.73	0.57
67	0.4	0.1	0.9	0.1	1.5	0.73	0.73
68	0.6	0.3	0.9	0.2	2.1	0.70	0.75
69	0.6	0.5	0.6	0.1	1.8	0.67	0.55
70	0.8	0.6	0.7	0.2	2.3	0.65	0.54
71	1.4	1.1	1.1	0.4	4.0	0.65	0.50
72	0.2	0.1	0.3	0.1	0.7	0.71	0.75
73	0.4	0.4	0.5	0.3	1.6	0.78	0.56
74	0.6	0.7	0.9	0.5	2.8	0.78	0.56
75	0.3	0.2	0.5	0.2	1.2	0.75	0.71
76	0.3	0.3	0.5	0.2	1.3	0.77	0.63
77	0.2	0.2	0.4	0.2	1.0	0.80	0.67
78	0.6	0.4	0.7	0.3	2.1	0.70	0.64
Mean	0.8	0.6	0.7	0.3	2.3	0.71	0.58

2-propenyl leaf glucosinolate content of the *B. juncea* parent, but significantly lower than for the *B. carinata* parent. Some F_1 plants had 2-propenyl leaf glucosinolate contents as low as the *B. juncea* parent. All F_1 plants of this backcross also contained 3-butenyl, 2-hydroxy 3-butenyl and 4-pentenyl and 2-hydroxy 4-pentenyl glucosinolates in their leaf tissues with an average concentration of 0.6, 0.7, and 0.3 micro moles g^{-1} leaf tissue, respectively.

The quotient Q1, as a measure of C3 →C4 glucosinolate precursor elongation capacity, was, on average, 0.71 for F_1 plants. Q1 values for F_1 plants were closer to Q1 values of the *B. juncea* parent and were significantly greater than for the *B. carinata* parent. Q2 values of F_1 plants were also high with an average of 0.58, indicative of strong 3-butenyl glucosinolate hydroxylation capacities.

3.3.5.2 Seed meal glucosinolate composition and glucosinolate quotients of F_2 plants of the backcross [(*B. carinata* x *B. juncea*) x *B. carinata*]

F_2 plants of the backcross [(*B. carinata* x *B.juncea*) x *B. carinata*] were highly male sterile and only four plants produced seed upon bud pollination. Two of these plants produced sufficient seed for glucosinolate analysis. The 2-propenyl glucosinolate contents of seed meal of the two F_2 backcross plants were 82.3 and 37.5 μ mole g^{-1} meal, respectively (Table 3.9). *B. carinata* seed meal was high in 2-propenyl glucosinolate content while *B. juncea* seed meal was low. The two F_2 backcross plants also contained 3-butenyl, 4-pentenyl and 2-hydroxy 3-butenyl glucosinolate. The 2-hydroxy 3-butenyl glucosinolate content was particularly high. *B. carinata* had a low 3-butenyl glucosinolate content while that of *B. juncea* was high. Both parents were low in 2-hydroxy 3-butenyl glucosinolate

Table 3.9 Seed meal glucosinolate content and quotients of parents and F_2 plants of the backcross [(*B. carinata* line C90-14 x *B. juncea* line J90-4253) x *B. carinata* line C90-14] grown in the greenhouse.

Parent and BCF2 plants	Glucosinolates μ moles g^{-1} meal						
	Pro [1]	But	Hbut	Pen	Total	Q1[2]	Q2[3]
Backcross F_2							
1	82.3	12.6	51.8	2.6	149.3	0.45	0.80
2	37.5	15.7	54.6	6.1	113.9	0.67	0.78
Parents							
B. carinata							
C90-14	133.0	0.7	3.0	0.0	136.7	0.03	0.81
B. juncea							
J90-4253	0.5	40.0	1.0	1.6	43.1	0.99	0.02

[1] Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl, glucosinolate.

$$^{2}\mathbf{Q1} = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}\mathbf{Q2} = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

content. Q1 values for the two backcross plants were 0.45 and 0.67, respectively while *B. carinata* had a low Q1 value and *B. juncea* had a high value. F_2 plants had high 3-butenyl glucosinolate hydroxylation levels as indicated by high Q2 values. The Q2 value for B. *carinata* was also high while that for *B. juncea* was low.

3.3.5.3 Seed glucosinolate composition and glucosinolate quotients of F_3 plants of the backcross [(*B. carinata* x *B. juncea*) x *B. carinata*]

The alkenyl glucosinolate content of four backcross F_2 plants which produced selfed seed was verified in F_3 progeny. Two F_3 plants derived from the backcross F_2 plant no.1, produced seed with glucosinolate contents and quotients similar to that of the F_2 plants (Table 3.10). Four F_3 plants, of backcross F_2 plant no. 2 had lower 2-propenyl glucosinolate contents similar to that of the F_2 plant. The 3-butenyl glucosinolate contents of F_3 plants varied but were lower than the levels in F_2 plants. The 2-hydroxy 3-butenyl glucosinolate contents of F_3 plants were high which was reflected in high Q2 values.

F_3 plants of backcross F_2 plant no. 3 contained higher levels of 2-propenyl glucosinolate than F_2 plants 1, 2 and 4. The 3-butenyl glucosinolate contents of the four plants varied and 2-hydroxy 3-butenyl glucosinolate contents were low for F_3 plant 3-1 and high for F_3 plants 3-2, 3-3 and 3-4. There was only one F_3 plant tested from F_2 plant no. 4. Parent glucosinolate contents for *B. carinata* and *B. juncea* were similar to those observed previously (Table 3.9 and Table 3.10).

Table 3.10 Whole seed glucosinolate composition and quotients of F_3 plants of the backcross [(*B. carinata* line C90-14 x *B. juncea* line J90-4253) x *B. carinata* line C90-14] grown in the greenhouse.

