The Application of Doubled Haploid Plants to Population Improvement in *Brassica rapa*

A Thesis Submitted to the

Faculty of Graduate Studies by

Holly A. Friesen

In Partial Fulfilment of the Requirements

for the Degree of Masters of Science

Department of Plant Science

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THE APPLICATION OF DOUBLED HAPLOID PLANTS TO POPULATION IMPROVEMENT IN *Brassica rapa*

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ABSTRACT

Doubled haploid line (DH) development is a breeding tool that has been shown to speed up the progress of plant breeding by producing homozygous individuals in a single generation. In the sporophytically self-incompatible *Brassica rapa*, the production of DH plants results in the expression of inbreeding depression and therefore diminishes their benefit.

This study investigated the improvements in the efficiency of DH applied to population improvement in *B. rapa*. Methods were investigated to overcome self-incompatibility in *B. rapa*, to improve agronomic performance of DH lines through the production of composite populations and to identify genetic variation between DH lines.

Field studies on *B. rapa* cv. Reward determined that 3 and 5 % NaCl solutions as well as bud pollination were effective at overcoming self-incompatibility. The NaCl solutions were found to be more efficient than bud pollination in overcoming self-incompatibility.

Composite populations were produced by random interpollination of 4, 8, 12 and 22 DH lines, developed from the *B. rapa* cv. Reward and the *B. rapa* breeding line DSC-3, for two generations. Several agronomic parameters were measured at one and two field locations for the DH lines and the composite populations, respectively. Intercrossing as few as 4 DH lines from the breeding line DSC-3 and 8 DH lines from the cv Reward improved population performance over that of the contributing DH lines. The development of composite populations showed that DH lines have potential for

application in *B. rapa* cultivar development.

Random amplified polymorphic DNA (RAPD) analysis successfully characterized genetic variation in DH lines produced from the cv. Reward and the breeding line DSC-3. RAPD analysis was performed on the DH lines and two generations of composite populations. RAPD analysis detected 40 to 83% polymorphic bands in the DH populations using five oligonucleotide primers and 17-53% and 27-47% polymorphic bands in the first composite and second composite populations, respectively.

NaCl spray solutions provide an efficient method of promoting self-pollination when increasing *B. rapa* DH. The application of DH lines to population improvement in *B. rapa* showed significant promise through the development of composite populations. RAPD analysis showed potential as a tool for characterizing the level of genetic variability among DH lines and determining optimal composite population size.

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1. INTRODUCTION

Brassica rapa cultivars represent approximately half of Canadian canola production (Askew, 1995). Biotechnology for the genetic manipulation of this species is not as well developed as that for *B. napus*. One method that has been applied to increase selection efficiency in breeding for improved varieties is doubled haploid (DH) line production. The development of DH in canola involves the production of haploid embryos through microspore culture followed by regeneration of haploid plants from these embryos in tissue culture. These haploid plants can spontaneously double or be treated with an antimicrotubule agent to become DH. This technique has been developed for *B. napus* and is currently being modified for use in *B. rapa*.

The usefulness of DH in breeding has been demonstrated in several self-pollinating crops including *B. napus* and wheat (Park et al., 1976; Winzeler et al., 1987; Bansal et. al., 1994). The application of DH development to the process of selection has the advantage of achieving homozygosity in a single generation in comparison to many generations of inbreeding using more traditional breeding techniques (Morrison and Evans, 1988). Undesirable alleles can be selected against in the first DH generation due to the absence of dominance interactions.

B. rapa reproduces via cross-pollination due to sporophytic self-incompatibility (SI). Traditional methods used to improve *B. rapa* populations have involved outcrossing between selected individual plants, such as mass selection, recurrent selection, development of synthetics and hybrid breeding. The resulting population is genetically variable and heterogeneous and can demonstrate improved agronomic

performance. Production of DH plants of *B. rapa* can lead to inbreeding depression, resulting in DH plants with poor agronomic performance, expressed in reduced seed and dry matter production. In order to exploit the benefits of DH development in population improvement, the performance of the original heterozygous donor *B. rapa* population must be restored.

Phenotype and genotype-based assays have been used to distinguish between cultivars (Demeke et al., 1996). Analysis of genomic DNA provides a method of characterizing genotypic variation that is not influenced by the environment and does not require the development of plants to maturity. Current DNA analysis procedures include restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) based markers. RFLP detects variation between genotypes using restriction endonucleases to fragment DNA along with blot hybridization to visualize the polymorphism. This procedure requires specialized equipment and is labour-intensive and time-consuming. A PCR based assay requires less DNA, equipment, labour and time to perform. However, PCR is limited in application due to the requirement for DNA sequence information.

Random amplified polymorphic DNA (RAPD) is a type of marker obtained from a PCR reaction that does not require sequence information. RAPD markers detect polymorphism using the occurrence of randomly amplified DNA sequences detected on an agarose gel (Williams et al., 1990). It has advantages over other methods in that no sequence information is required.

The objective of this project was to characterize DH lines using RAPD markers

and to determine the most efficient application of DH development to population improvement of *B. rapa* through the development of composite populations.

2. LITERATURE REVIEW

History of Canola

Production of *Brassica rapa* was recorded in India as long ago as 2000 BC. *B. rapa* had the widest distribution of all of the oilseed *Brassicas* which has led to discrepancy surrounding its centre of origin which has been reported in Asia, Europe and the Mediterranean (McNaughton, 1976). Botanical, linguistic and historical research have identified Europe as a centre of diversity for *B. rapa* (Reiner et al., 1995). An interspecific cross between the two diploid species, *B. rapa* and *B. oleracea,* is thought to have occurred in the region of southern Europe where they coexisted to produce the amphidiploid *B. napus* (Downey and Röbbelen, 1989). *B. rapa* and *B. napus* are the main *Brassica* oilseeds grown in Canada. The relationship of these species with other *Brassicas* is illustrated in U's triangle in Figure 2.1 (U, 1935).

Oilseed rape was introduced to Canada as *B. rapa* from Poland in 1936 (Bell, 1982). *B. napus* had been grown in Canada earlier as a forage crop and was introduced from Argentina. During World War II rapeseed oil was used as a marine engine lubricant after which its demand dropped. The desire to have a domestic supply of an oil suitable for human consumption and a meal for animal feed in Canada led to increased interest in oilseed rape production. Research led to the development of varieties with reduced levels of erucic acid which was found to be poorly digested and reduce growth in experimental animals (Sauer and Kramer, 1983). The first low-erucic

acid *B. napus* variety was Oro, registered in 1968, and the first low erucic acid *B. rapa* variety was Polar, registered in 1969. The next improvement was a reduction in glucosinolates. Glucosinolates hydrolyse to sulfur-containing compounds such as isothiocyanates which have adverse effects on growth in poultry and nonruminant animals (Sauer and Kramer, 1983). The first low erucic acid, low glucosinolate *B. napus* variety, Tower, was released in 1974 and the first 'double low' *B. rapa* variety, Candle, was released in 1977. These 'double low' oil and meal product varieties, with less than 5 % erucic acid and 3 mg/g glucosinolates, were given the designation 'canola' in 1978 (Eskin et al., 1996).

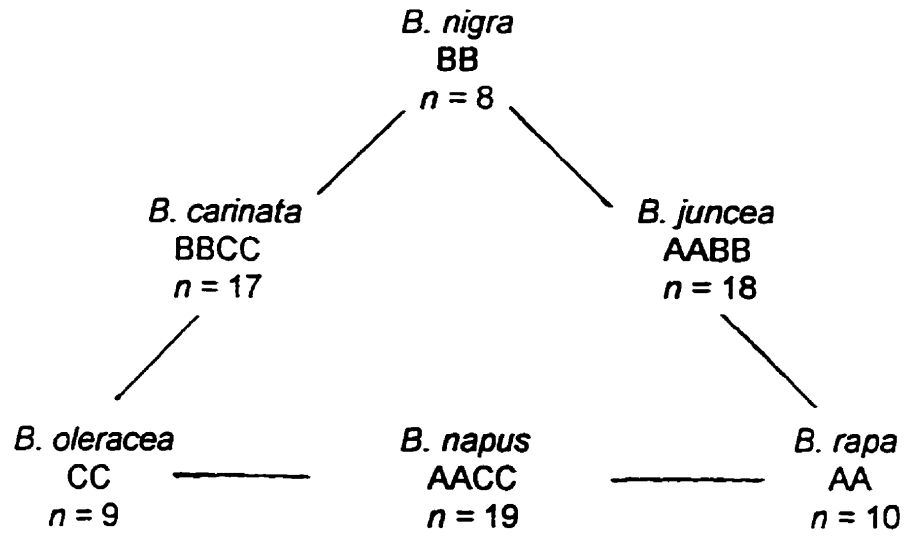

Figure 2.1. The genomic relationship of *Brassica* species based on U's triangle (Source: U, 1935). [A, B and C = genome; *n* = chromosome number]

Economic Importance and Distribution

Rapeseed oil is a major source for oils and fats, following soybean and palm oil in worldwide production (Askew, 1995). China, Europe, India and Canada are the major producers of canola/rapeseed (Kimber and McGregor, 1995). Canada produces the least of these countries but has shown the most dramatic increase in production by leaping from 0.4 % of world production in the early 1950's to 20 % in1994 (Eskin et al., 1996). Canada had the largest average annual export market from 1988-1992 for seed, followed by the European Union (Kimber and McGregor, 1995).

In 1993, *B. rapa* led canola production in western Canada over *B. napus* (Askew, 1995). *B. rapa* acreage is highest in Alberta, equivalent to *B. napus* acreage in Saskatchewan and is lower than *B. napus* in Manitoba (Eskin et al., 1996). *Brassica* crops are suitable for growth in western Canada due to their ability to germinate and grow at low temperatures. Kondra et al. (1983) found seed of *B. rapa* to be more sensitive to low soil temperatures during germination than *B. napus.* However, *B. rapa* is suited to production in the northern portion of the canola growing area because of its early maturity, which reduces the importance of early seeding that is critical for the later maturing *B. napus.* This makes *B. rapa* a desirable crop for production in northern regions. There are spring and winter forms of both species but the spring forms predominate in Canada due to the lack of a sufficiently winter hardy cultivar.

Reproductive Biology of *B. rapa*

Brassica plants flower indeterminately beginning with the lowest bud on the main raceme. *B. rapa* has a more compact bud arrangement than *B. napus* and unopened buds are sometimes found below opened flowers (Downey et al., 1980). Three to five flowers open per day on the main raceme. *Brassica* flowers have four petals, two pairs of stamens with long filaments and one pair with shorter filaments. Four nectaries occur at the base of the stamens and ovary. The stigma is receptive for pollination three days prior and three days after flowers open (Downey and Röbbelen, 1989). The flowering period of *B. rapa* is approximately 3 - 4 weeks.

B. napus is mainly self-pollinating with an approximate outcrossing rate of 20% in western Canada (Rakow and Woods, 1987). The diploid *B. rapa* relies on outcrossing for fertilization to occur, and has a self-incompatibility system that is controlled sporphytically by a multi-allelic S-locus. The number of S-alleles in *B. rapa* has been estimated to be approximately 100 (Nou et al, 1993). A population must have variability for these alleles in order for interpollination to occur. The main agents for pollination are wind and insects gathering nectar.

The S-locus in the pistil is active mainly in the papillar cells at the stigmatal surface and in the anthers sporophytically in the tapetal cells and gametophytically in microspores (Goring and Rothstein, 1992). Pollen tube growth is inhibited at the stigmatal surface when self-pollination occurs (Kandasamy et al., 1989). There are two

genes involved in recognizing and enforcing this incompatibility reaction, the S-locus glycoprotein (SLG) and S-locus receptor kinase (SRK). These genes share a high percentage of amino acid sequence homology, suggesting that there was selection pressure for both genes through evolution of this self-incompatibility system (Nasrallah and Nasrallah, 1993). The SLG accumulates mainly in the papillar cell walls where the reaction is thought to be stimulated when self-pollination occurs. The SLG is then modified and becomes competent to bind SRK (Nasrallah and Nasrallah, 1993). The SRK becomes activated when self-pollen contacts the papillar cells at the stigmatal surface. At the S-locus, S-alleles function as a dominance series determined by the pollen parent which makes it important to have the correct combination of alleles in the stigma and pollen grain for inhibition of self-pollination (Thompson and Taylor, 1966).

Variants have been found that result in self-compatibility expression. Mutations in S-alleles have been found in *B. oleracea* and *B. rapa* that reduce the amount of SLG in the stigma (Nasrallah, 1974; Nasrallah et al., 1992) or impair SRK transcription, eliminating the SI response (Nasrallah and Nasrallah, 1993). These mutated S-alleles are sometimes referred to as self-fertile (S_f) alleles.

The stigma is unable to developmentally recognize self- versus cross-pollen until one day prior to anthesis or flower opening. The incompatibility reaction can be overcome in *Brassica* through bud pollination which allows pollination before the stigma is biologically able to respond to self-pollen (Sun, 1938). This procedure is time-consuming and labour intensive. A less labour intensive method involves increasing levels of CO_2 which has been shown to inhibit the SI rejection response by inhibiting

the protein signal (O'Neill et al., 1984). NaCl spray treatments are also effective at overcoming SI with the breakdown of proteins that accumulate at the stigmatal surface (Fu et al., 1992).

Breeding Procedures

Traditional Breeding

Traditional breeding procedures in *B. rapa* have involved promoting outcrossing between desirable individuals to create an improved population through mass selection, recurrent selection, synthetic development and hybrid production. Mass selection involves genetic advancement through selection of desirable individuals and harvesting and bulking their seed to grow as the next generation (Poehlman and Sleper, 1995). Mass selection is not beneficial for traits with low heritability that can not be advanced quickly by visual selection. It is also difficult to control the pollen source in this procedure and therefore to predict how gene combinations will affect the next generation.

Recurrent selection involves repeated selections of desirable individuals based on progeny performance (Poehlman and Sleper, 1995). Selection and interpollination is repeated for several generations until a population is produced with improved gene combinations being expressed. Progeny tests allow greater control over the genetic constitution of the population in the next generation in comparison to mass selection.

There is potential for loss of desirable characteristics if not included in the initial selection criteria. Mass and pedigree selection are only useful in improving *B. rapa* if there is enough diversity within the selected populations to avoid inbreeding depression. Both of these procedures require many cycles of selection to obtain a stable population with desirable characteristics.

Synthetic varieties are developed by crossing parents selected based on their performance and general combining ability as determined by the performance of progeny from different combinations (Becker, 1988). The synthetic is established by mixing seed and allowing interpollination for a number of generations. Parents can be clones, inbred lines or small populations and must be maintained in isolation so that the variety can be reconstituted periodically. The number of parents selected to create the synthetic must be large enough to minimize inbreeding depression and small enough to incorporate only individuals that will maintain the mean performance of the population (Becker, 1988). The optimum number of generations of random interpollination will result in the maximum heterozygosity and potential for heterosis as shown in *B. napus* (Schuster, 1982).

Synthetic varieties have been developed in *B. rapa* to exploit heterosis for yield using two or more cultivars or inbred lines mixed in equal proportions and grown in isolation (Buzza, 1995). Development of synthetics is not currently used in *B. napus* breeding because it is difficult to predict the proportion of outcrossing between the parental lines based on the genetic variation and environmentally induced variation in outcrossing rates in *B. napus* (Rakow and Woods, 1987). Synthetics in *B. rapa* have

been more predictable in performance due to obligate outcrossing under SI. However, it is difficult and time consuming to overcome SI in the maintenance of the original parents so that the synthetic variety can be reconstituted.

Heterosis has also been exploited through hybrid production in *B. napus* and *B. rapa* (Brandle and McVetty, 1990; Schuler et al., 1992; Falk et al., 1994). Hybrids are produced by first developing highly homozygous parental lines and then intercrossing them. This first filial (F_1) generation is the hybrid and should result in the maximum expression of heterozygosity over any further generation produced by intercrossing of this population. A major obstacle in hybrid production is developing a method of reliable pollination control to minimize the amount of self-pollination occurring with each parent while maximizing cross-pollination between them.

Cytoplasmic male sterility (CMS) has been used successfully to promote cross-pollination in *B. napus*. This system involves the development of a CMS A-line (rr), a B-line with an identical nuclear genotype to the A-line (rr) and fertile cytoplasm (F) and a fertile R-line with fertility restorer gene(s) (RR) and sterile (S) or fertile (F) cytoplasm. The genotypes of these lines are demonstrated in Figure 2.2. The A-line is crossed as the female to the R-line pollinator to produce the fertile F_1 hybrid which is harvested as seed from the A-line plants in the hybrid production fields. Ideally this cross results in restoration of fertility and the expression of heterosis in the F_1 hybrid. The B-line is used to maintain the CMS A-line which carries the maternally inherited S cytoplasm. This system has been developed in *B. napus* using several cytoplasms including *nap*, *polima* and *ogura*, all of which require improvements either in their genetic stability or

their stability under different environments (Downey and Röbbelen, 1989). The CMS system is difficult to maintain in SI *B. rapa* where inbreeding depression is expressed.

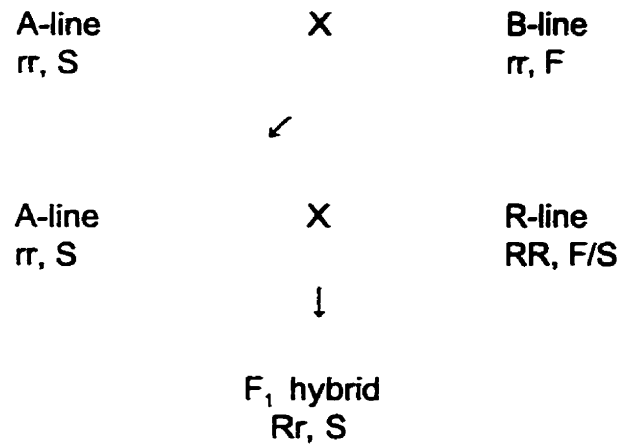

Figure 2.2. Production of F_1 hybrid in *Brassica*. S, sterile cytoplasm; F, fertile cytoplasm; R, dominant nuclear restorer gene; r, recessive nuclear restorer gene (Modified from: Buzza, 1995).