Family	Plant no.	Glucosinolates μ moles g^{-1} seed						
		Pro	But	Hbut	Pen	Total	Q1[2]	Q2[3]
Parents								
B. carinata								
C90-14		91.9	1.3	2.9	0.0	96.1	0.04	0.70
B. juncea								
J90-4253		0.8	35.1	3.2	1.6	40.7	0.98	0.04
Backcross								
F_2[1]	1	82.3	12.6	51.8	2.6	149.3	0.45	0.80
F_3	1-1	43.3	11.2	24.8	3.2	82.5	0.47	0.69
	1-2	52.2	6.0	25.0	1.2	84.4	0.38	0.81
F_2[1]	2	37.7	15.7	54.6	6.1	113.9	0.67	0.78
F_3	2-1	10.8	7.9	24.3	3.0	46.0	0.77	0.75
	2-2	15.6	12.8	19.1	3.8	51.3	0.69	0.60
	2-3	17.8	8.9	16.1	2.6	45.4	0.61	0.64
	2-4	11.2	5.9	25.6	2.7	45.4	0.75	0.82
F_2	3	not analysed						
F_3	3-1	52.7	24.5	5.5	0.8	83.5	0.37	0.18
	3-2	48.2	12.1	28.6	0.2	89.1	0.46	0.71
	3-3	40.2	6.5	24.2	1.2	72.1	0.44	0.79
	3-4	58.8	8.6	24.2	0.7	92.3	0.36	0.71
F_2	4	not analysed						
F_3	4	23.3	10.5	14.9	1.6	50.3	0.54	0.59

[1]Glucosinolate composition of F_2 of no. 1 and 2 plants was based on seed meal analysis.

Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl glucosinolates.

$$^{2}Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

3.3.6 Interspecific double cross BCF_1 [(*B. carinata* x *B. juncea*) x *B. carinata*] x BCF_1 [*B. carinata* x *B. napus*) x *B. carinata*]

Eight F_1 seeds were obtained from the cross BCF_1 [(*B. carinata* x *B. juncea*) x *B. carinata*] x BCF_1[(*B. carinata* x *B. napus*) x *B. carinata*]. The reciprocal double interspecific cross did not set any seed. Five F_1 seeds germinated and grew into plants with *B. carinata* plant morphology. A total of 171 F_2 seeds were produced by bud selfing from these five plants. The F_2 seed was planted, 171 germinated and grew into F_2 plants, and 99 of these produced selfed seed. F_3 seed from these F_2 plants was analysed for glucosinolate content.

3.3.6.1 Seed glucosinolate composition and glucosinolate quotients of F_2 plants of the double interspecific cross BCF_1 [(*B. carinata* x *B. juncea*) x*B. carinata*] x BCF_1 [*B. carinata* x *B. napus*) x *B. carinata*]

Seed of F_2 plants from the double interspecific cross contained, on average 42.5 μ moles of 2-propenyl, 10.0 μ moles 3-butenyl and 26.0 μ moles of 2-hydroxy 3-butenyl glucosinolates (Table 3.11, Figure 3.7a). The average total alkenyl glucosinolates content of F_2 plants was 79.4 μ moles g^{-1} seed. The level of 2-propenyl glucosinolate of individual F_2 plants ranged from 4.4 to 101.1 μ moles, 3-butenyl glucosinolate content varied from 0.0 to 24.5 μ moles (Figure 3.7b), 2-hydroxy 3-butenyl glucosinolate content varied from 0.8 to 61.8 μ moles g^{-1} seed (Figure 3.7c). Pentenyl glucosinolate contents were very low. The

Table 3.11 Seed glucosinolate content and quotients of F_2 plants from the double interspecific cross [(B. *carinata* line C90-14 x *B. juncea* line J90-4253) x *B. carinata* line C90-14] x [(*B. carinata* line C90-14 x *B. napus* cv. Westar) x *B. carinata* line C90-14] grown in the greenhouse.

F_2 Plant no.	Glucosinolates μ moles g^{-1} seed						
	Pro[1]	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
IC-101	33.0	1.3	5.0	0.9	40.2	0.18	0.80
IC-20	89.7	23.9	0.8	0.8	115.2	0.22	0.03
IC-92	79.2	24.3	0.8	0.3	104.6	0.24	0.03
IC-36	48.9	5.5	18.3	0.0	72.7	0.33	0.77
IC-2	53.6	4.1	24.2	0.4	82.3	0.35	0.86
IC-78	55.6	6.8	23.1	0.5	86.0	0.35	0.77
IC-40	21.0	0.0	7.8	4.1	32.9	0.36	1.00
IC-17	58.7	10.2	23.2	0.0	92.2	0.36	0.69
IC-6	53.6	4.8	28.7	0.0	87.1	0.38	0.86
IC-75	57.1	8.1	26.6	0.2	92.0	0.38	0.77
IC-109	57.1	8.1	26.6	0.2	92.0	0.38	0.77
IC-3	54.3	7.5	26.3	0.2	88.3	0.39	0.78
IC-10	58.2	6.6	30.0	0.2	95.0	0.39	0.82
IC-19	42.0	9.6	17.5	0.4	69.5	0.40	0.65
IC-47	49.3	5.4	27.6	0.5	82.8	0.40	0.84
IC-52	66.6	7.9	34.4	1.3	110.2	0.40	0.81
IC-76	45.6	9.2	22.9	0.0	77.7	0.41	0.71
IC-85	57.4	10.6	28.1	0.5	96.6	0.41	0.73
IC-36	33.4	5.8	16.8	0.3	56.3	0.41	0.74
IC-37	47.8	7.3	26.1	0.3	81.5	0.41	0.78
IC-12	34.0	5.1	18.5	1.1	58.7	0.42	0.78

[1] Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl, glucosinolate.

$$^{2}Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.11 Continued.

Plant no.	Glucosinolate μ moles g^{-1} seed						
	Pro	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
IC-43	78.5	9.9	47.1	0.6	136.1	0.42	0.83
IC-78	44.3	9.5	23.1	0.1	77.0	0.42	0.71
IC-205	44.3	9.5	23.1	0.1	77.0	0.42	0.71
IC-8	39.1	9.7	20.2	0.0	69.0	0.43	0.68
IC-15	44.8	6.7	27.0	0.7	79.2	0.43	0.80
IC-45	49.0	5.0	30.5	1.0	85.5	0.43	0.43
IC-83	48.8	10.5	26.1	0.2	85.6	0.43	0.71
IC-100	36.0	9.9	16.0	0.9	62.8	0.43	0.62
IC-4	45.2	8.3	26.1	1.3	81.0	0.44	0.76
IC-9	42.7	7.8	25.2	0.0	75.7	0.44	0.76
IC-11	40.6	10.0	21.3	0.2	72.1	0.44	0.68
IC-22	41.1	9.2	22.2	0.5	73.0	0.44	0.71
IC-65	44.8	8.1	26.4	0.3	79.6	0.44	0.77
IC-69	49.2	7.1	30.0	1.4	87.7	0.44	0.81
IC-98	41.4	7.5	25.5	0.1	74.5	0.44	0.77
IC-54	30.3	5.2	18.3	0.6	54.4	0.44	0.78
IC-18	44.2	9.9	25.4	0.0	79.5	0.44	0.72
IC-103	44.5	6.9	26.0	2.1	79.5	0.44	0.79
IC-1	30.1	8.4	16.3	0.0	54.8	0.45	0.66
IC-7	34.4	5.8	22.1	0.7	63.0	0.45	0.79
IC-13	41.2	12.5	19.8	0.8	74.3	0.45	0.61
IC-54	101.1	20.0	61.8	0.0	182.9	0.45	0.76
IC-72	20.1	4.0	11.6	0.7	36.4	0.45	0.74
IC-77	40.9	12.7	20.4	0.6	74.6	0.45	0.62
IC-95	32.3	8.3	18.1	0.1	58.8	0.45	0.69
IC-21	42.3	11.5	24.4	0.4	78.6	0.46	0.68
IC-44	35.5	4.7	24.8	0.7	65.7	0.48	0.84
IC-48	36.6	8.0	22.8	0.2	67.6	0.46	0.74
IC-71	36.4	5.2	24.9	1.3	67.8	0.46	0.83
IC-86	41.6	7.5	25.8	1.5	76.4	0.46	0.77
IC-202	34.2	8.6	19.9	0.2	62.9	0.46	0.70
IC-108	41.6	7.5	25.8	1.5	76.4	0.46	0.77
IC-5	26.5	7.9	15.4	0.1	49.9	0.47	0.66
IC-64	42.6	11.1	24.2	2.0	79.9	0.47	0.69
IC-73	31.3	6.1	19.5	2.0	58.9	0.47	0.76

Table 3.11 Continued.

Plant no.	Glucosinolate μ moles g^{-1} seed						
	Pro	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
IC-96	36.4	10.7	20.8	1.4	69.3	0.47	0.66
IC-102	36.7	23.5	7.5	2.7	70.4	0.48	0.24
IC-17	36.1	8.7	24.5	1.0	70.3	0.49	0.74
IC-41	24.6	7.3	16.5	0.0	48.4	0.49	0.69
IC-42	29.7	7.1	21.2	0.1	58.1	0.49	0.75
IC-50	51.0	15.6	32.2	0.4	99.2	0.49	0.67
IC-91	27.2	8.7	16.6	0.5	53.0	0.49	0.66
IC-84	35.1	6.9	25.2	1.1	68.3	0.49	0.79
IC-106	36.1	8.7	24.5	1.0	70.3	0.49	0.74
IC-23	41.6	8.2	31.9	0.8	82.5	0.50	0.80
IC-84	30.0	5.6	23.3	0.9	59.8	0.50	0.81
IC-99	36.6	10.8	25.1	0.3	72.8	0.50	0.70
IC-97	35.1	9.5	26.8	0.1	71.5	0.51	0.71
IC-39	38.5	9.4	30.1	0.0	78.0	0.51	0.76
IC-26	38.7	10.6	29.0	0.3	78.6	0.51	0.73
IC-200	39.5	6.3	31.3	4.4	98.0	0.52	0.83
IC-19	46.8	10.9	39.6	0.7	0.9	0.52	0.78
IC-34	12.6	5.8	8.5	0.0	26.9	0.53	0.59
IC-61	29.3	13.0	19.9	0.7	62.9	0.53	0.60
IC-68	39.8	8.9	34.7	0.4	83.8	0.53	0.80
IC-74	53.3	13.1	45.0	1.2	112.6	0.53	0.77
IC-80	58.6	11.1	52.8	1.9	124.4	0.53	0.83
IC-201	41.8	12.8	31.7	2.5	88.8	0.53	0.71
IC-204	37.3	11.1	30.3	0.7	79.4	0.53	0.73
IC-30	43.1	11.4	37.7	0.5	92.7	0.54	0.77
IC-67	31.6	14.3	22.7	1.0	69.6	0.53	0.61
IC-203	26.4	9.1	21.7	0.9	58.1	0.55	0.70
IC-55	4.4	1.4	4.1	0.2	10.1	0.56	0.75
IC-75	32.7	9.1	32.0	1.1	74.9	0.56	0.78
IC-18	31.1	13.7	25.0	2.0	71.8	0.57	0.65
IC-33	33.6	15.0	29.7	1.0	79.3	0.58	0.66
IC-79	29.5	8.1	32.3	0.8	70.7	0.58	0.80
IC-29	25.6	4.2	31.8	1.2	62.8	0.59	0.88
IC-82	31.4	16.3	26.9	1.2	75.8	0.59	0.62
IC-29	32.4	10.5	34.9	0.7	78.5	0.59	0.77

Table 3.11 Continued.