SI is a pollination control system which is naturally occurring in *B. rapa*. F_1 hybrid seed can be harvested from all plants in the hybrid production field under this system of pollination control. It is difficult and time-consuming to produce the inbred lines or even DH lines homozygous for an S allele to allow stable SI expression. While producing inbred *B. rapa* parents, inbreeding depression may result and progeny will express poor performance. A 4-way cross has been suggested by Kott (1995) to

overcome reduced vigour in the hybrid but this approach requires more time for crossing and identification of compatible genotypes . This problem may be minimized by the identification of S alleles in *B. rapa* using restriction fragment markers of SLG PCR products. Nishio et al. (1996) identified DNA band patterns that could be used as markers for S alleles.

DH Production

A critical step in any breeding program is the production of true breeding lines which are highly homozygous. Using traditional breeding procedures to establish complete homozygosity is a time consuming process that is confounded by the processes of segregation, recombination and independent assortment which create genetic diversity. Production of doubled haploid plants can be used to develop true breeding lines with complete homozygosity in months versus years with traditional breeding (Morrison and Evans, 1988). The absence of dominance interactions allows the expression of recessive alleles in the homozygous DH population, allowing accurate selection during phenotypic analysis of a population.

In a breeding program, DH plants can be produced from the F_1 or offspring from an initial cross between desirable parents. Complete homozygosity is produced in a single step. The DH plants represent a sample of the whole gametic array with distinct genetic contributions from the parents. Undesirable lines can be eliminated early in the selection process (Griffing, 1975; Powell et al., 1986). Superior DH lines can be

selected and selfed to create the next generation, with further selection for superior lines by testing phenotypic variation and environmental effects (Powell et al., 1990). Commercialization of an improved variety may be achieved in 5 years, as shown by the development of the *B. napus* cultivar Quantum from a DH-line (Stringham et al., 1995), compared to up to 9 years by traditional means (Morrison and Evans, 1988).

DH technology has been shown, in some self-pollinating crops such as barley (Bansal et al., 1994) and *B. napus* (Scarth et al., 1991), to improve selection efficiency compared to traditional breeding methods by providing a representative sample of variation available from a cross between parents. DH wheat lines have shown greater resistance to diseases such as powdery mildew and septoria blotch than those obtained by traditional breeding methods such as the pedigree system in which homozygosity is approached through repeated selections (Winzeler et al., 1987). This was due to the more precise disease assessment with the homozygous DH lines. DH populations have also been shown to produce a similar array of fatty acids to that obtained by single seed descent in *B. napus* (Chen and Beversdorf, 1990). The absence of dominance effects in DH plants may be especially useful in selection for quantitative traits. Snape et al. (1984) demonstrated that the number of genes segregating for a quantitative trait can be estimated more precisely by evaluating a DH generation rather than a segregating generation. This allows breeders more confidence in selection of desirable genotypes.

DH production is also useful for genetic studies such as RFLP mapping (Landry et. al.,1991; Ferreira et al., 1994) and study of physiological aspects of embryo

maturation and plant regeneration (Taylor et. al., 1993). Crop improvements through gene transfer (Huang, 1992) and mutagenesis of haploid cells have also been shown (Swanson et. al.,1989; Huang, 1992).

In *Brassica*, DH plants are usually produced through tissue culture of male gametes or microspores (Ferrie and Keller, 1995). Microspore culture in *Brassica* was first demonstrated by Lichter (1982). The procedure used to produce DH plants in canola involves the selection of buds containing late uninucleate to early binucleate microspores which have not undergone first pollen mitosis (Keller et al., 1975; Pechan and Keller, 1988). At this stage, microspores have the ability to switch from gametophytic to sporophytic control by undergoing symmetric instead of asymmetric division which occurs *in vivo* to promote gametogenesis (Zaki and Dickinson, 1990; 1991). Microspores can be characterized cytologically (Kott et al., 1988a) or using flow cytometry (Fuchs and Pauls, 1992). Buds are macerated to release microspores which are then cultured in a liquid medium to recover haploid embryos. Haploid plants are then regenerated from these embryos and chromosomes are doubled by submersing the plant roots in a diluted solution containing an antimicrotubule agent, such as colchicine. Colchicine has been applied in the microspore stage of culture to double chromosomes and even enhance embryogenesis in *B. napus* (Iqbal et al., 1994; Zaki and Dickinson, 1995; Zhao et al., 1996) and in anther culture of wheat (Barnabas et al., 1991; Navarro-Alvarez et al., 1994). When applied prior to the first pollen mitosis colchicine promotes symmetric cell division. Application of colchicine at the single cell stage avoids chimeric expression of different ploidy levels and associated poor seed

production (Mathias and Röbbelen, 1991). Herbicides have also been used to double chromosomes, such as trifluralin in *B. napus* (Zhao and Simmonds, 1995) and oryzalin and amiprophos-methyl in potato cell suspensions (Sree Ramulu et al., 1991)

Limitations exist in the application of the DH methodology in a breeding program. Some crops are recalcitrant to *in vitro* embryo production resulting in too few or weak DH plants recovered, such as anther-derived albinos in cereals (Day and Ellis, 1984). Recalcitrance may be genotype dependant (Ferrie et al., 1995) or due to conditions in DH production procedures. Factors that influence DH production include the conditions of the donor plant, culture media and culture environment.

Biotechnology for genetic manipulation of *B. rapa* is not as well developed as that for *B. napus*. The diploid *B. rapa* (AA) is more recalcitrant to tissue culture than the amphidiploid *B. napus* (AACC) (Baillie et al., 1992). It has been suggested that the A genome inhibits regeneration whereas the C genome contains shoot regeneration genes (Narasimhulu and Chopra, 1988). The evolution of ethylene from *B. rapa* cells or tissue in culture has also been suggested to cause low levels of regeneration (Chi et. al., 1991). It has been proposed that highly embryogenic genotypes have a high level of synchrony in the stages of microspore development (Kott et al., 1988b). Asynchronous cultures may inhibit embryogenesis of cells at the optimum stage of development due to endogenous toxins produced by the death of more mature cells (Kott et al., 1988b; Pechan and Keller, 1988).

Increasing frequency and speed of regeneration of *B. rapa* and *B. napus* microspore derived embryos enhances the production of DH plants (Coventry et. al.,

1988). This is accomplished by providing an *in vitro* culture environment for the haploid embryos similar to that provided by the embryo sac for zygotic embryos *in vivo*, for example, by inducing desiccation tolerance and dehydration of the embryos. *In vitro* embryos do not naturally develop desiccation tolerance or undergo dehydration or dormancy. To induce these conditions, abscisic acid (ABA), cold and heat treatments along with desiccation have been implemented to increase the frequency of conversion from embryos to plants (Kott and Beversdorf, 1990; Senaratna et al., 1991; Brown et al., 1993).

Self-pollination or inbreeding to produce homozygosity in obligate outcrossing crops leads to inbreeding depression, seen as a loss in population performance, due to the expression of deleterious homozygous recessive alleles. Inbreeding depression has been reported in DH lines of *B. rapa* expressed as poor germination rates, spindly branching, reduced height, late flowering, lack of pollen, low seed set and poor seed quality (Dewan et al., 1995). DH production does not avoid inbreeding by minimizing the repeated cycles of inbreeding. In order to exploit the benefits of DH development in cross pollinating crops, there must be a recovery of performance to at least the level of the original heterozygous population.

Genotype Analysis

Traditional plant breeding techniques are based on the selection of a genotype using phenotypic analysis. Phenotype is not an accurate indicator of genotype when

traits have low heritability, are quantitatively inherited or are influenced by the environment. Direct analysis of genotype is a more accurate method of selection. This could be accomplished through the identification of genetic markers linked to desirable traits (Tanhuanpää et al., 1996), development of genetic maps (Zhang et al., 1995) and establishment of genetic relationships between and among species (Mailer et al., 1994; Bagheri et al., 1995; Demeke et al., 1996). The use of genetic markers may help reduce the amount of field screening and therefore avoid the necessity of carrying plants to maturity which is often necessary with the use of morphological traits such as seed colour or seed quality. Genotype analysis used along with phenotypic or morphological information could reduce time required to develop a desirable variety.

Procedures such as restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) based assays have been used to characterize a genotype through DNA analysis.

Restriction Fragment Length Polymorphisms (RFLPs)

RFLP detects variation between genotypes using restriction endonucleases to fragment DNA along with blot hybridization to visualize polymorphism. RFLP analysis has been used successfully in the mapping of *B. napus* and *B. rapa* (Landry et al., 1991; Chyi, et al., 1992), determining genetic relationships within and among *Brassica* species (Song and Osborn, 1992; Diers and Osborn, 1994) and predicting heterosis in tetraploid alfalfa (Kidwell at al., 1994). RFLP markers segregate codominantly so that

heterozygotes as well as homozygotes can be identified. This is more informative for mapping than the dominant markers exhibited by some PCR based assays. RFLP assays detect polymorphisms in a larger area surrounding the probe than PCR based assays which detect only within the amplified fragment. However, RFLP analysis can not detect polymorphism in highly repetitive sequences and the technique requires specialized equipment and high quality and quantities of DNA. RFLP analysis is also labour-intensive and time-consuming, which diminishes its value in marker-assisted selection of large numbers of individual plants in a plant breeding program.

Polymerase Chain Reaction (PCR)

A PCR based assay requires less DNA, equipment, labour and time to perform than RFLP. However, some PCR based assays are limited in application due to the requirement for DNA sequence information to develop sequence specific primers. PCR-based markers include random amplified polymorphic DNA (RAPD), sequence tagged sites (STSs), microsatellites or simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLP).

RAPD primers, which are arbitrary sequenced oligonucleotides, randomly amplify DNA sequences using a PCR reaction. RAPD markers or polymorphisms are separated on an agarose gel and detected with ethidium bromide staining. The technique was developed independently by two laboratories (Welsh and McClelland, 1990 [AP-PCR]; Williams et al., 1990 [RAPD]). The RAPD primer binds to two sites on

opposite strands of template DNA and if they are within amplifiable distance from each other the DNA sequence between them is amplified. RAPD polymorphisms are usually based on base mutations within the amplified sequence. RAPD markers are dominant and therefore polymorphisms are detected by the presence or absence of a DNA product as a band.

STSs are unique sequences amplified by PCR using primers designed according to specific sequences of DNA (Olson et al., 1989). STSs focus on low copy number sequences and avoid highly repetitive DNA sequences that may be identified using the random primers with RAPD markers. The specific primers are usually longer than the random primers used with RAPD analysis and therefore have a tendency to be more stable under different PCR reaction conditions. STSs have also been shown to be more efficient than RFLP markers in genome analysis of wheat (Talbert et al., 1994). RAPD and RFLP markers can be converted into STSs. The disadvantage of STSs not present with RAPD markers is the requirement of sequence information for primer development.

Microsatellites or SSRs are tandem repeats of two to five nucleotide DNA sequences existing throughout eukaryotic genomes. Conserved DNA sequences flanking the SSR can be used as primers or to create primers for PCR amplification of the SSR. Polymorphic PCR products represent variation in the number of tandem repeats present in the genome. SSRs are abundant in plant genomes and high polymorphism can be detected compared to RFLP (Morgante and Olivieri, 1993; Zhang et al., 1995) and RAPD markers (Gupta et al., 1994). SSRs are codominant markers

and have been shown to be closely associated with regulation and expression of genes which makes them desirable genetic markers (Hamada et al., 1984). A disadvantage to using SSR in genotype analysis is that it is time-consuming to identify and sequence the regions flanking the SSR for primer development similar to the process of identifying probes useful for RFLP analysis. There is evidence that certain primers are able to amplify polymorphic SSR products from a wide range of plant species (Gupta et al., 1994). Development of a universal set of primers that can be used across a range of genomes would increase the usefulness of SSRs. SSRs have been used to detect genetic distance and predict heterosis in rice (Zhang et al., 1995) and enhance the genetic map in maize (Gupta et al., 1994).

Two cycles of PCR amplification are usually used to identify AFLP markers (Vos et al., 1995). The genomic DNA is cut with two restriction endonucleases and ligated to adaptors consisting of oligonucleotides with overlapping ends which anneal to the restricted DNA (Figure 2.3). Primers are designed with the sequence of the adaptors and arbitrary nucleotides attached to the 3' end. The number and type of nucleotides can be varied to produce different AFLP patterns. PCR amplification products are separated on a sequencing gel, producing a dominant marker. A higher proportion of the genome is assayed with each primer combination than with any other marker which makes it efficient for constructing genetic maps (van Eck et al., 1995).

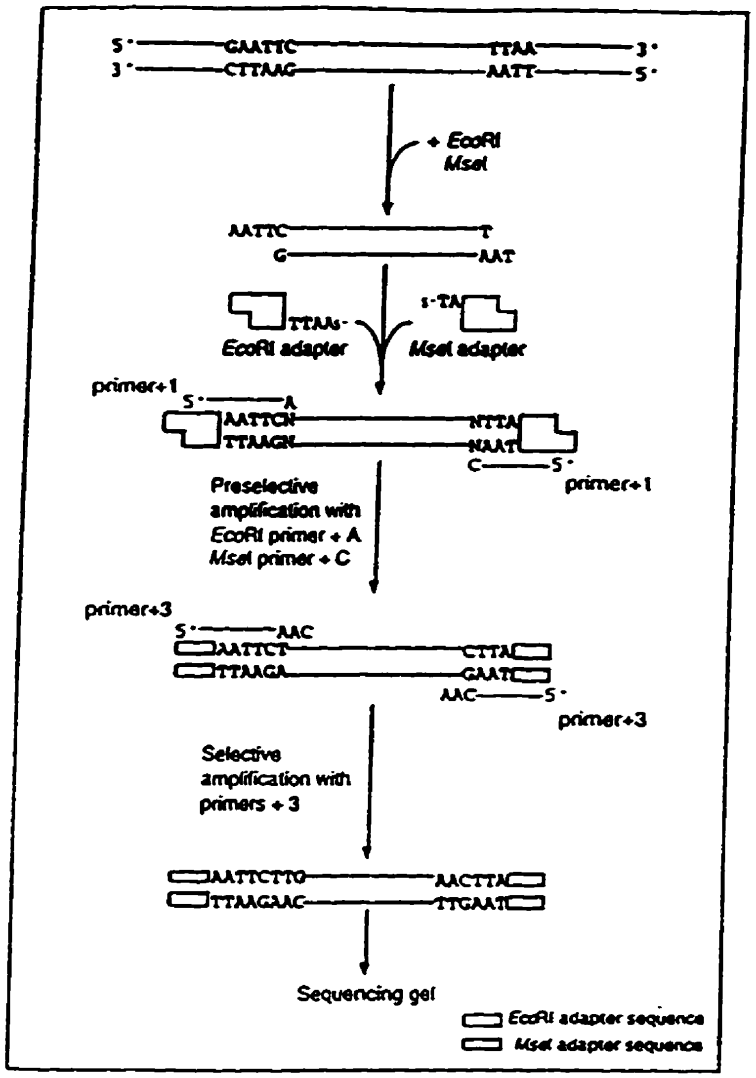

Figure 2.3. The AFLP procedure using two primer pairs. Genomic DNA is digested by EcoRI and Mse I restriction endonucleases in this example. (Source: CIMMYT, Int., 1996)

Application of RAPDs

PCR conditions can be manipulated to influence the RAPD banding pattern. These conditions include the temperatures controlled by the thermal cycling device responsible for PCR automation. This device acts by heating matt black metal surfaces followed by air cooling. Optimum temperatures at different stages of the PCR reaction vary based on the primer and genomic DNA being used. The first action of the thermal cycler is to dissociate the double stranded template DNA with heat in the presence of primers, a thermostable DNA polymerase, dNTPs, $MgCl_2$ and a PCR buffer. The primers are then annealed to the template DNA by reduction of the temperature to 37 - 65°C, depending on the melting temperature (Tm) of the primers. The primers are then extended, aided by a thermostable DNA polymerase which adds the dNTPs. These steps are repeated for at least 20 cycles. The number of cycles and the duration of each step influence the number, specificity and reproducibility of the RAPD bands. It is important to optimize the number of PCR cycles to avoid amplifying background products which may amplify preferentially. The last extension time is usually increased by several minutes to complete the synthesis of all strands (Newton and Graham, 1994).

PCR conditions may be varied in a number of ways. Primers of 20 - 30 nucleotides allow reasonably high Tm which minimizes the amplification of non-specific products. It is desirable for primer sequences to have at least 50 % guanine and cytosine and avoid repetitive sequences to minimize amplification of primer-dimers and

non-specific artifacts which affect reproducibility of RAPD analysis (Saiki , 1990). When the primers have annealed to the template DNA, a thermostable polymerase is required for the extension of the sequence. *Taq* DNA polymerase is a common thermostable DNA polymerase used in PCR reactions. It exhibits 5' --> 3' exonuclease activity, which removes nucleotides ahead of the growing sequence, and has an extension rate of about 75 nucleotides per second at 70 - 80°C (Abramson, 1995). The amount of dNTPs that should be included in the reaction mix depends on $MgCl_2$ concentration, reaction stringency, primer concentration, length of amplified product and the number of PCR cycles. RAPD production usually requires 100 μM of each of the four bases (Williams et al, 1993). The Mg ions present in the PCR reaction form complexes with dNTPs for incorporation on to the DNA template, stimulate polymerase activity, increase the annealing temperature of the double stranded DNA and therefore influence primer/template interaction. Concentrations of $MgCl_2$ can range from 0.5 - 5.0 mM. $MgCl_2$ concentration affects specificity and yield of RAPD product. Low concentrations result in low product yield whereas high concentrations lead to non-specific products (Newton and Graham, 1994). The PCR buffer usually contains Tris-HCl, KCl and gelatin.