	Glucosinolate μ moles g^{-1} seed						
Plant no.	Pro	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
IC-31	30.6	15.0	29.7	1.0	73.6	0.60	0.66
IC-46	18.7	13.9	15.3	0.6	48.5	0.61	0.52
IC-49	26.2	13.3	26.1	0.7	66.4	0.61	0.66
IC-16	26.2	8.3	33.2	1.9	69.6	0.62	0.80
IC-28	31.4	8.9	41.4	2.4	84.1	0.63	0.82
IC-70	17.9	4.6	25.4	1.1	49.0	0.63	0.85
IC-44	21.1	24.5	31.9	0.0	77.5	0.73	0.57
Mean	42.5	10.0	26.0	1.3	79.4	0.47	0.72

total alkenyl glucosinolate contents of F_2 plants ranged from 10.1 to 182.9 μ moles g^{-1} seed (Figure 3.7d). Glucosinolate precursor chain elongation varied greatly among F_2 plants as indicated by Q1 values that ranged from 0.18 to 0.73 with average of 0.47. All F_2 plants exhibited 3-butenyl glucosinolate hydroxylation except for plants no. IC-20 and IC-92 which had no hydroxylation capacity and were similar in this respect to the non hydroxylating *B. juncea* parent (Table 3.11 and Figure 3.7e). The Q2 values serve as an indicator for 3-butenyl glucosinolate hydroxylation (Figure 3.7f).

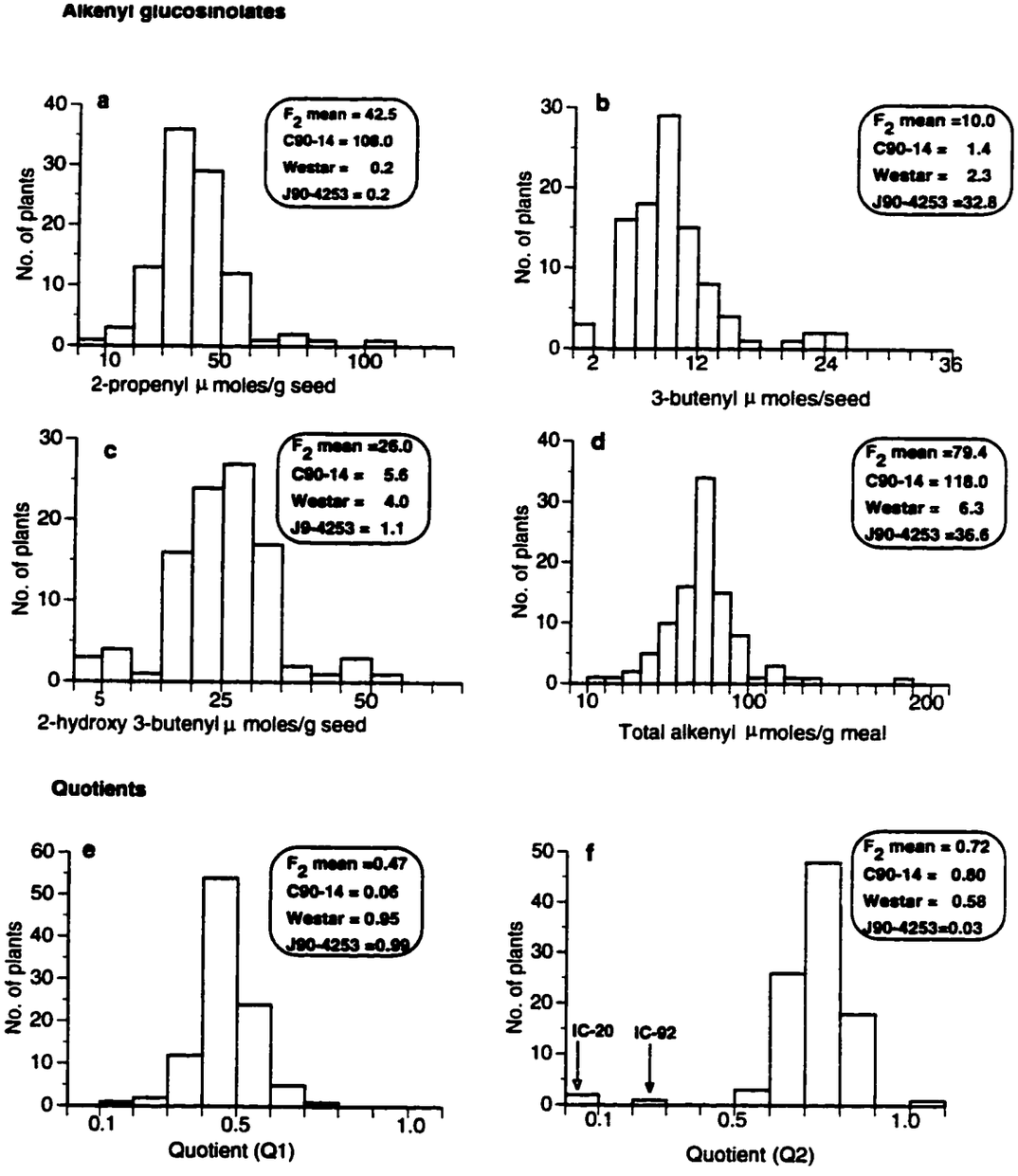

Figure 3.7 Frequency distribution of alkenyl glucosinolates contents and quotients in seed of F_2 plants from the double interspecific cross BCF_1 [(*B. carinata* C90-14 x *B. juncea*] x *B. carinata*] xBCF_1 [(*B. carinata* C90-14 x *B. napus*) x *B. carinata*] grown in the greenhouse

3.3.6.2 Seed glucosinolate composition and glucosinolate quotients of F_3 plants of the double interspecific cross BCF_1 [(*B. carinata* x *B. juncea*) x *B. carinata*] x BCF_1 [(*B. carinata* x *B. napus*) x *B. carinata*]

The 2-propenyl glucosinolate content of F_3 plants from four (IC-16, IC-41, IC-40, and IC-72) F_2 plants with low 2-propenyl glucosinolate contents were similar to those in plants. F_3 plants derived from F_2 plant IC-21 had a higher 2-propenyl glucosinolate content (Table 3.12). Also, F_3 progeny from the F_2 plant IC-55 (4.4 µ moles of 2-propenyl glucosinolate) had 2- propenyl glucosinolate levels similar to those of F_3 plants from other F_2 plants. All F_3 plants contained 3-butenyl, 2-hydroxy 3-butenyl and 4-pentenyl glucosinolate in concentrations similar to those in F_2 plants. Total alkenyl glucosinolate contents of F_3 plants ranged from 64.4 to 105.8 µ moles.

Table 3.12 Individual seed glucosinolate content of F_3 intercross plants from six F_2 families of the cross [(B. *carinata* line C90-14 x *B. juncea* line J90-4253) x *B. carinata* line C90-14] x [(*B. carinata* line C90-14 x *B. napus* cv. Westar) x *B. carinata* line C90-14] (Inter cross -IC) grown in the greenhouse.

Generation	Plant no.	Glucosinolate μ moles g^{-1} seed						
		Pro[1]	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
F_2	IC-16	26.2	8.3	33.2	1.9	69.6	0.62	0.80
F_3	IC-16-1	36.2	7.8	27.2	1.0	72.3	0.50	0.78
F_2	IC-21	42.3	11.5	24.4	1.1	78.6	0.46	0.70
	IC-21-1	46.8	8.0	38.7	2.1	97.4	0.52	0.83
	IC-21-2	53.7	9.0	40.3	2.2	105.8	0.50	0.82
	IC-21-3	50.7	8.7	29.1	0.4	100.0	0.35	0.27
	IC-21-4	46.9	8.6	38.7	1.0	95.9	0.51	0.41
	IC-21-5	42.6	6.0	41.7	2.0	92.7	0.54	0.46
	IC-21-6	35.4	4.9	25.8	1.9	68.9	0.48	0.37
	IC-21-7	51.0	8.4	30.6	0.4	90.7	0.44	0.34
F_2	IC-40	21.0	0.0	7.8	0.0	32.9	0.36	1.00
F_3	IC-40-1	55.1	6.6	24.5	0.2	86.5	0.36	0.70
	IC-40-2	67.2	5.0	20.6	0.1	93.2	0.28	0.80
	IC-40-3	53.6	7.6	19.9	0.2	81.4	0.34	0.72
	IC-40-4	50.4	4.4	18.0	0.1	73.2	0.31	0.80
	IC-40-5	64.7	7.8	16.5	0.3	89.6	0.28	0.68
	IC-40-6	49.6	7.2	16.0	0.3	73.2	0.32	0.69
	IC-40-7	56.8	6.8	11.9	0.2	75.9	0.25	0.64
	IC-40-8	53.3	8.3	14.4	0.6	76.7	0.30	0.63

[1] Pro= 2-propenyl, But= 3-butenyl, Hbut= 2-hydroxy 3-butenyl, Pen=4-pentenyl glucosinolates.

$$^{2}Q1 = \frac{\textbf{Total alkenyl minus 2-propenyl}}{\textbf{Total alkenyl}}$$

$$^{3}Q2 = \frac{\textbf{2-hydroxy 3-butenyl}}{\textbf{3-butenyl + 2-hydroxy 3-butenyl}}$$

Table 3.12 Continued.

Generation	Plant no.	Glucosinolate μ moles g^{-1} seed						
		Pro	But	Hbut	Pen	Total alk	Q1[2]	Q2[3]
F_2	IC-41	24.6	7.3	16.5	0.0	48.5	0.49	0.69
F_3	IC-41-1	34.1	7.7	27.3	0.4	69.1	0.51	0.78
	IC-41-2	40.7	2.7	21.3	0.2	64.4	0.37	0.89
	IC-41-3	52.8	3.4	22.7	0.1	79.3	0.33	0.87
F_2	IC-55	4.4	0.2	4.1	0.2	10.1	0.51	0.95
F_3	IC-55-1	45.1	7.8	26.7	0.8	80.7	0.44	0.77
	IC-55-2	44.3	5.4	31.4	1.8	83.6	0.47	0.85
	IC-55-3	37.3	8.7	30.7	1.7	78.8	0.52	0.78
F_2	IC-72	20.1	4.0	11.6	0.7	36.5	0.45	0.74
F_3	IC-72-1	41.3	7.3	26.1	1.0	76.1	0.45	0.78
	IC-72-3	66.3	5.5	26.2	2.8	100.1	0.34	0.83
	IC-72-3	35.7	9.3	22.4	1.4	69.9	0.48	0.71
	IC-72-4	39.0	7.3	26.4	1.8	74.5	0.48	0.78

3.4 DISCUSSION: DEVELOPMENT OF LOW GLUCOSINOLATE *B. CARINATA* LINES

The development of low glucosinolate *B. carinata*, in addition to zero erucic acid, is a prerequisite for the development of canola quality forms in this species. The level of glucosinolate in *B. carinata* seed must be reduced to very low levels in order for the meal to be used as a high protein feed without detrimental effects to monogastric animals. The development of low glucosinolate *B. carinata* requires the removal of the predominant 2-propenyl glucosinolate, which accounts for 98% of its total alkenyl glucosinolate content (Getinet *et al.* 1996). Without exception the *Brassica* species that contain B genome chromosomes all accumulate 2-propenyl glucosinolates in their seed, including *B. nigra* (BB), *B. juncea* (AABB) and *B. carinata* (BBCC) (Gland *et al.* 1981). Almost all genotypes of these three species that have been analysed contained only 2-propenyl glucosinolate. Therefore, 2-propenyl glucosinolate synthesis is strongly linked to genetic factors residing on B genome chromosomes of these species. However, *B. juncea* genotypes have been identified that also accumulate 3-butenyl glucosinolate in addition to 2-propenyl glucosinolate (Vaughan *et al.* 1963, Love *et al.* 1990b). Selections within genotypes containing both 2-propenyl and 3-butenyl glucosinolates, resulted in the identification of lines containing 3-butenyl glucosinolate only.