Template DNA does not have to be as high quality or quantity as that required for RFLP analysis but it is important that it does not contain inhibitors to the PCR reaction. RFLP analysis requires 2 - 10 μg or DNA per isolate whereas RAPD analysis only requires about 25 ng (Williams et al., 1993). Crude methods of extraction, for example from leaf discs, have been used successfully in the isolation of DNA for RAPD

analysis (Edwards et al., 1991). The extraction procedure uses ionic detergents, such as SDS or CTAB, which should be removed by phenol extraction because they inhibit *Taq* polymerase activity. Residual traces of phenol, which also inhibits PCR, should be removed by chloroform : isoamyl alcohol (24:1) extraction or by ethanol precipitation of the DNA (Newton and Graham, 1994).

Concerns have been expressed regarding the reliability of RAPD markers. These concerns are based on the fact that random primers do not bind 100 % to target DNA, as is the case with primers based on specific sequence information in other PCR assays. The accuracy of random primers in RAPD analysis compared to RFLP analysis was tested in the detection of genetic relationships within and among cruciferous species (Thormann et al., 1994). They found a discrepancy for interspecific but not intraspecific relationships between RFLP and RAPD data due to the false scoring of non-homologous amplified sequences as homologous.

DNA extraction procedures can affect repeatability of RAPD markers as well. It is important to use consistent methods to optimize the template concentration relative to the primer to minimize competition for primer sites. Thermocyclers have been tested for reliability and different machines have produced repeatable RAPD products (Smith and Chin, 1992; Weeden et al., 1992; Mailer et al., 1994). Various concentrations of *Taq* polymerase and numbers of cycles of PCR also produce repeatable RAPD products (Smith and Chin, 1992). Methods of scoring RAPD products may also result in non-repeatable results. Faint bands tend to be less repeatable and therefore should not be scored as RAPD products unless tested repeatedly. The optimization and

maintenance of DNA extraction procedures and PCR conditions should minimize concerns about repeatability of RAPD markers.

The advantages of using RAPD markers have been shown in the identification of useful genetic markers and estimating genetic relationships or diversity in several crop species. RAPD markers are dominant and therefore detect less polymorphism per locus than RFLPs, only two versus multiple alleles in RFLP, but they also do not require as much DNA and time as RFLP assays. This has led to suggestions that RAPD analysis be used in mapping cultivar genomes, such as rice, where the wide hybridizations required to produce enough polymorphism for RFLP analysis often result in sterility or poor growth (Mackill, 1995). Williams et al. (1990) also showed that RAPD markers could saturate the soybean map by filling in areas not detected with RFLP analysis. RAPD markers linked to specific traits can serve as an alternative to morphological markers, allowing earlier selection for disease resistance in rice varieties (Naqvi, et al., 1995) and for outcrossed tetraploid alfalfa plants (Gjuric and Smith, 1996).

RAPD markers have also been used to distinguish genetic relationships among cultivars in *B. napus* (Mailer et al., 1994), pea (Bagheri et al., 1995) and potato (Demeke et al., 1996) and between species in *Brassica* (Demeke et al., 1992) . Similar genetic relationships using RAPD and RFLP analysis have been reported in *B. napus* breeding lines (Hallden et al., 1994) and *B. oleracea* genotypes (dos Santos et al., 1994). These studies came after reports that RAPD markers were not as reliable as RFLP in detecting genetic relationships between cruciferous species (Thormann et al.,

1994). RAPD information is useful for variety protection or germplasm collection as well. In these cases it is critical to optimize PCR conditions and DNA extraction techniques so that RAPD fingerprints are repeatable across different labs and germplasm. RAPD analysis will decrease the number of samples required to ensure that a broad range of variability is sampled in germplasm by confirming that observed phenotypic variation is due to genotype (Ren et al., 1995). Detection of genetic diversity using RAPD markers may also allow selection of diverse parents for hybridization so that inbreeding can be avoided (Demeke et al., 1996).

Comparison of Bud Pollination and Salt (NaCl) Spray Treatments in Overcoming Self-Incompatibilty of *Brassica rapa*

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ABSTRACT

Field studies were conducted to determine the effectiveness of NaCl solutions in overcoming self-incompatibility in *Brassica rapa* as compared to bud pollination. All treatments promoted compatibility. NaCl solutions of 3% and 5% were significantly more efficient than bud pollination in overcoming self-incompatibility.

Key words: bud pollination, salt spray, self-incompatibility, *Brassica rapa*

INTRODUCTION

Production of doubled haploid (DH) plants improves selection efficiency in *Brassica napus* by providing completely homozygous individuals (Scarth et al., 1991) and speeds up commercialization of improved varieties as demonstrated with the development of the cultivar Quantum (Stringam et al, 1995). DH lines are difficult to maintain in *B. rapa* which limits their application to population improvement. Selfed seed is difficult to obtain when inbreeding naturally cross-pollinating crops, such as sporophytically self-incompatible *B. rapa*.

The traditional approach to producing selfed seed from *B. rapa* is through bud pollination (Sun, 1938). Bud pollination involves emasculating an immature bud to expose the stigma, 2-3 days before flower opening. An anther from the same plant is used to pollinate the stigma. Bud pollination allows pollination before the stigma is biologically able to respond to self-pollen which is 1 day prior to flower opening. However, bud pollination is time consuming and labour intensive. Ferrie and Keller (1995) found that exposing *B. rapa* plants to elevated CO_2 levels produced more seed than bud pollination or NaCl spray treatment, but the CO_2 treatment is expensive. NaCl spray treatment is a potentially useful, inexpensive method of producing selfed seed that can be applied under field conditions.

Incompatible pollination in *B. rapa* results from callose accumulation on stigmatal surfaces preventing penetration of the pollen tube through the papillar cell (Kanno and Hinata, 1969). NaCl solutions overcome this barrier in self-incompatible

B. napus by causing the collapse of the callose (Fu et. al., 1992). The purpose of this study was to determine the effectiveness of NaCl solutions as compared to bud pollination in overcoming self-incompatibility in *B. rapa*.

MATERIALS AND METHODS

B. rapa cv Reward plants were studied under field conditions in 1995. The experiment was seeded on May 29 with 100 seeds per row. Plant rows were 3 m long and spaced 1 m apart. The mean day/night temperature from seeding to maturity was 27/15°C.

Pollination treatments were applied to four randomly selected rows. Each row represented a replication. Within each row five plants were selected for each treatment. Treatments included bud pollination, a 3% w/v NaCl solution and a 5% w/v NaCl solution. NaCl solutions of 3% and 5% were prepared with filtered water and then sprayed on the stigma and stamen of newly opened flowers using a mist bottle. Polyethylene bags were placed over individual plants. Spray treatments took place every 3 to 4 d during the flowering period. Bud pollination was also applied. This involved emasculating buds approximately 2 to 3 days prior to opening and then pollinating the stigma with pollen from the same plant.

The duration of treatment application and seed set were recorded. The efficiency index (EFI), compatibility index (CI) and economic index (EI) were then calculated for each treatment as follows:

$$\text{Efficiency Index} = \frac{\text{total number of seeds set}}{\text{no. of minutes of treatment}}$$

$$\text{Compatibility Index} = \frac{\text{total no. of seeds set}}{\text{total no. of flowers pollinated}}$$

$$\text{Economic Index (\%)} = \frac{\text{no. of seeds produced per treated plant}}{\text{no. of seeds produced per bud pollinated plant}}$$

Treatment results from the four replicates were averaged and then analyzed using a means comparison test with $P>0.1$.

RESULTS AND DISCUSSION

The EFI of both NaCl solutions was significantly higher than the EFI of bud pollination (Table 3.1). The NaCl treatments produced approximately equal EFI values.

The CI of both NaCl treatments and the bud pollination treatment did not differ significantly (Table 3.1). All treatments promoted a similar level of self-compatibility.

The EI of both NaCl treatments was not significantly different from the bud pollination treatment (Table 3.1). Approximately equal numbers of plants were required to produce the same amount of seed when using bud pollination or NaCl solution treatments.

All treatments overcame self-incompatibility and promoted self-pollination to a similar degree. The NaCl solution treatments were efficient, producing more seed with less time (EFI). Bud pollination is a time consuming, labour intensive process that is not any more effective at achieving seed set than NaCl solution treatments. This study

shows that the 3% and 5% NaCl solutions are equally effective in overcoming self-incompatibility in *B. rapa*. The 3% NaCl spray treatment was used successfully in the maintenance of DH *B. rapa* lines in further studies of *B. rapa* population improvement (Friesen, 1997).

Table 3.1. EFI[1], CI[2] and EI[3] and seeds produced after bud pollination, 3% and 5% NaCl solution treatment

Treatment	EFI[1]	CI[2]	EI[3]	No. of seeds per plant
Bud	16.85b	7.97a	1.00a	31.75a
3% NaCl	116.00a	9.74a	1.32a	38.97a
5% NaCl	116.70a	9.07a	1.14a	36.30a

[1] Efficiency Index (seeds per minute of treatment)

[2] Compatibility Index (seeds per flower pollinated)

[3] Economic Index [seeds per treated plant divided by seeds per bud-pollinated plant (%)]

a-b Same letters following the values indicate no significant difference between values in the same column as tested with Duncan's means test

Application of doubled haploid development to population improvement of *Brassica rapa*

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ABSTRACT

The production of doubled haploid (DH) plants in *Brassica rapa* results in inbreeding depression. In order to exploit the benefits of DH plants in population improvement, agronomic performance must be recovered to that of the original donor population. The application of DH plants in population improvement of *B. rapa* was studied by randomly intercrossing 4, 8, 12 and 22 DH lines developed from the *B. rapa* cultivar Reward and the *B. rapa* breeding line DSC-3 for two generations to constitute composite populations. The composite populations and the DH plants used in their development were evaluated for agronomic performance at two locations in the field in 1996. In the breeding line DSC-3, intercrossing as few as 4 DH lines in a composite population improved population performance over that of the contributing DH lines. In the cultivar Reward, interpollination of 8 DH lines improved composite population performance over that of the contributing DH lines to a level similar to the original Reward donor population. This study indicates that the number of individual DH lines required to contribute to the composite population is relatively small. This is encouraging for the application of DH lines in *B. rapa* cultivar development.

Keywords: *Brassica rapa*, composite population, doubled haploids

INTRODUCTION

Brassica rapa represents approximately half of canola production in Canada (Askew, 1995). Traditional breeding methods with this sporophytically self-incompatible (SI) species have involved mass selection, hybridization or the development of synthetics, all of which rely on outcrossing. Doubled haploid (DH) line development is a breeding tool that has been shown to speed up the breeding process by achieving homozygosity in a single generation and avoiding repeated generations of inbreeding traditionally required to fix desirable traits (Chen and Beversdorf, 1990).

The production of DH plants in *B. rapa* results in inbreeding depression. In order to make use of DH plants in population improvement, agronomic performance must be restored. Inbreeding through DH production provides the opportunity to fix desirable alleles in homozygous condition while subsequent production of cross-pollinated composites can be used to restore vigour. The production of a composite population involves the intercrossing and recombining of two or more open-pollinating inbreds, DH lines or cultivars and is maintained simply by further random interpollination. Intercrossing promotes the maintenance of heterozygosity and heterogeneity in these populations which have been shown to improve yield and yield stability in *B. napus* hybrids (Léon, 1991).

Optimum population size is an important consideration in composite production. In *B. rapa* populations it is critical to have sufficient variation at the multi-allelic S-locus, which controls SI, to ensure an adequate number of compatible mates. The

population should be large enough to reduce the chance of mating between close relatives and minimize the occurrence of inbreeding depression (Mirando-Filho and Chaves, 1991; Byers and Meagher, 1992). If the population is too large, the probability increases of having below optimum combining ability and therefore depressed agronomic performance. Assessment and selection of agronomically superior DH lines prior to recombination should enhance the performance of the resulting composite populations.

The purpose of this study was to determine the most efficient application of DH development to population improvement of *B. rapa* through production of composite populations. An optimum population size was determined for the production of composites using the DH lines, in order to reconstitute the agronomic performance of the original donor population.

MATERIALS AND METHODS

The seed sources for the DH lines were second generation selfed DH lines from Reward and second generation selfed DH lines from DSC-3, a breeding line from Agriculture and Agri-Food Canada. The DH lines were produced using the microspore culture protocol reported by Ferrie and Keller (1995), and the first generation seed was provided by Dr. Allison Ferrie (PBI, Saskatoon).

Five single plants from the DH seed provided were sown in 6-inch pots and grown in growth chambers with a 18 h photoperiod, 580 μeinsteins $m^{-2}s^{-1}$ light intensity

and a day/night temperature of 15/10°C. These plants were covered with polyethylene isolation bags with 1-mm holes from about 1 d prior to flowering until the end of the flowering period. Plants were sprayed with 3 % NaCl spray every 3 d after the first flowers were newly opened until flowering was complete. After each spray treatment, flowers were left to dry and then shaken gently within the isolation bags to facilitate self-pollination. The NaCl spray treatment was compared to bud pollination and was found to be more time efficient with the same ability to overcome SI in *B. rapa* (Friesen, 1997). Selfed seed was harvested from 52 DSC-3 and 60 Reward DH lines and was used for the 1995 field study.

DH lines were evaluated in the field in 1995 as randomized complete blocks (RCB) which consisted of two replicates with 3-m rows sown with 100 seeds per row for each DH line. Check rows of cv. Reward were sown between each DH line to control competition between rows. DH lines were characterized for performance according to the parameters: days to flowering, seed quantity, fertility index, lodging, disease index (DI) relating to white rust (*Albugo candida*), days to maturity and height at maturity (data not shown for 1995).

The days to flowering were determined as the number of days from when 50 % of the plants had emerged until 50% of the plants in a row had begun flowering. The fertility index was determined by visually rating plants in a row under field conditions according to a scale of 1-5 where a 5 indicated the plants had pod and seed development similar to the check cv. Reward. Lodging was measured according to a scale of 1-5 where a 5 indicated good stalk strength with upright growth. DI was a

measure of the percentage of infection of a DH line, calculated based on a rating of about 10 plants inoculated on the cotyledons with spores of white rust *Albugo candida* race 7A and grown under controlled conditions (Williams, 1985). The Williams scale of 1-9 was used to rate plants, where the higher number represents a more severe infection of white rust. Days to maturity was determined as the number of days from when 50 % of the plants had emerged until 50% of the plants in a row were physiologically mature. The height (cm) was measured as the average of all plants in a row once flowering had finished.

In Winnipeg, the average daily temperature and total precipitation from the time of seeding on May 29 to the end of June in 1995 was 28.4°C and 25.6 mm and from the time of seeding on May 28 to the end of June in 1996 was 25.7°C and 31.7 mm, respectively (Appendix 1 and 2). Field conditions in 1995 subsequent to seeding led to poor seedling emergence and establishment. Assessment of the parameters: days to flowering, lodging and days to maturity was not possible due to the poor establishment. Therefore, DH lines were selected for composite development based on the availability of at least 0.6 g of seed which was required for further field study, a mean fertility index of at least 2 and a mean DI of at most 82 % (Appendix 3).

Populations of 4, 8, 12 and 22 of the selected lines (C_{0-4}, C_{0-8}, C_{0-12}, C_{0-22}) from each of the DSC-3 and Reward DH populations were grown in the greenhouse inside isolation tents. The DH lines included in C_{0-4} were also included in all larger populations of C_{0-8}, C_{0-12} and C_{0-22}. The DH lines in C_{0-8} and C_{0-12} were also included in the larger populations. Cross-pollination was encouraged by brush pollinating the

plants within each tent using a feather duster every other day. Two tent replicates were grown for each of DSC-3 and Reward DH populations. The position of the plants in the pollination tent was rotated every 4 days after flowering began. Seed produced was harvested from single plants and an equal amount from each plant was composited to produce the first generation composite (C_1). From each of these C_1 populations, 60 plants were seeded in individual pots and randomly intercrossed to produce the second generation composite (C_2). The C_2 was produced from 60 individual C_1 plants to ensure adequate seed production for the 1996 field season.

All C_1 and C_2 populations were grown at two field locations in 1996 as separate C_1 and C_2 RCB with 6 and 4 replications in Winnipeg and Carman respectively. The original DH lines with sufficient seed were evaluated in Winnipeg in two replications. In Winnipeg 3-m rows were planted with 0.3 g of seed and in Carman 5-m rows were planted with 0.5 g of seed with 60-cm spacing between each row. Reward check rows were planted to alternate with the DH lines, C_1 or C_2 plant rows. These check rows were used in the data analysis in the calculation of the Reward donor population mean. There was no donor information for the DSC-3 breeding line.

Agronomic performance of C_0, C_1 and C_2 populations was evaluated by measuring number of plants emerged per metre of row, days to flowering, days to maturity, height at maturity, lodging, seed yield, harvest index (HI) and seed oil content (Appendix 4-9). Plant rows representing DH lines and replicates of C_1 and C_2 were hand harvested, tied and stooked in the field. Each plant row was weighed when dry (moisture content was not determined) and threshed in the field when dry using a

stationary thresher. Seed yield was determined by weighing seed harvested from each plant row. HI was calculated as the seed yield per total biomass yield measured for each row. Seed oil content was measured for each row from 20 g of seed using a Nuclear Magnetic Resonance spectrometer (Robertson et al., 1979). Data was analyzed through nearest neighbour analysis of variance (NNA) using Agrobase/4™ (Mulitze, 1992) statistical program. A standard error (SE) value was calculated for each parameter using a complete data set.

RESULTS

Seedling Emergence

In 1996, the Reward DH lines showed a lower mean level of emergence than the Reward donor population (Table 4.1; Appendix 10a). DSC-3 DH lines also expressed a low level of seedling emergence (Table 4.2). Poor emergence could be attributed to the poor seed quality obtained through self-pollination of the individual DH lines that expressed inbreeding depression. Individual Reward and DSC-3 DH lines showed variation in the level of seedling emergence (Table 4.3).

There was no difference in seedling emergence between any of the population sizes in the Reward C_1 or between the C_1 and the donor population (Table 4.4). The Reward C_2 populations all had higher numbers of seedlings emerging than the Reward donor population (Table 4.5). There were no differences between any of the DSC-3 C_1 and C_2 populations in seedling emergence (Table 4.6 and Table 4.7).

Days to Flowering

Reward DH lines took longer mean days to flower than the Reward donor population with a range of 16.8 - 29.0 days to flowering (Table 4.1; Appendix 10b). The individual DSC-3 DH lines expressed a range of 15.5 - 27.0 days to flowering (Table 4.3).