3.4.1 The working hypothesis

The objective of this study was the complete removal of 2-propenyl glucosinolate from *B. carinata* seed. An interspecific crossing and selection scheme was designed that should allow the development of low glucosinolate *B. carinata*.

The crossing scheme is based on the genome interrelationships of *B. carinata* (BBCC) with the two amphidiploid species *B. napus* (AACC) and *B. juncea* (AABB). Since it was expected that 2-propenyl glucosinolate was synthesized by genes of B genome chromosomes of *B. carinata*, the *B. juncea* genotype chosen for the interspecific cross was a 3-butenyl (non2-propenyl) glucosinolate form. This choice of B genome donor should have allowed the transfer of genes to the B genome of *B. carinata* which did not synthesise 2-propenyl glucosinolate. Since genes for 2-propenyl glucosinolate synthesis could also be present in C genome chromosomes of *B. carinata*, an interspecific cross with *B. napus* was was made with the objective of introducing genes from the C genome of *B. napus* into C genome chromosomes of *B. carinata* which would indirect glucosinolate syntheis from 2-propenyl to 3-butenyl glucosinolate. The intercrossing of the plants from the two interspecific crosses should combine the B and C genome sources for low 2-propenyl glucosinolate in one genome thus creating *B. carinata* germplasm free of 2-propenyl glucosinolate.

The *B. napus* and *B. juncea* genotypes used for interspecific crosses contained the Bronowski genetic block which limits the accumulation of alkenyl glucosinolates to a very low concentration. The introduction of this genetic block into *B. carinata* serves to limit the synthesis of 3-butenyl, 2-hydroxy 3-butenyl, 4-pentenyl and 2-hydroxy 4-pentenyl

glucosinolate; thus ensuring that the *B. carinata* germplasm to be developed is not only low in 2-propenyl glucosinlate content, but has also low concentrations of the other alkenyl glucosinolates.

3.4.2 Glucosinolate content of parents and interspecific F_1 generation

Glucosinolate analysis of seed of the *B. carinata* parent C90-14 indicated that 96% of its total alkenyl glucosinolate content was 2-propenyl glucosinolate (Table 3.2). The *B. napus* parent cv. Westar and *B. juncea* parent J90-4253 were non-2-propenyl glucosinolate types that contained a low concentration of 3-butenyl, 2-hydroxy 3-butenyl, 4-pentenyl and 2-hydroxy 4-pententyl glucosinolates and were thus suitable as C and B genome donors respectively, for non2-propenyl glucosinolate synthesis.

Interspecific F_1 seed from the interspecific crosses between *B. carinata* x *B. napus* and *B. carinata* x *B. juncea* and backcross seed from backcrosses of *B. carinata* was obtained with ease and without embryo rescue or ovary culture. However, it was evident that the moderate temperature in the growth cabinet and vigorous plant growth improved hybrid seed set (Ayotte *et al.* 1985, 1986, Rashid *et al.* 1994). As expected seed set in backcrosses to *B. carinata* was better than seed set in interspecific crosses.

Since F_1 plants of both interspecific crosses were highly sterile, seed could not be produced in sufficient quantities for glucosinolate analysis and the plants had to be backcrossed to *B. carinata* to increase fertility. The hybrid nature of F_1 plants was confirmed on the basis of plant morphological characteristics and by leaf glucosinolate analysis. Leaf tissue of F_1 plants of both interspecific crosses had much lower 2-propenyl

glucosinolate contents than leaf tissue of the *B. carinata* parent. Leaf tissue of interspecific plants also contained 3-butenyl, 2-hydroxy 3-butenyl, 4-pentenyl and 2-hydroxy 4-pentenyl glucosinolates which were not present in *B. carinata* leaf tissue thus clearly characterizing the plants as interspecific hybrids. The glucosinolate content and composition of leaf tissue has been shown to be an indicator of seed glucosinolate content and composition in *B. juncea*. McGregor and Love (1987) demonstrated a relationship between leaf and seed glucosinolates in *B. juncea*, and Magrath *et al.* (1993, 1994) and Parkins *et al.*(1994) made similar observations in glucosinolate composition in *B. napus*. It was therefore logical to assume a similar relationship in *B. carinata*.

3.4.3. Glucosinolate content of seed of F_2 and F_3 generations of the backcross [(*B. carinata* x *B. napus*) x *B. carinata*] cross

Seed of backcross F_2 plants of the interspecific backcross [(*B. carinata* BBCC) x *B. napus* AACC) x *B. carinata* BBCC)] contained primarily 2-propenyl glucosinolate and levels ranged from 75 to 190 μ moles g^{-1} meal of individual plants (Figure 3.6a). This cross was designed to introgress genes for non2-propenyl glucosinolate synthesis from the C genome of *B. napus* (AACC) into the C genome of *B. carinata* (BBCC). The high level of 2-propenyl in F_2 seed of backcross plants indicated that no reduction in 2-propenyl glucosinolate accumulation capacity was achieved by C genome introgression and that almost the total amount of 2-propenyl glucosinolate found in F_2 seed was contributed by genes from the B genome of *B. carinata*. Backcross F_2 plants contained 3-butenyl glucosinolate which was only present in trace amounts in the *B. carinata* parent indicating

the transfer of genes for 3-butenyl glucosinolate synthesis from C genome chromosomes of *B. napus* into C genome chromosomes of *B. carinata*. The frequency distribution of 3-butenyl glucosinolate contents in BCF_2 plants was clearly bimodal indicating that genes carrying different 3-butenyl glucosinolate synthesis capacities must have been transferred from *B. napus* cv. Westar (Figure 3.6b). There were gene(s) that contributed less than 6 μ moles per g^{-1} meal of 3-butenyl glucosinolate, and another or additional gene(s) involved in 3-butenyl glucosinolate synthesis that contributed from 6 to 20 μ moles of 3-butenyl glucosinolate.