The Reward C_1 and C_2 populations took more days to flower than the donor population (Table 4.4 and 4.5). Among the C_1 populations, C_{1-4} at Carman was the latest to flower. Among the C_2 populations, C_{2-4} and C_{2-12} both took more days to flowering in Carman and were also ranked the latest in Winnipeg. In Carman C_{2-22} flowered earlier than any other C_2 population.

In Carman, the DSC-3 C_{1-22} and C_{2-22} were earliest to flower within each C_1 and C_2 population respectively (Table 4.6 and 4.7). There were no differences in days to flowering between the C_1 and C_2 populations in Winnipeg.

Days to Maturity

The Reward donor population took longer to mature than the mean of the DH lines (Table 4.1; Appendix 10c). Individual DSC-3 DH lines showed variation for days to maturity (Table 4.3).

The Reward C_1 populations expressed no difference from the donor population for the days to maturity. The Reward C_2 populations took longer to mature than the donor population in Winnipeg. There were no differences in the days to maturity between the DSC-3 C_1 or C_2 populations.

Height

The mean height of the Reward donor population was greater than the mean of the DH lines (Table 4.1). Individual Reward and DSC-3 DH lines showed variation for height (Table 4.3; Appendix 10d).

There were no differences in height between Reward C_1 and C_2 populations or the donor population (Table 4.4 and 4.5). There was no difference in height between any DSC-3 C_1 and C_2 populations (Table 4.6 and 4.7).

Lodging

There was no difference in the level of lodging shown by the mean of the Reward DH lines and the Reward donor population (Table 4.1; Appendix 10e). Variation for lodging was expressed by individual Reward and DSC-3 DH lines (Table 4.3).

No difference in the level of lodging occurred between the Reward C_1 and C_2 populations or the donor population (Table 4.4 and 4.5). There was also no difference in the level of lodging between any of the DSC-3 C_1 and C_2 populations (Table 4.6 and 4.7).

Seed Yield

The Reward DH lines had a lower mean seed yield than the Reward donor population (Table 4.1). The variation in seed yield performance between the individual DH lines that contributed to the C_{1-4}, C_{1-8}, C_{1-12} and C_{1-22} populations is shown in Fig. 4.1a-d. The individual DSC-3 DH lines expressed low seed yields with a range of 2.50 - 116 g (Table 4.3).

In Carman, Reward C_{1-8} and C_{1-12} yielded more seed than the donor and other C_1 populations (Table 4.4). This ranking was also observed in the C_2 generation where the donor population was lower yielding than all of the C_2 populations except C_{2-4} (Table 4.5). In Winnipeg, the Reward donor and C_{1-8} population ranked the highest for seed yield but were only significantly higher than C_{1-4} and C_{1-22}. At this location, there were no differences between the Reward C_2 and donor Reward populations. There was no difference in seed yield between the DSC-3 C_1 populations. There was no consistent ranking of the DSC-3 C_2 populations.

Harvest Index

The mean HI of the Reward DH lines was lower than that of the Reward donor population (Table 4.1; Appendix 10f). Individual Reward and DSC-3 DH lines showed variation for HI (Table 4.3).

There were no differences in HI between the Reward C_1 and C_2 populations or the donor population (Table 4.4 and 4.5). The DSC-3 C_1 and C_2 populations showed no differences in HI (Table 4.6 and 4.7).

Seed Oil Content

The Reward DH lines demonstrated lower mean seed oil content in comparison to the Reward donor population (Table 4.1; Appendix 10g). Individual Reward and DSC-3 DH lines showed variation for seed oil content (Table 4.3).

Reward C_1, C_2 and the Reward donor population had similar seed oil content (Table 4.4 and 4.5). The DSC-3 C_1 and C_2 produced similar seed oil content as well (Table 4.6 and 4.7).

DISCUSSION

Intercrossing DH lines in *B. rapa* through the production of composite populations led to the recovery of population performance for some traits. There were improvements in seedling emergence, height, seed yield and HI of the C_1 and C_2 populations of both Reward and DSC-3 above that expressed by the mean of the DH lines (Table 4.1 and 4.2). In Reward, C_1 and C_2 population performance was similar to the original Reward donor population with the exception of increased seedling emergence and days to maturity in all C_2 populations, as well as higher seed yield of C_{1-8} and C_{1-12}, C_{2-8}, C_{2-12} and C_{2-22} at Carman. In Reward, interpollination of 8 DH lines (C_{1-8}) resulted in a population with agronomic performance better than any of the individual DH lines and better than the original Reward donor population at one location, Carman. There was no consistent ranking between the DSC-3 C_1 or C_2 populations in yield performance or HI. The DSC-3 C_2 generation showed improvement in agronomic parameters in comparison to the mean of the DH lines.

The C_{1-4} and C_{2-4} Reward composite populations were significantly lower in seed yield than the C_{1-8}, C_{1-12} and C_{2-8} and C_{2-12} populations respectively. Genetic characterization using randomly amplified polymorphic DNA (RAPD) analysis showed that the 4 DH lines chosen for the C_{0-4} population had collectively less heterogeneity than C_{0-8}, C_{0-12} and C_{0-22} (Friesen, 1997). This C_{0-4} population may not have had enough variability to avoid mating between close relatives and inbreeding depression.

The Reward C_2 populations had higher seedling emergence than their C_1

counterparts. The extra generation of outcrossing may be beneficial by increasing heterogeneity in these populations. This was supported by RAPD analysis that showed more genetic variation present in $C_{2\text{-}4}$ and $C_{2\text{-}22}$ than in $C_{1\text{-}4}$ and $C_{1\text{-}22}$ populations, respectively (Friesen, 1997). However, this was not apparent in the RAPD analysis of $C_{2\text{-}8}$ and $C_{2\text{-}12}$ or with the other agronomic parameters that did not vary significantly between C_1 and C_2 populations. Therefore increased heterogeneity apparently contributes to improved performance of the C_2 populations but may not be the sole cause.

The DH lines involved in the development of the C_1 populations were selected based on the phenotype of the individual DH lines and not on combining abilities expressed in progeny of controlled crosses. C_1 and C_2 yields therefore may not represent the potential yield attainable if combining ability was tested before interpollination to identify the optimum combination of DH lines.

Another useful method of identifying desirable DH lines through heterogeneity is DNA analysis. This has been used to individually characterize *B. napus* cultivars (Mailer et al. 1994) and identify genetic diversity in DH lines of *B. rapa* (Friesen, 1997) in the form of RAPD analysis. RAPD analysis is a quick method of characterizing plant genotypes and measuring the potential for hybrid vigour to be expressed when they are intercrossed.

The Reward composite populations took longer to reach first flower than the original Reward donor population but the days to maturity were not different. Therefore, the period of seed filling was reduced. Thurling (1991) demonstrated the

importance of the time between anthesis and maturity by introducing genes for early flowering into a *B. napus* line and a *B. rapa* population. Thurling (1991) concluded that the earlier flowering resulted in a longer period of dry matter accumulation and greater seed yields. The later flowering did not adversely affect the performance of the composite populations in this study with the exception of the Reward C_{2-4} which was later flowering in Carman. There was a positive correlation between days to flowering and seed yield for the Reward C_2 population in Carman (Table 4.8). A negative correlation between days to flowering and seed yield was found with the Reward and DSC-3 DH lines and DSC-3 C_1 and C_2 populations in Carman (Table 4.8).

CONCLUSION

The production of composite populations from DH lines has the potential of speeding up cultivar development. As a breeding tool, improvements can be made in *B. rapa* populations by selecting DH lines with fixed traits followed by random interpollination to recover performance. This method avoids the short comings of traditional breeding systems such as the time required for repeated generations of inbreeding and selection seen with mass selection, the difficulty in producing a reliable pollination control system for hybrid production and the maintenance of original entries for reconstitution of a population as a synthetic.

The individual DH lines do not exceed donor population performance but, when crossed to establish a composite, population performance can be recovered. This

composite population can either be used in further selections or established as a cultivar itself. This study shows that only a small, maintainable population is required to recover population performance. To optimize this method further, the selection of DH lines can be made by determining their combining abilities or genetic variation through DNA analysis. This would help to optimize the recovery of heterogeneity in the composite populations produced from interpollination of DH lines.

Table 4.1. Mean parameter values for DH lines (C_0) produced from *B. rapa* cv. Reward and comparison to mean values of the check Reward donor population in Winnipeg, 1996

Parameter	Means		SE	$LSD_{0.05}$	CV
	DH lines	Donor			(%)
Seedling emergence[1]	8.68	15.4	0.83	2.34	44.7
Days to flowering (d)	21.0	18.6	0.61	1.73	21.5
Days to maturity (d)	57.6	59.5	0.26	0.87	0.88
Height (cm)	90.0	99.3	2.35	6.66	17.0
Lodging (1-5)	4.76	4.64	0.11	0.32	16.5
Seed yield per row (g)	82.5	316.0	10.7	30.3	32.9
Harvest index[2]	0.18	0.30	0.01	0.03	27.8
Seed oil content (%)	42.6	46.8	0.50	1.42	7.65

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

Table 4.2. Mean parameter values for DH lines (C_0) produced from *B. rapa* breeding line DSC-3 and comparison to mean values of the check Reward donor population in Winnipeg, 1996

Parameter	Means		SE	$LSD_{0.05}$	CV
	DH lines	Donor			(%)
Seedling emergence[1]	6.31	14.9	1.05	3.00	56.0
Days to flowering (d)	20.0	16.9	0.71	2.04	23.0
Days to maturity (d)	56.4	57.5	0.47	1.34	4.93
Height (cm)	84.0	100.9	2.48	4.36	2.75
Lodging (1-5)	4.47	4.20	0.18	0.37	4.97
Seed yield per row (g)	43.1	362.9	11.3	32.3	30.6
Harvest index[2]	0.16	0.30	0.01	0.03	28.7
Seed oil content (%)	42.4	45.6	0.48	1.36	6.44

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

Table 4.3. Range parameter values for DH lines (C_0)produced from *B. rapa* cv. Reward and breeding line DSC-3 in Winnipeg, 1996

	DH lines							
	Reward				DSC-3			
Parameter	Range	SE	$LSD_{0.05}$	CV (%)	Range	SE	$LSD_{0.05}$	CV (%)
Seedling emergence[1]	0.15 - 16.5	0.35	5.78	26.7	2.00 - 15.5	4.12	8.37	37.1
Days to flowering (d)	16.8 - 29.0	0.24	3.99	12.0	15.7 - 26.9	0.27	3.90	12.4
Days to maturity (d)	55.5 - 61.5	0.17	2.90	2.90	56.0 - 58.0	2.17	4.40	3.80
Height (cm)	66.4 - 121	0.71	11.7	7.21	55.0 - 100	8.68	17.6	9.39
Lodging (1-5)	3.60 - 5.70	0.05	0.85	10.7	3.04 - 6.06	0.09	1.37	18.4
Seed yield per row (g)	19.5 - 176	40.9	82.3	18.3	2.50 - 116	3.66	34.0	13.9
Harvest index[2]	0.09 - 0.28	0.03	0.06	11.9	0.05 - 0.26	0.03	0.07	14.5
Seed oil content (%)	36.1 - 47.7	1.09	2.20	2.42	37.7 - 49.6	1.06	2.14	2.40

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

Table 4.4. Mean values for parameters characterizing the cv. Reward donor and C_1 populations produced from 4, 8, 12 or 22 DH lines of *B. rapa* cv. Reward, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Population	EMER[1]		DTF (d)		DTM (d)		HT (cm)		LOD (1-5)		YLD (g)		HI[2]		OIL (%)	
	[3]L1	[4]L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Donor	14.5	15.9	22.3	18.9	69..0	59.5	120	103	4.45	4.70	328	292	0.24	0.28	42.4	46.7
C_{1-4}	12.5	14.0	30.1	21.4	70.2	57.5	119	105	4.55	4.82	277	216	0.18	0.30	41.7	45.2
C_{1-8}	11.9	15.7	27.4	22.6	69.6	60.0	127	108	4.99	5.00	463	271	0.17	0.32	42.0	45.7
C_{1-12}	18.6	18.7	27.5	21.9	69.8	59.7	122	113	4.59	4.89	411	251	0.23	0.28	42.2	45.2
C_{1-22}	13.8	14.5	28.1	22.4	69.9	59.8	120	103	4.63	4.65	307	198	0.20	0.26	43.7	47.9
CV (%)	23.0	36.6	2.23	10.0	0.73	3.58	3.21	3.54	6.78	5.32	15.3	18.7	29.3	11.9	1.67	1.86
$LSD_{0.05}$	5.72	6.70	0.95	3.48	0.87	3.64	6.71	6.33	0.53	0.43	75.9	58.0	0.11	0.04	1.23	1.48
SE	0.75	3.35	0.12	0.49	0.11	0.51	0.87	0.88	0.07	0.06	37.0	28.7	0.01	0.02	0.16	0.21

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

[3] L1 = Carman

[4] L2 = Winnipeg

Table 4.5. Mean values for parameters characterizing the cv. Reward donor and C_2 populations produced from 4, 8, 12 or 22 DH lines of *B. rapa* cv. Reward, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Population	EMER[1]		DTF (d)		DTM (d)		HT (cm)		LOD (1-5)		YLD (g)		HI[2]		OIL (%)	
	L1[3]	L2[4]	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Donor	15.2	15.4	23.0	16.2	69.5	57.1	121	101	4.29	4.63	287	251	0.21	0.28	41.3	46.8
C_{2-4}	21.3	20.5	30.5	26.2	69.9	63.5	122	103	4.76	4.97	353	213	0.20	0.25	42.8	46.2
C_{2-8}	22.0	24.1	26.1	23.5	69.9	62.2	126	104	4.72	5.02	469	252	0.21	0.29	42.5	46.7
C_{2-12}	23.9	29.1	27.5	26.7	70.4	64.9	124	105	4.85	4.71	432	231	0.19	0.26	43.0	45.5
C_{2-22}	18.0	26.1	24.5	24.2	70.0	64.4	122	102	4.94	4.82	378	255	0.21	0.28	43.1	46.2
CV (%)	13.3	18.5	2.62	15.2	0.60	3.29	2.11	3.19	6.93	5.93	13.9	9.10	10.3	8.37	1.63	1.80
$LSD_{0.05}$	4.07	6.70	1.12	3.57	0.73	3.37	4.45	5.56	0.54	0.48	80.6	38.1	0.04	0.04	1.18	1.43
SE	0.53	0.86	0.15	1.76	0.09	0.47	0.58	0.77	0.07	0.07	10.5	5.31	0.005	0.006	0.15	0.20

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

[3] L1 = Carman

[4] L2 = Winnipeg

Table 4.6. Mean values for parameters characterizing the cv. Reward donor and C_1 populations produced from 4, 8, 12 or 22 DH lines of the *B. rapa* breeding line DSC-3, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Population	EMER[1]		DTF (d)		DTM (d)		HT (cm)		LOD (1-5)		YLD (g)		HI[2]		OIL (%)	
	L1[3]	L2[4]	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Donor	16.8	17.7	21.2	18.4	46.9	59.1	118	102	3.99	4.50	362	335	0.19	0.42	41.3	46.5
C_{1-4}	13.6	15.3	28.3	19.2	41.0	56.2	118	96.7	4.02	4.34	70.4	103	0.14	0.23	40.0	43.0
C_{1-8}	15.1	15.3	28.3	18.7	40.9	56.2	118	103	3.87	4.48	116	94	0.15	0.21	40.5	42.1
C_{1-12}	13.2	15.3	28.0	18.5	40.7	56.3	124	105	3.78	5.04	178	127	0.16	0.24	40.9	43.9
C_{1-22}	12.8	19.7	27.3	17.7	41.2	56.0	121	100	3.60	4.63	163	112	0.18	0.22	41.6	43.6
CV (%)	26.5	35.8	13.2	16.1	2.87	5.13	6.21	3.52	11.5	6.93	22.0	28.5	29.3	111	1.55	1.75
$LSD_{0.05}$	7.13	7.15	4.60	3.47	0.67	3.46	4.24	3.54	0.78	0.54	103	78.0	0.09	0.43	1.10	1.34
SE	0.93	3.54	2.24	1.72	0.09	1.71	1.67	0.59	0.10	0.07	13.4	38.6	0.01	0.21	0.14	0.19

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

[3] L1 = Carman

[4] L2 = Winnipeg

Table 4.7. Mean values for parameters characterizing the cv. Reward donor and C_2 populations produced from 4, 8, 12 or 22 DH lines of the *B. rapa* breeding line DSC-3, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Population	EMER[1]		DTF (d)		DTM (d)		HT (cm)		LOD (1-5)		YLD (g)		HI[2]		OIL (%)	
	L1[3]	L2[4]	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
Donor	15.7	18.1	22.2	20.1	68.5	61.2	120	100	4.53	4.46	415	309	0.21	0.30	41.6	47.1
$C_{2\text{-}4}$	18.0	19.3	27.3	20.7	68.3	58.1	117	101	3.98	4.73	365	139	0.21	0.25	40.9	43.9
$C_{2\text{-}8}$	15.0	18.3	28.7	22.3	68.8	61.2	119	102	4.40	4.88	131	131	0.14	0.28	39.9	45.1
$C_{2\text{-}12}$	18.0	17.0	26.5	21.4	68.0	59.7	118	99.0	4.92	4.55	197	115	0.14	0.21	40.5	44.2
$C_{2\text{-}22}$	13.8	21.8	25.5	21.4	69.1	61.1	119	101	4.83	4.86	232	174	0.14	0.28	41.0	45.5
CV (%)	23.2	23.8	6.24	9.15	1.02	2.94	1.74	1.58	13.6	6.77	32.4	12.6	28.2	16.4	1.61	1.13
$LSD_{0.05}$	6.26	5.15	2.61	3.24	1.23	3.04	3.65	2.71	1.09	0.53	183	50.3	0.09	0.05	1.17	0.89
SE	2.98	2.55	1.24	0.45	0.16	0.42	0.47	0.38	0.14	0.07	87.3	7.01	0.01	0.03	0.15	0.12

[1] Number of plants per row

[2] Seed yield (g) divided by total above ground biomass (g)

[3] L1 = Carman

[4] L2 = Winnipeg

Table 4.8. Correlation of days to flowering with seed yield (g) in *B. rapa* cv. Reward and breeding line DSC-3 at two locations in 1996.

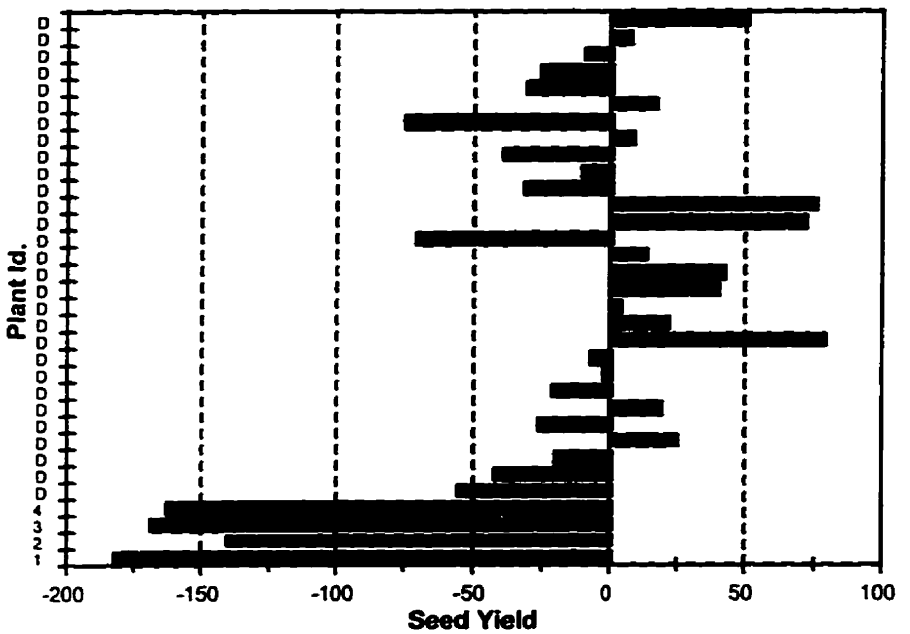
Origin	Population	Location	[1]r	Student's t
Reward	C_0	Winnipeg	-0.391	4.07 *
	C_1	Carman	0.092	0.56 NS
		Winnipeg	-0.161	1.15 NS
	C_2	Carman	0.494	3.41 *
		Winnipeg	-0.068	0.48 NS
DSC-3	C_0	Winnipeg	-0.420	3.82 *
	C_1	Carman	-0.740	6.60 *
		Winnipeg	0.011	0.08 NS
	C_2	Carman	-0.699	4.99 *
		Winnipeg	-0.019	0.13 NS

[1] Correlation coefficient = r

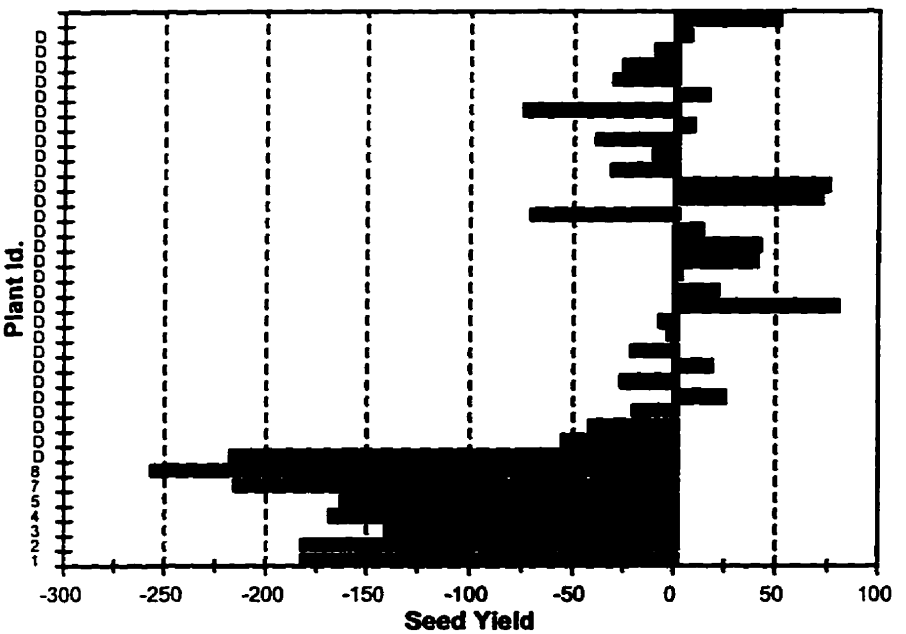
*, Significant at P = 0.05; NS, nonsignificant

Fig. 4.1. The mean seed yield of individual *B. rapa* cv. Reward DH lines available for field study, contributing to C_{1-4} (a), C_{1-8} (b), C_{1-12} (c) and C_{1-22} (d), and donor (D) checks relative to the overall mean yield of the Reward donor checks, 1996. Yield was not measured for individual DH lines 6,10, 20 and 22.

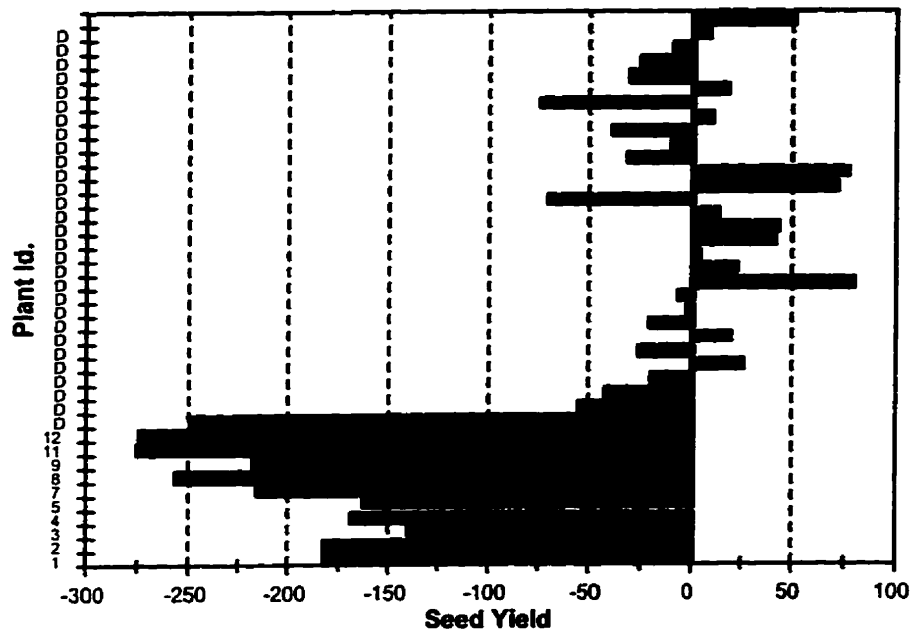
a


Plant Id.
-200
-150
-100
-50
0
50
100
Seed Yield

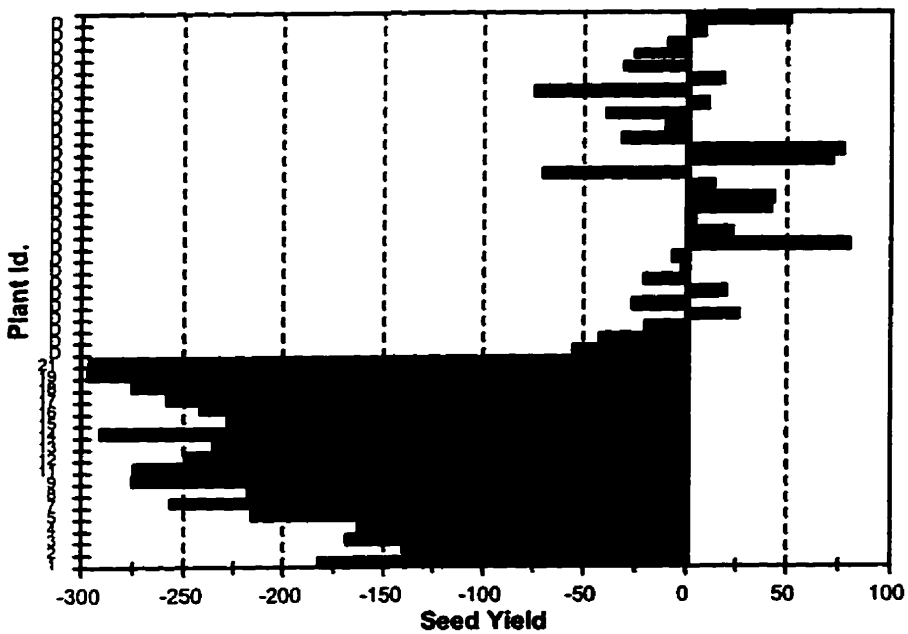
b


Plant Id.
-300
-250
-200
-150
-100
-50
0
50
100
Seed Yield

c


Plant Id.
-300
-250
-200
-150
-100
-50
0
50
100
Seed Yield

d


Plant Id.
-300
-250
-200
-150
-100
-50
0
50
100
Seed Yield

Detection of Genetic Variation in *Brassica rapa* using random amplified polymorphic DNA analysis

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ABSTRACT

Production of doubled haploid (DH) plants has been used as a tool to shorten the time frame of population improvement in several self-pollinating species. In *Brassica rapa*, the production of DH plants leads to inbreeding depression and therefore diminishes their usefulness. This study examined the ability of random amplified polymorphic DNA (RAPD) to detect genetic variation among DH lines produced from *B. rapa* populations. RAPD analysis was performed on DH lines and composite populations developed after two generations of outcrossing within a collection of 4, 8,12 and 22 individual DH lines. RAPD analysis detected 40 to 83% polymorphic bands between the DH lines using five oligonucleotide primers. RAPD analysis detected 17-53% and 27-47% polymorphic bands in the first composite and second composite populations, respectively. The highest level of genetic variation was shown by the composite population produced from 22 DH lines. RAPD analysis efficiently characterized the genotypic variation present in DH and composite populations. RAPD analysis may be useful as a tool in the reestablishment of heterogeneity and recovery of agronomic performance in *B. rapa* composite populations derived from DH lines by characterizing the level of genetic variability among DH lines and determining optimal population size.

Key words: *Brassica rapa*, genetic variation, heterosis, PCR, RAPD

INTRODUCTION

The production of doubled haploid (DH) plants is a tool that is being used to shorten the time required to improve *Brassica* populations. A completely homozygous plant can be obtained in a single generation. This leads to a decrease in the time required for selection of desirable plants over traditional breeding (Chen and Beversdorf, 1990). The production of DH plants in *Brassica rapa* leads to inbreeding depression and therefore diminishes their usefulness (Dewan et al., 1995). It is necessary to recover at least the level of agronomic performance and vigour that existed in outcrossing *B. rapa* populations in order for the development of DH plants to lead to improved *B. rapa* cultivars.

Breeding of naturally outcrossing populations requires the establishment and maintenance of genetic variation to maintain vigour through heterosis. This is particularly important in the sporophytically self-incompatible *B. rapa* where traditional breeding methods have involved mass selection, hybridization or development of synthetics, all of which rely on outcrossing.

Genetic variability can be detected by examining the phenotype of individuals in a population. However, phenotype is influenced by the environment and its interaction with a particular genotype. Genetic analysis has been applied to detect genetic variation in various crops using molecular DNA techniques such as restriction fragment length polymorphisms (RFLP) (Kidwell et al., 1994) and random amplified DNA (RAPD) (Demeke et al., 1996; Mailer et al., 1994). RFLP analysis is a time-consuming,

expensive and labour intensive process which requires large quantities of high integrity genomic DNA. RAPD analysis requires less DNA, time and equipment than RFLP analysis. Unlike some polymerase chain reaction (PCR) assays, RAPD analysis identifies polymorphism independent of prior DNA sequence knowledge. RAPD analysis is based on PCR, where DNA sequences are amplified using arbitrary primers and then separated by electrophoresis to detect polymorphisms (Williams et al., 1990). RAPD analysis provides a method of characterizing genotypic variation between individuals without the influence of environment. Genotypes can be selected to maximize genetic variation in a population and this heterogeneity may contribute to the agronomic performance when plants are crossed.

This study reports on the application of RAPD analysis to detect genetic variation in populations of DH lines in *B. rapa*. The maintenance of this variation was investigated through two generations of outcrossing populations consisting of different numbers of individual DH lines.

MATERIALS AND METHODS

The DH lines were produced using the microspore culture protocol reported by Ferrie and Keller (1995), and the first generation seed was provided by Dr. Allison Ferrie (PBI, Saskatoon). DH lines were second generation selfed DH lines from Reward and second generation selfed DH lines from DSC-3, a breeding line from Agriculture and Agri-Food Canada.

DH lines (C_0) were grouped into populations of 4, 8, 12 and 22 lines with one

plant from each line. The DH plants were randomly intercrossed in isolation tents covered with clear plastic containing 2-mm holes and brush pollinated every other day with a feather duster in the greenhouse for two generations to produce composite populations (C_1 and C_2). There were two replicates grown for each of DSC-3 and Reward DH populations. The position of the plants in the pollination tent was rotated every 4 days after flowering began. Seed was harvested from single plants and an equal amount from each plant was composited to produce the first generation composite (C_1). From each of these C_1 populations, 60 plants were seeded in individual pots and randomly intercrossed to produce the second generation composite (C_2). The composite population size was set at 60 individual plants after one generation of intercrossing regardless of their original size to ensure adequate seed production for the 1996 field season. The Reward C_1 and C_2 populations were evaluated in the field (1996) and single plants were sampled and analyzed for genetic variation.

Plant Materials

RAPD analysis was performed on populations of 22 and 23 DH lines derived from breeding line DSC-3 and cv Reward, respectively.

At the 4 -5 leaf stage, all leaves from 10 - 15 plants of each DH line were excised and pooled. The DH lines were grown under controlled conditions in a growth chamber. At the 5 - 6 leaf stage, 4-5 leaves from randomly selected single plants were excised in the field and individually prepared for DNA analysis. Each individual plant

represented one sample from C_1 and C_2.

DNA Extraction

Leaf material was frozen with liquid nitrogen, lyophilized in a freeze drier for a minimum of 48 hours and then stored at - 20°C. DNA was isolated from the lyophilized tissue (200 mg) suspended in a buffer solution containing cetyltrimethylammonium bromide (CTAB). This procedure was based on the method reported by Kidwell and Osborn (1992). Two chloroform extractions were performed to remove insoluble debris and soluble proteins. DNA was precipitated with ethanol (-20°C) and a pellet was recovered and washed twice with 75% ethanol (-20°C) and 10 mM ammonium acetate. The pellet was dried and dissolved in 300 μl 10 mM Tris/ 1 mM EDTA (TE) buffer.

DNA extracted from plants grown under controlled and field conditions yielded approximately 114 to 2280 μg and 105 to 2164 μg of DNA respectively. The amount of time required to dissolve the DNA samples obtained from the field samples was several hours compared to several minutes with growth chamber plants. The field samples were taken at a more advanced stage of plant development than the growth chamber samples and therefore they may have contained more impurities, such as carbohydrates, as demonstrated by some 260/280 ratios recorded with the UV spectrophotometer falling outside of the acceptable range of 1.8-2.0. Reduced DNA yields from *Brassica* were also found by Kidwell and Osborn (1992) when they sampled leaf tissue of different maturity and stress levels.

The DNA concentration was measured using an ultraviolet spectrophotometer at

260 nm. To check for DNA degradation, the samples were separated electrophoretically on ethidium bromide stained 0.75% agarose gels in 0.5 x TAE buffer (tris/sodium/acetate/EDTA pH 8.0). Isolated DNA was stored in TE buffer at -20°C.

Polymerase Chain Reaction (PCR)

A set of 30 primers (oligonucleotides), obtained from the University of British Columbia (UBC), was tested to detect polymorphism between the donor populations of DCS-3 and Reward. PCR was performed using 50 ng/μl of genomic DNA in 25 μl volumes using a PTC-100™ (MJ Research, Inc.), as reported by Mailer et al. (1994). RAPD products were separated by electrophoresis on ethidium bromide stained 1.4 % agarose gels in 1x TAE buffer. Genomic DNA from *B. napus* and a negative control were exposed to the PCR conditions with the analysis of each new primer. Lambda DNA digested with *Hind*III (Pharmacia Biotech) was included as the size marker.

RAPD Analysis

Primers that expressed polymorphism between DSC-3 and Reward donor populations were selected from the initial primer set. These primers, shown in Table 5.1, were tested against each of the DH lines produced from the two donor populations.

UBC primers 329 and 338 were selected for RAPD analysis because they resulted in the highest amount of informative bands being expressed among all of the primers used to analyze the DH lines (Table 5.2). This was determined by calculating

the percent of polymorphism present, which equals the number of bands that were polymorphic divided by the total number of bands. Primer 318 was not considered because it produced only monomorphic bands in the DSC-3 DH lines.

The number of individuals analyzed in C_1 and C_2 was determined by the frequency of bands expressed in the DH lines (Table 5.3). The lowest band frequency (p) with a specific primer was calculated by dividing the number of individuals with the least frequently expressed band by the total number of individual lines in the DH population. Then the number of plants (N) sampled from C_1 and C_2 was calculated to give a 95% probability (P) that the least frequent allele (p) would be represented, as shown in the following equation (Jasieniuk et al., 1996):

$$N = \frac{\ln(1 - P)}{\ln(1-p)}$$

RAPD fragment sizes were estimated from the gels in relation to the size marker. Data matrix tables were compiled where bands were recorded as either present (1) or absent (0), as shown in Table 5.4 and Appendix 11-14.

RESULTS AND DISCUSSION

RAPD Analysis

Polymorphism was detected for 9 of the original 30 primers tested between the DSC-3 and Reward donor populations (data not shown). When tested on the DH lines in both populations, 5 primers produced bands that expressed adequate intensity to

distinguish between their presence or absence. These primers and their sequences are shown in Table 5.1.