Plants that contained higher levels of 3-butenyl glucosinolate contained less 2-propenyl glucosinolate and vise versa (Table 3.6). This indicated that with the incorporation of genes for 3-butenyl glucosinolate into *B. carinata*, two glucosinolate biosynthetic pathways were active in these plants and were competing for the same precursor. The reduction in 2-propenyl glucosinolate in plants with increased 3-butenyl glucosinolate synthesis did not indicate a genetic reduction of 2-propenyl glucosinolate but rather were indicative of a compensatory effect. The frequency distribution for Q1 quotients among BCF_2 plants, indicative of their glucosinolate precursor chain elongation capacities, was separated into four classes indicating discrete genetic factors for chain elongation capacity that were introgressed from *B. napus*.

Backcross F_2 plants did not synthesize 4-pentenyl glucosinolate because it would have been expected that genes for 4-pentenyl glucosinolate synthesis could have been introgressed from *B. napus*. It is postulated that the reason for this must be that C4 → C5 glucosinolate precursor chain elongation were located on A genome chromosomes of *B. napus* which were

not introgressed into *B. carinata*. This explanation is based on the observation that *B. rapa* (AA) has a much stronger C4 → C5 chain elongation system than *B. napus* (Gland *et al.* 1981) and genes for 4-pentenyl glucosinolate synthesis might therefore be primarily located on A genome chromosomes of *B. napus*.

The *B. carinata* and *B. napus* parents both expressed very strong 3-butenyl glucosinolate hydroxylation capacities (Figure 3.6f). High concentrations of 3-butenyl glucosinolate in some backcross plants resulted in increased 2-hydroxy 3-butenyl glucosinolate levels simply because of the availability of a greater pool of precursor molecules for their synthesis. However, the frequency distribution of 2-hydroxy 3-butenyl glucosinolate in backcross plants was not continuous and there was an indication for two maxima, one at less than 5 μ moles and the other at approximately 20 μ moles (Figure 3.6c). This then could indicate segregation for 3-butenyl glucosinolate hydroxylation which is also evident from the varying levels of 3-butenyl and 2-hydroxy 3-butenyl glucosinolate contents in individual backcross plants where concentration for these two glucosinolates did not vary in concert. The explanation for this observation is that the C genome chromosome of *B. carinata* and *B. napus*, which carry different genes for 3-butenyl glucosinolate hydroxylation, were recombined in backcross plants resulting in different hydroxylation capacities.

The BCF_2 plant no. 139 contained 2-propenyl and 3-butenyl glucosinolates and no 2-hydroxy 3-butenyl glucosinolate. This plant must be the result of cross over events between of C genome chromosomes of *B. carinata* and *B. napus* that resulted in the removal of glucosinolate hydroxylation from chromosomes of both C genomes.

3.4.4 Glucosinolate content of seed of F_2 and F_3 generations of the backcross [(*B. carinata* x *B. juncea*) x *B. carinata*] cross

There were only two BCF_2 plants from the [(*B. carinata* x *B. juncea*) x *B. carinata*] cross and the seed contained primarily 2-hydroxy 3-butenyl and 2-propenyl glucosinolates (Table 3.9). This cross was intended to introgress genes for C3 → C4 glucosinolate precursor elongation thus converting glucosinolate synthesis from 2-propenyl to 3-butenyl glucosinolate. This was achieved to a certain extent in that the two backcross plants synthesized 12.6 and 15.7 μ moles of 3-butenyl glucosinolate, respectively, which resulted in an indirect reduction of 2-propenyl glucosinolate to 82.3 and 37.5 μ moles, respectively (Table 3.9). A complete redirection of glucosinolate synthesis from 2-propenyl to 3-butenyl was not achieved. This could be due to the fact that only two backcross plants were obtained and analysed, and that the analysis of additional plants might have provided more genetic variation. Four F_3 plants of BCF_2 plant no. 2 (Table 3.10) were analysed for glucosinolate content and it was found that this plant possessed the highest level of C3 → C4 glucosinolate precursor chain elongation capacity of the four BCF_3 progenies analysed (Table 3.10). These plants contained only from 10 to 15 μ moles of 2-propenyl glucosinolate while BCF_3 plants of BCF_2 plant no. 3 were less efficient in 3-butenyl glucosinolate synthesis indicating genetic differences between the two plants in their ability to synthesize and accumulate different quantities of 2-propenyl and 3- butenyl glucosinolates. To completely remove 2-propenyl glucosinolate from *B. carinata*, selections in progenies of BCF_3 family no. 2 should be carried out which might yield the desired non2-propenyl glucosinolate phenotype.

Another possibility to remove 2-propenyl glucosinolate completely from *B. carinata* would be a second cross with non-2-propenyl glucosinolate *B. juncea* to introduce additional genetic factors for C3 → C4 precursor chain elongation to shift glucosinolate synthesis away from 2-propenyl glucosinolate and into 3-butenyl glucosinolate. Once a complete removal of 2-propenyl glucosinolate from *B. carinata* has been achieved, the remaining levels of other alkenyl glucosinolates in *B. carinata* could be reduced by introgression of genes for low alkenyl glucosinolate content using the Bronowski block.

The backcross plants of the *B. juncea* cross had high 2-hydroxy 3-butenyl glucosinolate contents. This can be explained by the fact that *B. carinata* C genome chromosomes contain genes for 3-butenyl hydroxylation (Getinet *et al.* 1995) which are now fully expressed because of the availability of sufficient quantities of 3-butenyl glucosinolate precursors. The degree of 3-butenyl glucosinolate hydroxylation in BCF_3 plants varied greatly (Table 3.10), indicating genetic variability in 3-butenyl glucosinolate hydroxylation among different BCF_3 plants. The genetic variation must already have been present in the *B. carinata* parent but could not be observed because of low concentrations of 3-butenyl glucosinolate precursor in *B. carinata*. Since the *B. juncea* parent was a non-hydroxylating type it could not have contributed to genetic variation in 3-butenyl glucosinolate hydroxylation and *B. carinata* is therefore the sole source for this variation.