A total of 98 RAPD products were visualized, including 57 polymorphic and 41 monomorphic bands. The number of bands produced per primer ranged from 4 to 15.

Polymorphism was expected between the DSC-3 and Reward donor populations. Primers used in this test differed in their ability to detect genetic variation in each of the collection of DH lines (Table 5.2). Bands expressed when DSC-3 was amplified with primer 318 (UBC) were monomorphic for the DH lines. All other primers resulted in polymorphic expression of bands in the DH lines from both donor populations (Fig 5.1).

Application of RAPD analysis

Genetic variation and therefore the RAPD pattern expressed in a population is affected by the number of individual plants in the population and the number of generations of inbreeding occurring (Falconer and Mackay, 1996). RAPD analysis demonstrated that as the number of individual DH lines included to create each composite population increased, there was more genetic variability expressed in C_0 (Table 5.5). After one and two generations of interpollination, all C_1 and C_2 populations expressed less polymorphism than the corresponding DH lines (C_0) used in their production. This is expected because of the restricted number of lines used to produce C_1 and C_2. Interpollination results in eventual fixation or loss of alleles. The level of polymorphism that was reached in C_1 and C_2 was similar for all population sizes. The lowest level of polymorphism was expressed when only 4 DH lines contributed to C_1

and C_2 and a similar level of polymorphism was observed over the two generations of interpollination. The smaller C_1 and C_2 populations reached a fixed level of polymorphism faster because they were closer to this fixation of alleles initially.

Using RAPD analysis as an indication of genetic variation leads to the conclusion that the expression of heterogeneity would be greatest with interpollination of 22 DH lines which had the greatest level of genetic variation initially (C_0) (Table 5.5). Starting with larger numbers of individual DH lines should therefore optimize the recovery of agronomic performance of *B. rapa*. However, agronomic field trials performed in 1996 on the Reward C_1 and C_2 demonstrated that sufficient variability existed between 8 DH lines to recover population performance equal to and greater than that of the donor population (Friesen, 1997). Field evaluation and RAPD analysis were in agreement in the assessment of consistent agronomic performance and genetic variation after the two generations of outcrossing (C_1 and C_2).

RAPD analysis provides an effective method of characterizing the genotypic variation present in DH and composite populations. This method has an advantage over morphological evaluation of genotypic variation in saving time and reducing the environmental effect on expression of the characters. It is also a sampling method which may be used to analyze a character or genetic variation in plants at a young vegetative stage or plants which are limited in their ability to produce seed. RAPDs provide the ability to optimize the amount of variation present in a population, reducing the effects of inbreeding and maximizing the recovery of vigour and agronomic performance of *B. rapa*. This provides assistance in using DH plants as a tool in population improvement in *B. rapa*.

Table 5.1. Primers used to characterize genetic variation in *B. rapa* DH populations.

UBC primer	Nucleotide sequence (5' -> 3')
312	ACG GCG TCA C
318	CGG AGA GCG A
329	GCG AAC CTC C
337	TCC CGA ACC G
338	CTG TGG CGG T

Table 5.2. Polymorphic levels in C_1 and C_2 populations determined by RAPD analysis.

Primer (UBC)	DH lines	Polymorphism[1] (%)
312	DSC-3	22
	Reward	72
329	DSC-3	56
	Reward	53
337	DSC-3	28
	Reward	50
338	DSC-3	38
	Reward	63

[1] Polymorphism (number of polymorphic bands divided by total number of bands)

Table 5.3. The number of single plants of C_1 and C_2 sampled from the four sizes (4, 8, 12, 22) of DH populations of *B. rapa* for RAPD analysis.

Primer (UBC)	DH lines	DH line population	N range[1]
329	Reward	4	12
		8	12
		12	12
		22	15
338	Reward	4	12
		8	7
		12	6
		22	8

[1] Number of plants sampled [logarithm of 1 minus the percent probability that the least frequent allele is present (95%) divided by the logarithm of 1 minus the lowest allele frequency]

Table 5.4. Data matrix of RAPD products for the DSC-3 donor population (D) and DH lines with primer 318 and 337 (UBC).

Primer (UBC)	Marker size (kb)	DH lines																						
		D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
318	2.32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.66	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
337	1.74	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0
	1.62	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1
	1.55	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	1
	1.35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	1.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	0.72	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 5.5. Proportion of polymorphic loci detected in four population sizes (4, 8, 12, 22) of *B. rapa* cv. Reward developed from DH lines (number of polymorphic loci).

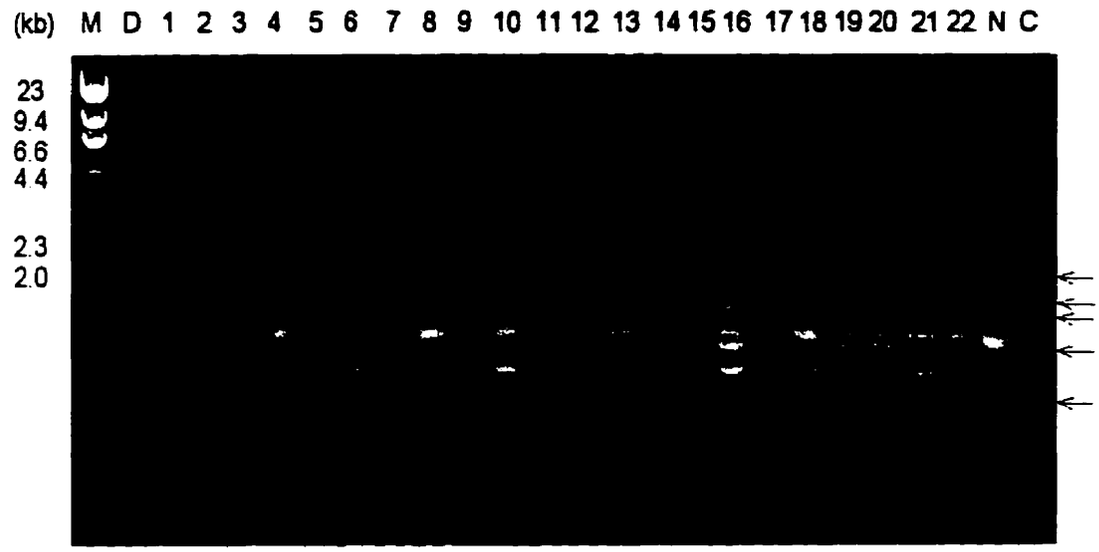
	Generation											
Primer	4			8			12			22		
(UBC)	C_0[1]	C_1[2]	C_2[3]	C_0	C_1	C_2	C_0	C_1	C_2	C_0	C_1	C_2
329	40	40	47	53	53	47	60	40	41	60	29	33
	(6)	(6)	(7)	(8)	(9)	(8)	(9)	(6)	(7)	(9)	(4)	(5)
338	50	17	36	50	33	36	67	33	27	83	46	43
	(3)	(2)	(4)	(3)	(4)	(4)	(4)	(4)	(3)	(5)	(6)	(6)

[1]DH lines

[2]First composite population

[3]Second composite population

Fig. 5.1. **RAPD profiles from the DSC-3 donor (D) population and DH lines (1-22) expressed as polymorphic RAPDs with primer 337 (UBC). (M, size marker; N, *B. napus*;** C, negative control)



6. GENERAL DISCUSSION AND CONCLUSION

The purpose of this project was to determine if DH lines can be effectively applied to improve *B. rapa* populations. This project involved the development of composite populations by interpollinating DH lines to reduce the effects of inbreeding depression.

The project required a method of obtaining or increasing enough seed from the DH plants for field and greenhouse study. Traditionally, the labour-intensive process of bud pollination is used in *B. rapa* to overcome SI. This involves bud emasculation and forced self-pollination prior to flowering while the stigma is still receptive to self-pollen. A more efficient method of overcoming the SI barrier was discovered with NaCl spray solutions of 3 and 5 %. All treatments were effective at promoting self-compatibility but the NaCl solution treatments produced more seed with less time and lower material requirements than the bud pollination treatment.

The *B. rapa* population improvement study involved the interpollination of 4, 8, 12 and 22 DH lines from the cv. Reward and the breeding line DSC-3 to develop composite populations . The composite populations demonstrated agronomic performance exceeding that of the individual DH lines from both sources. The number of DH lines required to contribute to the composite population was small. Only four DH lines from the breeding line DSC-3 and eight DH lines from the cv. Reward were required to improved population performance over that of the contributing DH lines.

RAPD analysis was studied to determine its effectiveness in detecting genetic variation between the composite populations and the DH lines used in their

development. RAPD analysis provided a rapid method of successfully detecting polymorphism between composite populations and the DH lines. This method showed potential in supplementing morphological evaluation of genotypic variation because it avoids the influence of environmental effects on the observed variation.

In order to obtain the benefits of using DH plants in the improvement of *B. rapa* an efficient system of DH production is required. *B. rapa* is more recalcitrant to tissue culture than *B. napus* and therefore methodology for DH production is not as well developed (Baillie et al., 1992). Genotype, conditions of the donor plant, culture media and culture environment influence the efficiency of DH regeneration from tissue culture. Modifications, such as increased sucrose concentrations in media and shorter incubation periods, have been made in microspore culture protocols for *B. napus* to promote embryogenesis in *B. rapa* (Ferrie and Keller, 1995). This project intended to study further modifications for regeneration of *B. rapa* plants from microspore-derived embryos with the objective of increasing efficiency of DH plant production. The modification that may have been useful at improving plant regeneration was promoting dormancy and desiccation in microspore-derived embryos which occurs naturally in zygotic embryos. However, embryo yields from the microspore culture were too low to continue with desiccation. Currently, the inability to produce DH lines from all genotypes imposes a major constraint on the general application of DH technology in *B. rapa* breeding.

This study suggests that the development of composite populations has potential in speeding up cultivar development. DH technology allows the fixation of traits in

B. rapa DH lines. Selected DH lines can then be randomly interpollinated into composite populations to improve the agronomic performance of the population. It may be useful to identify and select optimum DH lines for development of the composite populations by determining combining abilities expressed in controlled crosses. However, this would be time consuming and labour intensive. It may be necessary to determine the optimum number of DH lines required for interpollination in the development of composite populations for different populations.

RAPD analysis may provide a rapid, easy method of characterizing genotypes for optimization of composite population development by identifying groups of DH lines that are heterogeneous. Incorporation of these lines may maximize the heterotic effect or minimize the level of inbreeding that could be obtained through interpollination of the lines. RAPD analysis determined that the populations produced from 22 DH lines should have led to the greatest level of heterogeneity and potentially the most improvement in agronomic performance above the DH lines. This interpretation differed from the results of the field study of composite development which determined that interpollination of only 8 Reward DH lines was required to improve the population performance above that of the DH lines. Contradiction between these results may be the result of the limitations in the field study which encompassed only one year and two locations.

RAPD analysis may also be useful in future studies for predicting the level of heterosis that may be expressed when DH lines are chosen and intercrossed based on their RAPD profile. To further optimize interpollination between individual DH lines

when developing the composite population it may be useful to identify which S-alleles are acting in the individuals. This is a labour-intensive process but this information is becoming available with the study of genetic markers.

DH technolcgy has the potential to shorten the time frame for population improvement in a breeding program where recurrent cycles of selection are traditionally required to obtain high levels of homozygosity. DH technology has not been as widely applied in SI *B. rapa* as in self-pollinating crops such as *B. napus*, due to the effects of inbreeding depression that occur when DH plants are regenerated from *B. rapa*. The development of composite populations and the use of RAPD analysis show potential in contributing to the progress of cultivar development. Further study is required to confirm these results.

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8. APPENDIX

Appendix 1. Meteorological report from seeding date in May to August 1995 in Winnipeg.

Appendix 2. Meteorological report from seedling date in May to August 1996 in Winnipeg and Carman.

Appendix 3. Measurements of fertility index and disease index (DI) for DH lines in 1995.

Appendix 4. Field measurements for parameters characterizing the cv. Reward DH lines (Plant Id. 1-19) and the Reward checks (Plant Id. 20-48) in Winnipeg, 1996.

Appendix 5. Field measurements for parameters characterizing the DSC-3 DH lines (Plant Id. 1-16) and the Reward checks (Plant Id. 17-36) in Winnipeg, 1996.

Appendix 6. Field measurements for parameters characterizing the Reward C_1 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996.

Appendix 7. Field measurements for parameters characterizing the Reward C_2 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996.

Appendix 8. Field measurements for parameters characterizing the DSC-3 C_1 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996.

Appendix 9. Field measurements for parameters characterizing the DSC-3 C_2 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996.

Appendix 10. The mean performance of *B. rapa* cv. Reward DH lines (Id. 1 - 19) and donor checks (Id. 20 - 48) relative to the overall mean performance of the Reward donor checks for parameters: seedling emergence (a), days to flowering (b), days to maturity (c), height (d), lodging (e), harvest index (f) and seed oil content (g), 1996.

Appendix 11. Data matrix of RAPD products for the DSC-3 donor population (D) and DH lines

Appendix 1. Meteorological report from seeding date in May to August 1995 in Winnipeg.

Month	Day	Temperature			Precipitation
		Mean	Min	Max	(mm)
5	29	21.58	10.56	30.12	0.00
5	30	24.90	15.19	33.92	0.00
5	31	24.70	15.34	32.03	0.00
6	1	21.23	13.35	28.38	0.00
6	2	21.00	13.27	29.09	0.00
6	3	21.74	15.01	30.83	1.02
6	4	24.39	15.03	31.83	0.00
6	5	24.67	15.55	31.05	0.25
6	6	17.42	7.31	27.62	1.02
6	7	9.83	7.15	14.19	0.00
6	8	12.85	3.19	19.48	0.00
6	9	17.27	7.68	25.89	0.00
6	10	15.61	9.32	20.95	0.00
6	11	17.58	7.33	25.65	0.00
6	12	21.41	8.56	29.70	0.00
6	13	23.15	14.26	30.66	0.00
6	14	22.99	16.57	32.08	4.32
6	15	26.75	17.94	34.92	0.00
6	16	29.01	23.50	34.54	0.00
6	17	31.26	23.31	38.41	0.00
6	18	30.12	24.20	36.63	0.00
6	19	27.87	19.31	35.59	0.00
6	20	26.02	18.78	36.00	11.43
6	21	24.17	17.85	31.88	0.00
6	22	24.13	18.94	31.78	0.25
6	23	21.06	17.13	26.11	0.25
6	24	17.46	16.26	18.79	0.51
6	25	18.29	14.01	22.41	0.00
6	26	20.34	15.06	24.97	0.00
6	27	23.03	17.26	27.47	0.00
6	28	23.08	17.37	29.16	0.00
6	29	14.13	11.67	17.37	3.05
6	30	14.36	11.12	19.00	3.56
7	1	16.47	9.14	22.36	0.00
7	2	19.21	10.54	26.79	0.00
7	3	19.55	14.40	25.80	0.00
7	4	19.11	15.43	24.02	0.00
7	5	15.36	13.62	17.25	4.83
7	6	18.02	12.81	23.28	0.00
7	7	19.21	11.17	28.31	0.76
7	8	21.16	13.99	26.12	0.00
7	9	23.01	13.92	30.38	0.00
7	10	22.84	16.65	27.58	0.00
7	11	24.07	16.67	31.91	3.05
7	12	27.45	21.47	32.74	0.00
7	13	24.82	19.50	30.67	0.00
7	14	22.73	17.16	28.13	0.00
7	15	21.24	14.41	29.70	14.22
7	16	18.84	16.53	22.16	7.62
7	17	19.77	15.74	24.21	0.00

Appendix 1. (continued)

Month	Day	Temperature			Precipitation
		Mean	Min	Max	(mm)
7	18	20.35	13.80	26.04	0.00
7	19	19.82	16.44	24.81	1.02
7	20	20.72	13.98	26.77	0.00
7	21	20.84	16.63	25.67	0.00
7	22	20.04	13.85	26.10	0.00
7	23	22.99	17.49	29.19	0.00
7	24	18.62	14.42	22.24	0.76
7	25	18.80	11.97	25.70	0.00
7	26	21.96	14.36	28.19	0.00
7	27	22.89	17.70	29.16	0.00
7	28	21.11	15.23	25.83	0.00
7	29	21.01	11.02	28.56	0.00
7	30	23.77	14.81	29.77	0.51
7	31	18.25	13.60	24.23	0.00
8	1	21.28	13.69	28.58	0.00
8	2	22.37	16.50	30.79	0.00
8	3	18.73	12.59	24.54	0.00
8	4	20.96	11.97	28.82	0.00
8	5	23.82	13.32	31.92	0.00
8	6	24.13	17.40	30.75	0.00
8	7	26.02	19.36	33.57	0.00
8	8	24.17	18.16	31.44	2.79
8	9	20.76	14.67	27.11	0.00
8	10	18.17	12.78	23.54	0.00
8	11	17.64	10.48	24.07	0.00
8	12	20.00	12.97	26.53	0.00
8	13	21.19	13.09	31.18	2.29
8	14	18.02	13.77	23.86	0.00
8	15	23.37	12.72	33.20	0.00
8	16	24.93	17.97	31.35	0.00
8	17	26.34	20.11	33.33	0.00
8	18	20.83	14.02	24.54	75.90
8	19	19.52	13.04	26.67	0.00
8	20	21.13	14.52	27.80	0.00
8	21	19.37	12.09	26.40	0.00
8	22	19.84	13.85	28.64	17.02
8	23	16.28	12.40	21.81	0.00
8	24	18.08	14.92	22.68	0.00
8	25	17.98	14.19	24.30	0.25
8	26	18.89	11.06	27.75	0.00
8	27	20.06	14.74	27.65	3.81
8	28	18.39	14.04	23.55	0.00
8	29	20.66	11.88	28.06	25.40
8	30	20.34	14.05	23.21	1.27
8	31	16.99	11.74	23.22	0.25

Appendix 2. Meteorological report from date of seeding in May to August 1996 in Winnipeg and Carman.

Location	Month	Day	Temperature			Precipitation
			Mean	Min	Max	(mm)
Winnipeg	5	28	18.17	7.84	25.93	0.00
	5	29	18.31	8.23	26.04	0.00
	5	30	18.71	9.19	25.00	0.00
	5	31	16.19	13.95	19.59	12.95
	6	1	17.96	13.48	25.27	3.30
	6	2	11.34	6.18	14.89	0.00
	6	3	11.45	5.55	19.69	0.00
	6	4	17.78	7.22	25.13	0.00
	6	5	—	—	—	—
	6	6	—	—	23.24	0.00
	6	7	19.72	9.15	26.47	0.00
	6	8	22.96	15.32	29.36	0.00
	6	9	25.27	16.71	32.86	0.00
	6	10	24.53	16.79	33.86	1.27
	6	11	24.19	15.41	34.28	0.00
	6	12	21.65	14.59	26.22	0.00
	6	13	21.84	12.49	29.47	0.00
	6	14	24.69	16.30	32.39	0.00
	6	15	22.56	15.06	28.47	0.25
	6	16	19.23	13.18	25.22	0.00
	6	17	22.54	13.35	30.10	0.00
	6	18	23.12	16.40	29.84	0.00
	6	19	17.57	12.31	21.22	4.57
	6	20	19.20	12.44	24.83	0.00
	6	21	13.86	8.32	19.01	0.00
	6	22	10.84	6.66	16.41	0.00
	6	23	10.17	5.77	13.14	2.54
	6	24	15.29	9.19	21.51	0.00
	6	25	14.88	8.42	21.74	3.05
	6	26	20.24	15.98	23.05	0.00
	6	27	28.49	20.02	35.35	0.00
	6	28	27.61	23.15	32.95	2.79
	6	29	23.43	18.67	30.47	1.02
	6	30	20.73	14.49	26.37	0.00
	7	1	21.55	15.36	28.07	0.00
	7	2	23.42	13.70	30.75	0.00
	7	3	23.49	13.29	30.41	0.00
	7	4	23.99	17.54	30.29	0.00
	7	5	24.38	20.62	29.72	0.25
	7	6	22.33	16.65	26.13	0.00
	7	7	16.85	12.36	24.85	11.68
	7	8	15.62	13.09	20.21	4.57
	7	9	17.74	9.57	24.89	0.00
	7	10	19.96	10.35	26.41	6.60
	7	11	18.44	15.08	24.33	5.33
	7	12	19.89	15.32	25.49	0.00
	7	13	21.34	14.37	29.40	0.00
	7	14	21.10	16.06	26.64	0.00
	7	15	21.17	14.04	27.88	0.00
	7	16	21.41	14.12	26.74	12.95
	7	17	24.74	19.05	30.26	0.00

Appendix 2. (continued)

Location	Month	Day	Temperature			Precipitation
			Mean	Min	Max	(mm)
Winnipeg	7	18	23.21	18.93	28.27	0.51
	7	19	20.65	17.12	25.97	0.00
	7	20	19.53	14.45	25.73	14.99
	7	21	21.23	15.09	27.87	0.51
	7	22	18.37	12.04	23.89	0.00
	7	23	17.71	14.89	21.14	0.00
	7	24	18.52	14.36	23.73	2.03
	7	25	19.22	12.57	25.70	0.00
	7	26	17.74	12.17	24.83	0.00
	7	27	19.08	14.47	24.68	0.00
	7	28	17.01	13.23	20.96	0.00
	7	29	19.21	14.58	26.54	8.13
	7	30	20.05	13.68	25.88	0.00
	7	31	20.65	12.79	27.06	0.00
	8	1	21.08	12.87	28.16	0.00
	8	2	22.63	16.44	28.33	0.00
	8	3	23.38	16.98	29.09	0.00
	8	4	22.33	18.85	28.97	77.00
	8	5	22.99	16.65	28.72	0.00
	8	6	21.31	16.10	24.35	0.00
	8	7	17.67	14.07	23.50	9.65
	8	8	15.68	12.21	19.43	0.00
	8	9	17.14	12.67	22.67	0.00
	8	10	20.06	11.20	27.77	0.00
	8	11	21.31	14.58	25.50	0.00
	8	12	19.10	10.50	26.09	0.00
	8	13	19.16	14.83	24.86	0.00
Carman	5	23	9.90	5.33	14.46	0.00
	5	24	9.77	1.89	16.01	0.00
	5	25	11.59	2.91	19.52	0.00
	5	26	15.20	5.33	21.71	0.00
	5	27	16.44	6.45	23.51	0.00
	5	28	16.11	5.37	24.09	0.00
	5	29	17.16	6.42	25.44	0.00
	5	30	17.75	7.61	24.90	0.00
	5	31	14.97	13.30	18.88	15.20
	6	1	17.27	10.79	24.00	0.00
	6	2	9.49	6.23	12.75	0.20
	6	3	12.40	5.98	19.35	0.00
	6	4	16.34	6.16	23.62	0.00
	6	5	13.25	11.02	16.11	14.00
	6	6	15.57	10.06	21.75	0.20
	6	7	18.44	7.38	26.26	0.00
	6	8	21.85	11.72	29.43	0.00
	6	9	24.86	14.57	33.67	0.00
	6	10	24.09	15.75	31.35	4.60
	6	11	23.18	13.32	33.01	0.00
	6	12	20.98	13.09	25.40	0.00
	6	13	20.86	9.92	28.07	0.00
	6	14	22.82	11.95	30.19	6.40
	6	15	20.23	14.48	24.80	0.00
	6	16	18.03	10.07	24.74	0.00

Appendix 2. (continued)

Location	Month	Day	Temperature			Precipitation
			Mean	Min	Max	(mm)
Carman	6	17	21.15	10.45	28.48	0.00
	6	18	22.15	14.54	28.01	0.00
	6	19	15.72	11.53	21.87	30.00
	6	20	17.88	10.64	23.65	0.00
	6	21	12.83	9.37	17.04	0.00
	6	22	9.68	6.66	14.03	0.60
	6	23	9.25	7.24	10.67	4.80
	6	24	13.24	8.89	18.91	0.00
	6	25	13.34	8.19	17.08	8.00
	6	26	18.98	14.67	23.49	0.00
	6	27	25.69	18.77	32.67	0.00
	6	28	25.12	21.23	29.75	0.00
	6	29	23.37	17.78	28.72	0.20
	6	30	19.50	12.34	25.09	0.00
	7	1	18.89	11.43	25.33	1.60
	7	2	19.58	8.65	27.92	0.00
	7	3	21.18	12.44	27.99	0.00
	7	4	22.50	15.83	29.43	0.00
	7	5	22.07	17.16	26.77	0.40
	7	6	20.44	12.54	24.70	0.00
	7	7	15.35	8.96	23.32	8.80
	7	8	14.88	10.36	18.82	0.60
	7	9	15.66	8.21	22.25	0.00
	7	10	17.38	7.62	24.35	3.00
	7	11	16.75	13.73	21.43	1.60
	7	12	19.03	13.85	23.49	2.20
	7	13	20.10	13.87	25.51	0.40
	7	14	17.59	12.75	22.68	32.40
	7	15	19.05	10.68	25.51	0.00
	7	16	19.87	12.54	25.75	1.80
	7	17	22.44	18.83	26.54	0.00
	7	18	21.86	17.28	26.49	13.60
	7	19	19.90	15.86	24.69	0.00
	7	20	19.72	15.51	25.10	22.60
	7	21	18.96	11.89	25.54	0.00
	7	22	17.60	11.94	22.93	0.00
	7	23	16.53	14.46	21.32	5.00
	7	24	17.36	13.86	21.55	0.20
	7	25	17.28	11.85	22.81	0.00
	7	26	15.45	11.42	20.22	3.20
	7	27	16.59	13.97	20.93	0.40
	7	28	15.61	12.52	17.93	0.00
	7	29	16.93	12.52	22.04	13.00
	7	30	17.69	12.11	24.01	0.00
	7	31	18.30	11.42	23.97	0.00
	8	1	18.30	11.11	24.83	0.00
	8	2	20.60	13.73	26.54	0.00
	8	3	21.87	17.46	27.58	0.20
	8	4	20.13	15.60	27.15	15.00
	8	5	20.57	11.90	27.32	0.00
	8	6	18.67	12.47	23.12	0.00
	8	7	16.71	11.87	22.46	1.00
	8	8	15.07	10.72	19.47	0.00

--- = missing data

Appendix 3. Measurements of fertility index and disease index (DI) for DH lines in 1995.

Genotype	DH line	Replicate	Fertility Index (1-5)	DI (%)
DSC-3	1	1	5	0
		2	5	0
	2	1	4	0
		2	5	0
	3	1	5	0
		2	4	0
	4	1	3	0
		2	5	0
	5	1	5	82
		2	4	22
	6	1	3	90
		2	5	0
	7	1	—	100
		2	5	44
	8	1	2	0
		2	5	0
	9	1	4	0
		2	4	6
	10	1	4	96
		2	4	0
	11	1	4	0
		2	3	0
	12	1	—	6
		2	4	0
	13	1	5	0
		2	3	0
	14	1	4	0
		2	3	0
	15	1	4	100
		2	3	30
	16	1	4	93
		2	2	36
	17	1	4	0
		2	1	0
	18	1	3	0
		2	3	0
	19	1	3	85
		2	3	60
	20	1	2	0
		2	3	0
	21	1	2	0
		2	3	0
	22	1	3	83
		2	2	13
Reward	1	1	5	10
		2	5	0
	2	1	5	20
		2	5	0
	3	1	5	31
		2	5	17
	4	1	5	36
		2	5	53

Appendix 3. (continued)

Genotype	DH line	Replicate	Fertility Index (1-5)	DI (%)
	5	1	5	0
		2	4	0
	6	1	4	67
		2	5	44
	7	1	10	5
		2	69	3
	8	1	4	0
		2	4	0
	9	1	4	0
		2	4	0
	10	1	4	0
		2	4	0
	11	1	4	0
		2	4	0
	12	1	4	96
		2	4	58
	13	1	3	0
		2	4	0
	14	1	3	0
		2	4	0
	15	1	3	0
		2	4	0
	16	1	3	0
		2	4	0
	17	1	3	9
		2	4	13
	18	1	4	13
		2	3	33
	19	1	3	63
		2	4	67
	20	1	4	0
		2	2	0
	21	1	3	0
		2	3	0
	22	1	3	0
		2	—	0

— = missing data

Appendix 4. Field measurements for parameters characterizing the cv. Reward DH lines (C (Plant Id. 1-19) and the Reward checks (Plant Id. 20-48) in Winnipeg, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
1	1	7.0	24.0	56.0	105.0	5.0	53.0	0.133	36.6
1	2	6.0	24.0	60.0	130.0	5.0	63.0	0.105	35.6
2	1	10.0	17.0	57.0	105.0	3.0	69.0	0.197	44.8
2	2	6.0	17.0	56.0	65.0	5.0	80.0	0.229	43.8
3	1	16.0	26.0	58.0	115.0	5.0	52.0	0.116	41.0
3	2	11.0	21.0	57.0	120.0	5.0	67.0	0.134	41.9
4	1	5.0	21.0	59.0	95.0	5.0	51.0	0.093	41.4
4	2	4.0	22.0	60.0	85.0	5.0	31.0	0.078	43.1
5	1	7.0	17.0	57.0	80.0	5.0	107.0	0.153	39.5
5	2	4.0	18.0	56.0	60.0	5.0	70.0	0.175	40.0
6	1	1.0	22.0	57.0	70.0	4.0	12.0	0.160	—
6	2	3.0	21.0	57.0	105.0	5.0	70.0	0.233	46.4
7	1	11.0	21.0	57.0	95.0	5.0	151.0	0.232	45.9
7	2	7.0	21.0	59.0	110.0	5.0	156.0	0.240	46.0
8	1	2.0	26.0	59.0	85.0	5.0	38.0	0.127	40.0
8	2	4.0	17.0	59.0	85.0	4.0	13.0	0.087	—
9	1	15.0	18.0	56.0	95.0	5.0	128.0	0.233	45.6
9	2	10.0	19.0	56.0	110.0	5.0	140.0	0.200	42.6
10	1	16.0	17.0	56.0	75.0	5.0	12.0	0.120	—
10	2	12.0	19.0	56.0	65.0	5.0	28.0	0.187	46.5
11	1	4.0	29.0	59.0	80.0	5.0	12.0	0.080	—
11	2	8.0	28.0	59.0	80.0	5.0	27.0	0.108	36.0
12	1	14.0	23.0	56.0	75.0	4.0	70.0	0.140	41.3
12	2	12.0	22.0	56.0	70.0	5.0	92.0	0.153	41.3
13	1	12.0	19.0	56.0	95.0	5.0	181.0	0.278	47.8
13	2	13.0	24.0	60.0	105.0	5.0	171.0	0.263	47.5
14	1	9.0	22.0	58.0	95.0	5.0	80.0	0.200	41.1
14	2	16.0	23.0	57.0	85.0	5.0	121.0	0.269	40.6
15	1	9.0	22.0	57.0	80.0	4.0	89.0	0.223	43.3
15	2	8.0	23.0	56.0	70.0	4.0	108.0	0.309	41.8
16	1	9.0	17.0	56.0	90.0	5.0	155.0	0.258	47.0
16	2	10.0	19.0	56.0	80.0	5.0	140.0	0.311	46.4
17	1	8.0	25.0	57.0	100.0	5.0	53.0	0.076	36.5
17	2	7.0	13.0	58.0	100.0	5.0	81.0	0.095	38.6
18	1	10.0	16.0	57.0	85.0	4.0	152.0	0.253	46.7
18	2	7.0	17.0	56.0	85.0	4.0	128.0	0.284	44.8
19	1	6.0	24.0	58.0	90.0	5.0	38.0	0.127	43.6
19	2	11.0	24.0	58.0	100.0	5.0	46.0	0.131	44.6
20	1	16.0	19.0	60.0	90.0	4.0	309.0	0.309	47.4
20	2	16.0	19.0	60.0	100.0	5.0	213.0	0.266	47.5
21	1	19.0	15.0	56.0	90.0	4.0	283.0	0.314	45.1
21	2	16.0	19.0	60.0	100.0	5.0	265.0	0.252	46.5
22	1	17.0	22.0	63.0	105.0	5.0	347.0	0.315	46.9
22	2	8.0	19.0	60.0	110.0	5.0	245.0	0.306	48.9
23	1	15.0	22.0	63.0	95.0	4.0	343.0	0.327	47.3
23	2	24.0	26.0	66.0	100.0	4.0	338.0	0.270	46.5
24	1	16.0	19.0	60.0	90.0	4.0	323.0	0.294	46.5
24	2	9.0	15.0	56.0	100.0	5.0	257.0	0.257	44.9

Appendix 4. (continued)

Plant Id.	Replicate	EMG	DTF (d)	LOD (d)	DTM (cm)	HT (1-5)	YLD (g)	HI	OIL (%)
25	1	13.0	15.0	56.0	100.0	5.0	367.0	0.367	47.8
25	2	15.0	15.0	56.0	90.0	3.0	302.0	0.263	45.6
26	1	22.0	19.0	60.0	95.0	5.0	299.0	0.272	46.6
26	2	11.0	22.0	63.0	100.0	4.0	291.0	0.265	45.9
27	1	18.0	19.0	60.0	90.0	5.0	260.0	0.306	47.7
27	2	13.0	15.0	56.0	110.0	5.0	367.0	0.294	47.3
28	1	13.0	16.0	57.0	105.0	5.0	218.0	0.291	46.8
28	2	19.0	19.0	60.0	105.0	4.0	400.0	0.333	45.8
29	1	20.0	22.0	63.0	105.0	5.0	373.0	0.311	47.5
29	2	15.0	16.0	56.0	95.0	4.0	417.0	0.334	46.7
30	1	12.0	22.0	63.0	100.0	4.0	358.0	0.298	46.9
30	2	13.0	19.0	60.0	100.0	3.0	317.0	0.276	46.7
31	1	17.0	19.0	60.0	100.0	4.0	310.0	0.282	46.4
31	2	17.0	16.0	56.0	100.0	5.0	328.0	0.285	47.5
32	1	13.0	15.0	56.0	105.0	5.0	363.0	0.346	47.1
32	2	20.0	19.0	60.0	110.0	4.0	349.0	0.268	45.5
33	1	17.0	19.0	60.0	95.0	5.0	350.0	0.259	47.5
33	2	15.0	22.0	63.0	100.0	5.0	366.0	0.318	46.9
34	1	21.0	20.0	60.0	100.0	4.0	352.0	0.306	47.9
34	2	17.0	19.0	60.0	100.0	5.0	306.0	0.291	47.9
35	1	13.0	20.0	60.0	115.0	5.0	227.0	0.303	48.3
35	2	13.0	19.0	60.0	85.0	4.0	264.0	0.293	40.8
36	1	11.0	15.0	56.0	95.0	3.0	430.0	0.319	46.4
36	2	5.0	20.0	60.0	90.0	5.0	345.0	0.276	47.0
37	1	21.0	22.0	63.0	95.0	4.0	446.0	0.319	46.9
37	2	17.0	15.0	56.0	90.0	4.0	336.0	0.305	45.9
38	1	18.0	15.0	56.0	100.0	5.0	296.0	0.296	47.4
38	2	18.0	19.0	60.0	115.0	5.0	273.0	0.237	44.5
39	1	14.0	20.0	60.0	105.0	5.0	293.0	0.308	46.0
39	2	14.0	16.0	56.0	110.0	5.0	318.0	0.277	45.8
40	1	15.0	20.0	60.0	95.0	5.0	271.0	0.301	47.8
40	2	11.0	19.0	60.0	100.0	5.0	283.0	0.226	47.6
41	1	19.0	19.0	60.0	90.0	5.0	326.0	0.310	47.5
41	2	18.0	15.0	56.0	105.0	5.0	323.0	0.281	48.0
42	1	18.0	20.0	60.0	100.0	5.0	236.0	0.295	48.6
42	2	14.0	20.0	60.0	100.0	5.0	247.0	0.291	48.0
43	1	13.0	20.0	60.0	95.0	5.0	317.0	0.334	47.7
43	2	21.0	19.0	60.0	110.0	5.0	348.0	0.303	47.4
44	1	15.0	19.0	60.0	90.0	5.0	260.0	0.306	46.7
44	2	19.0	23.0	64.0	110.0	5.0	311.0	0.311	47.0
45	1	10.0	16.0	56.0	90.0	5.0	295.0	0.328	46.8
45	2	16.0	15.0	56.0	100.0	5.0	286.0	0.318	46.9
46	1	11.0	20.0	60.0	100.0	5.0	248.0	0.292	46.0
46	2	15.0	19.0	60.0	105.0	5.0	365.0	0.261	46.3
47	1	17.0	16.0	56.0	95.0	5.0	381.0	0.318	46.0
47	2	20.0	19.0	60.0	95.0	5.0	265.0	0.279	48.1
48	1	2.0	19.0	60.0	90.0	5.0	426.0	0.328	46.4
48	2	19.0	19.0	60.0	105.0	5.0	307.0	0.279	46.7