3.4.5 Double interspecific cross

The objective of the double interspecific cross was to combine genetic factors for C3 → C4 glucosinolate precursor chain elongation from the B genome of *B. juncea* and C

genome of *B. napus*. This hypothesis was based on the assumption that genes for C3 → C4 glucosinolate chain elongation were present on chromosomes of both genomes. Results of the *B. juncea* interspecific backcross to *B. carinata* indicated that B genome chromosomes of *B. juncea* carried genetic factors for C3 → C4 glucosinolate chain elongation which resulted in an indirect reduction of 2-propenyl glucosinolate content in *B. carinata*. On the other hand, the *B. napus* interspecific backcross to *B. carinata* indicated that C genome chromosomes of *B. napus* did not carry any genetic factors involved in C3 → C4 glucosinolate chain elongation. The *B. napus* derived plants can therefore be considered being normal 2-propenyl glucosinolate *B. carinata* plants. The cross between *B. juncea* derived *B. carinata* and *B. napus* derived *B. carinata* referred to as the double interspecific cross was in reality a second backcross of the *B. juncea* derived *B. carinata* with normal 2-propenyl glucosinolate *B. carinata* in relation to C3 → C4 glucosinolate precursor chain elongation. This resulted in an increase in 2-propenyl glucosinolate content rather than a decrease (Table 3.12). This is also the explanation why the " double interspecific" crossing approach did not yield the anticipated zero 2-propenyl glucosinolate *B. carinata*.

The genetic variation observed in individual F_2 and F_3 plants of the double interspecific cross was the result of introgression of genes for C3 → C4 glucosinolate precursor chain elongation from the C genome of *B. napus* into *B. carinata*. Also, strong 3-butenyl glucosinolate hydroxylation was introgressed from *B. napus* into these plants.

It was concluded that the development zero 2-propenyl glucosinolate *B. carinata* can be achieved through further selection in segregating backcross generations of *B. juncea* derived *B. carinata* populations.

4 .0 SUMMARY AND CONCLUSIONS

Brassica carinata is a high yielding well adapted, disease and pest tolerant oilseed crop in the Ethiopian highlands. Very little work has been done in this species, and there is a broad range of genetically diverse germplsam available from recent collections that could be utilized in the breeding of improved *B. carinata* cultivars. There are only two registered cultivars available for production in Ethiopia, these are Dodolla (yellow seeded) and S-67 (brown seeded). *B. carinata* seed oil contains approximately 40% erucic acid and the meal remaining after oil extraction is high in 2-propenyl glucosinolate content. The development of canola quality *B. carinata* forms is an important research objective of the National Oilseeds Research program in Ethiopia. Zero erucic acid *B. carinata* has recently been developed, however, no low glucosinolate germplasm is available that could be used for the development of canola quality cultivars.

This study had the objective to investigate the inheritance of erucic acid in *B. carinata* and to develop low glucosinolate forms.

4.1 Inheritance of erucic acid.

The erucic acid content in two *B. carinata* cultivars Dodolla and S-67 is controlled by two alleles at each of two loci which act in an additive manner with each allele contributing about 10% erucic acid to the total content of 40% erucic acid. The erucic acid content is

under the genetic control of the developing embryo and not the female sporophyte. The erucic acid alleles have equal strength and are similar to those in *B. napus*. The development of zero erucic acid *B. carinata* cultivars can easily be accomplished by crossing and backcrossing of the zero erucic acid trait into well adapted, high yielding *B. carinata* cultivars and breeding populations. The use of the half-seed technique in combination with backcrossing and selection of heterozygotes in every backcross generation will significantly speed up the development of zero erucic acid *B. carinata* cultivars.

4.2 Development of low glucosinolate *B. carinata*

B. carinata seed meal contains primarily 2-propenyl glucosinolate which accounts for 98% of its oil total alkenyl glucosinolate content. The 2-propenyl glucosinolate is also the dominating glucosinolate in *B. nigra* and *B. juncea*, the other two species which have the B genome in common with *B. carinata*. No low 2-propenyl glucosinolate mutants have been identified in *B. carinata*. The objective of this study was to introgress B and C genome chromosomes from *B. juncea* and *B. napus* respectively, that do not synthesise 2-propenyl glucosinolate, into B and C genomes of *B. carinata*, and thus develop non2-propenyl glucosinolate *B. carinata*.

Interspecific crosses were made between *B. carinata* x *B. juncea* and *B. carinata* x *B. napus* to accomplish this objective. Backcross F_2 and F_3 segregation data of the *B. juncea* backcross indicated that B genome chromosomes of *B. juncea* that lack 2-propenyl glucosinolate synthesis were successfully introgressed into the *B. carinata* B genome resulting in a reduction of 2-propenyl glucosinolate content in *B. carinata* from 150 μ moles

to approximately 15 μ moles. Further selections in F_4 and F_5 backcross generations might yield the desired non2-propenyl glucosinolate *B. carinata.*

The [(*B. carinata* x *B. napus*) x *B. carinata*] backcross did not yield plants with reduced 2-propenyl glucosinolate contents. It is therefore concluded that genes for 2-propenyl glucosinolate synthesis in *B.carinata* are located on B genome chromosomes and not on chromosomes of its C genome.

When non2-propenyl glucosinolate *B. carinata* is developed this trait can then be incorporated into elite germplasm by backcrossing, and be combined with the zero erucic acid trait, thus developing canola quality *B. carinata* which will have a major impact on oilseed production in Ethiopia.

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