— = missing data

Appendix 5. Field measurements for parameters characterizing the DSC-3 DH lines (C0) (Plant Id. 1-16) and the Reward checks (Plant Id. 17-36) in Winnipeg, 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
1	1	10.0	19.0	56.0	80.0	5.0	24.0	0.096	39.9
1	2	6.0	19.0	56.0	105.0	3.0	50.0	0.167	40.3
2	1	12.0	17.0	56.0	85.0	5.0	102.0	0.255	43.8
2	2	8.0	19.0	56.0	85.0	3.0	74.0	0.211	43.6
3	1	9.0	19.0	56.0	75.0	5.0	27.0	0.135	44.3
3	2	9.0	21.0	56.0	60.0	4.0	39.0	0.195	43.0
4	1	5.0	21.0	56.0	105.0	4.0	63.0	0.180	43.2
4	2	9.0	20.0	56.0	80.0	4.0	80.0	0.267	45.3
5	1	12.0	27.0	57.0	60.0	5.0	4.0	0.080	42.6
5	2	9.0	27.0	56.0	50.0	5.0	1.0	0.020	42.6
6	1	3.0	22.0	56.0	100.0	4.0	35.0	0.140	42.0
6	2	1.0	25.0	58.0	100.0	2.0	13.0	0.087	42.0
7	1	13.0	27.0	58.0	90.0	5.0	34.0	0.136	37.7
7	2	7.0	27.0	57.0	85.0	5.0	34.0	0.136	37.6
8	1	4.0	16.0	56.0	75.0	5.0	47.0	0.235	49.9
8	2	7.0	17.0	56.0	70.0	3.0	84.0	0.280	49.2
9	1	2.0	21.0	56.0	100.0	5.0	27.0	0.108	40.0
9	2	2.0	20.0	56.0	90.0	5.0	36.0	0.144	40.9
10	1	3.0	16.0	56.0	85.0	4.0	10.0	0.100	45.2
10	2	2.0	15.0	56.0	70.0	5.0	11.0	0.073	42.6
11	1	3.0	20.0	56.0	70.0	5.0	23.0	0.153	38.3
11	2	1.0	16.0	56.0	75.0	5.0	15.0	0.150	38.3
12	1	7.0	20.0	57.0	90.0	5.0	40.0	0.160	43.8
12	2	5.0	22.0	57.0	90.0	5.0	32.0	0.107	41.5
13	1	8.0	17.0	56.0	90.0	5.0	60.0	0.150	42.7
13	2	2.0	17.0	56.0	70.0	5.0	7.0	0.280	42.7
14	1	4.0	17.0	56.0	100.0	4.0	27.0	0.108	40.6
14	2	2.0	16.0	56.0	80.0	3.0	57.0	0.190	41.8
15	1	20.0	22.0	60.0	70.0	5.0	100.0	0.250	42.9
15	2	11.0	18.0	56.0	80.0	5.0	131.0	0.238	42.1
16	1	4.0	22.0	56.0	100.0	5.0	55.0	0.122	42.6
16	2	2.0	19.0	56.0	90.0	5.0	37.0	0.185	42.8
17	1	13.0	15.0	56.0	120.0	5.0	456.0	0.338	46.4
17	2	15.0	23.0	64.0	90.0	4.0	321.0	0.279	46.3
18	1	12.0	16.0	56.0	100.0	4.0	448.0	0.280	45.3
18	2	15.0	15.0	56.0	95.0	3.0	337.0	0.250	45.6
19	1	25.0	15.0	56.0	95.0	5.0	294.0	0.346	47.7
19	2	10.0	17.0	56.0	110.0	3.0	327.0	0.273	46.2
20	1	8.0	16.0	56.0	100.0	5.0	383.0	0.319	47.0
20	2	15.0	16.0	56.0	95.0	4.0	384.0	0.307	45.9
21	1	13.0	20.0	60.0	110.0	5.0	405.0	0.261	46.2
21	2	6.0	15.0	56.0	90.0	4.0	388.0	0.259	41.8
22	1	8.0	19.0	60.0	100.0	5.0	480.0	0.343	46.3
22	2	16.0	16.0	56.0	90.0	4.0	363.0	0.316	45.6
23	1	21.0	15.0	56.0	100.0	5.0	313.0	0.313	45.8
23	2	17.0	15.0	56.0	100.0	4.0	359.0	0.299	45.5
24	1	10.0	20.0	60.0	120.0	5.0	377.0	0.290	46.6
24	2	20.0	16.0	56.0	95.0	5.0	459.0	0.340	44.7

Appendix 5. (continued)

Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
25	1	12.0	15.0	56.0	100.0	5.0	290.0	0.305	45.2
25	2	22.0	26.0	67.0	100.0	4.0	419.0	0.289	43.1
26	1	15.0	15.0	56.0	100.0	4.0	329.0	0.274	46.2
26	2	14.0	15.0	56.0	105.0	5.0	305.0	0.321	46.8
27	1	12.0	19.0	60.0	100.0	3.0	442.0	0.305	45.6
27	2	11.0	15.0	56.0	110.0	5.0	421.0	0.324	46.1
28	1	16.0	19.0	60.0	95.0	4.0	354.0	0.295	44.5
28	2	20.0	19.0	60.0	105.0	4.0	407.0	0.291	46.2
29	1	14.0	16.0	56.0	100.0	4.0	301.0	0.287	46.0
29	2	10.0	19.0	60.0	105.0	3.0	377.0	0.279	45.5
30	1	16.0	15.0	56.0	105.0	5.0	294.0	0.346	46.9
30	2	9.0	16.0	57.0	100.0	3.0	407.0	0.291	44.2
31	1	14.0	15.0	56.0	85.0	3.0	400.0	0.296	45.8
31	2	16.0	16.0	56.0	95.0	5.0	378.0	0.280	46.1
32	1	16.0	16.0	56.0	100.0	5.0	258.0	0.287	46.2
32	2	19.0	20.0	60.0	95.0	3.0	409.0	0.273	42.4
33	1	17.0	15.0	56.0	100.0	5.0	272.0	0.286	45.2
33	2	20.0	20.0	60.0	100.0	3.0	418.0	0.279	45.2
34	1	22.0	15.0	56.0	100.0	4.0	385.0	0.321	46.4
34	2	17.0	15.0	56.0	95.0	5.0	349.0	0.332	46.1
35	1	12.0	15.0	56.0	100.0	5.0	284.0	0.299	46.1
35	2	18.0	16.0	56.0	95.0	4.0	286.0	0.249	44.4
36	1	9.0	15.0	56.0	110.0	5.0	316.0	0.263	47.0
36	2	22.0	19.0	60.0	90.0	3.0	319.0	0.290	43.4

Appendix 6. Field measurements for parameters characterizing the Reward C1 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Carman	C1-4	1	14.0	30.0	71.0	100.0	4.0	364.0	0.187	42.2
	C1-4	2	10.0	30.0	70.0	125.0	5.0	276.0	0.184	43.2
	C1-4	3	15.0	31.0	70.0	125.0	5.0	236.0	0.163	41.6
	C1-4	4	10.0	29.0	70.0	120.0	5.0	231.0	0.159	40.4
	C1-8	1	11.0	28.0	72.0	120.0	5.0	477.0	0.220	41.9
	C1-8	2	19.0	29.0	69.0	130.0	5.0	459.0	0.224	43.2
	C1-8	3	17.0	27.0	69.0	125.0	5.0	462.0	0.260	41.9
	C1-8	4	11.0	27.0	68.0	125.0	5.0	455.0	0.190	42.6
	C1-12	1	17.0	27.0	70.0	125.0	4.0	500.0	0.200	42.0
	C1-12	2	15.0	28.0	68.0	120.0	4.0	394.0	0.232	43.3
	C1-12	3	10.0	27.0	72.0	120.0	5.0	418.0	0.194	43.0
	C1-12	4	27.0	27.0	70.0	125.0	5.0	331.0	0.170	40.0
	C1-22	1	20.0	28.0	70.0	120.0	3.0	268.0	0.199	43.9
	C1-22	2	13.0	28.0	69.0	125.0	5.0	389.0	0.229	42.7
	C1-22	3	9.0	27.0	71.0	120.0	5.0	425.0	0.193	44.5
	C1-22	4	10.0	29.0	69.0	125.0	5.0	147.0	0.105	44.1
	5	1	13.0	22.0	69.0	115.0	4.0	353.0	0.220	43.6
	5	2	20.0	22.0	68.0	120.0	4.0	304.0	0.760	43.2
	5	3	11.0	23.0	70.0	120.0	5.0	308.0	0.293	43.1
	5	4	9.0	22.0	68.0	125.0	5.0	365.0	0.187	42.6
	6	1	16.0	23.0	70.0	100.0	4.0	353.0	0.220	43.6
	6	2	13.0	22.0	68.0	120.0	5.0	364.0	0.214	42.5
	6	3	14.0	22.0	70.0	120.0	5.0	305.0	0.277	41.6
	6	4	19.0	22.0	69.0	120.0	5.0	274.0	0.157	41.1
	7	1	20.0	23.0	69.0	120.0	3.0	360.0	0.212	41.9
	7	2	18.0	22.0	69.0	125.0	5.0	292.0	0.225	44.1
	7	3	10.0	23.0	70.0	125.0	5.0	301.0	0.232	41.9
	7	4	15.0	22.0	69.0	125.0	5.0	252.0	0.163	40.0
	8	1	9.0	22.0	69.0	115.0	3.0	353.0	0.220	42.3
	8	2	15.0	22.0	68.0	125.0	4.0	395.0	0.226	42.5
	8	3	20.0	23.0	70.0	125.0	4.0	332.0	0.221	41.1
	8	4	14.0	22.0	70.0	120.0	5.0	302.0	0.178	41.8
	9	1	10.0	22.0	70.0	120.0	4.0	409.0	0.195	43.6
	9	2	16.0	23.0	70.0	120.0	4.0	354.0	0.186	42.7
	9	3	13.0	22.0	69.0	125.0	4.0	387.0	0.201	41.1
	9	4	22.0	23.0	69.0	120.0	5.0	255.0	0.232	42.4
	10	1	14.0	22.0	69.0	105.0	5.0	291.0	0.243	42.4
	10	2	12.0	22.0	68.0	120.0	4.0	299.0	0.214	43.8
	10	3	10.0	22.0	68.0	120.0	5.0	394.0	0.202	42.7
	10	4	14.0	22.0	68.0	125.0	5.0	264.0	0.189	42.1
Winnipeg	C1-4	1	30.0	29.0	66.0	125.0	5.0	191.0	0.220	45.4
	C1-4	2	11.0	20.0	58.0	120.0	5.0	209.0	0.250	44.9
	C1-4	3	7.0	19.0	57.0	115.0	5.0	268.0	0.240	42.4
	C1-4	4	20.0	23.0	63.0	110.0	4.0	284.0	0.270	44.8
	C1-4	5	22.0	25.0	60.0	100.0	5.0	317.0	0.290	47.0
	C1-4	6	22.0	19.0	57.0	110.0	5.0	235.0	0.240	45.5

Appendix 6. (continued)

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Winnipeg	C1-8	1	25.0	30.0	67.0	100.0	5.0	187.0	0.290	49.2
	C1-8	2	11.0	23.0	60.0	100.0	4.0	228.0	0.290	47.4
	C1-8	3	9.0	19.0	56.0	110.0	5.0	217.0	0.270	46.9
	C1-8	4	15.0	19.0	56.0	110.0	5.0	173.0	0.250	46.5
	C1-8	5	14.0	20.0	60.0	100.0	5.0	218.0	0.270	48.8
	C1-8	6	13.0	24.0	60.0	105.0	4.0	166.0	0.260	47.5
	C1-12	1	10.0	21.0	57.0	105.0	5.0	191.0	0.290	46.5
	C1-12	2	23.0	24.0	61.0	95.0	5.0	251.0	0.280	44.8
	C1-12	3	19.0	20.0	57.0	110.0	5.0	234.0	0.260	44.7
	C1-12	4	13.0	21.0	57.0	105.0	5.0	199.0	0.250	46.2
	C1-12	5	9.0	22.0	57.0	105.0	5.0	314.0	0.290	46.5
	C1-12	6	10.0	19.0	56.0	100.0	4.0	109.0	0.200	43.2
	C1-22	1	16.0	30.0	67.0	105.0	5.0	254.0	0.270	44.2
	C1-22	2	7.0	23.0	60.0	110.0	5.0	315.0	0.300	47.6
	C1-22	3	16.0	21.0	60.0	110.0	5.0	260.0	0.210	46.7
	C1-22	4	26.0	23.0	60.0	110.0	5.0	279.0	0.280	45.3
	C1-22	5	12.0	19.0	56.0	105.0	5.0	273.0	0.300	45.8
	C1-22	6	17.0	19.0	56.0	110.0	5.0	243.0	0.290	45.8
	5	1	17.0	25.0	66.0	95.0	4.0	312.0	0.310	47.4
	5	2	13.0	20.0	60.0	110.0	5.0	283.0	0.260	44.8
	5	3	23.0	20.0	60.0	105.0	5.0	350.0	0.270	46.6
	5	4	17.0	16.0	56.0	105.0	5.0	355.0	0.310	46.5
	5	5	15.0	19.0	60.0	100.0	5.0	216.0	0.230	45.6
	5	6	17.0	20.0	60.0	105.0	5.0	201.0	0.270	47.1
	6	1	13.0	20.0	60.0	110.0	5.0	221.0	0.210	47.5
	6	2	15.0	22.0	64.0	95.0	5.0	231.0	0.290	46.8
	6	3	12.0	19.0	60.0	105.0	5.0	360.0	0.330	47.6
	6	4	16.0	19.0	60.0	105.0	5.0	342.0	0.300	47.0
	6	5	17.0	16.0	56.0	110.0	5.0	278.0	0.280	47.8
	6	6	20.0	16.0	56.0	95.0	4.0	263.0	0.310	48.3
	7	1	10.0	19.0	60.0	105.0	5.0	333.0	0.300	47.5
	7	2	22.0	22.0	63.0	95.0	4.0	301.0	0.320	47.2
	7	3	11.0	15.0	56.0	110.0	5.0	373.0	0.340	47.5
	7	4	10.0	19.0	60.0	105.0	4.0	231.0	0.290	46.6
	7	5	22.0	19.0	60.0	110.0	5.0	343.0	0.310	47.3
	7	6	15.0	20.0	60.0	105.0	5.0	267.0	0.300	47.4
	8	1	10.0	25.0	66.0	100.0	4.0	357.0	0.300	47.7
	8	2	25.0	19.0	60.0	95.0	5.0	299.0	0.300	48.3
	8	3	13.0	19.0	60.0	105.0	5.0	348.0	0.290	43.0
	8	4	27.0	22.0	63.0	110.0	4.0	240.0	0.300	48.7
	8	5	17.0	16.0	57.0	105.0	5.0	168.0	0.180	44.7
	8	6	10.0	16.0	56.0	100.0	4.0	252.0	0.280	45.9
	9	1	16.0	16.0	56.0	100.0	5.0	373.0	0.310	46.3
	9	2	13.0	16.0	56.0	95.0	4.0	254.0	0.280	45.2
	9	3	16.0	22.0	63.0	105.0	5.0	368.0	0.310	46.3
	9	4	16.0	19.0	60.0	105.0	5.0	304.0	0.290	47.9
	9	5	16.0	15.0	56.0	100.0	4.0	307.0	0.310	46.2
	9	6	14.0	16.0	56.0	100.0	5.0	229.0	0.240	46.0

Appendix 7. Field measurements for parameters characterizing the Reward C2 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Carman	C2-4	1	28.0	31.0	71.0	125.0	5.0	372.0	0.200	43.8
	C2-4	2	22.0	31.0	70.0	115.0	5.0	391.0	0.210	42.9
	C2-4	3	18.0	30.0	70.0	125.0	5.0	387.0	0.220	42.3
	C2-4	4	20.0	30.0	70.0	120.0	5.0	269.0	0.180	42.1
	C2-8	1	30.0	27.0	71.0	125.0	5.0	557.0	0.280	44.0
	C2-8	2	24.0	26.0	69.0	125.0	4.0	536.0	0.210	43.7
	C2-8	3	18.0	25.0	70.0	125.0	5.0	438.0	0.220	41.8
	C2-8	4	19.0	26.0	69.0	125.0	4.0	250.0	0.130	41.5
	C2-12	1	25.0	29.0	70.0	125.0	5.0	492.8	0.220	44.1
	C2-12	2	21.0	26.0	70.0	125.0	5.0	499.0	0.210	43.4
	C2-12	3	24.0	26.0	71.0	125.0	5.0	370.0	0.190	41.5
	C2-12	4	22.0	29.0	70.0	123.0	5.0	404.0	0.160	41.4
	C2-22	1	17.0	24.0	70.0	123.0	5.0	450.0	0.230	44.9
	C2-22	2	21.0	26.0	70.0	125.0	4.0	391.0	0.200	41.9
	C2-22	3	15.0	24.0	70.0	120.0	5.0	390.0	0.190	42.6
	C2-22	4	17.0	24.0	70.0	125.0	5.0	332.0	0.200	43.5
	5	1	9.0	23.0	70.0	120.0	5.0	276.0	0.180	41.2
	5	2	17.0	23.0	69.0	120.0	3.0	297.0	0.240	41.4
	5	3	9.0	23.0	70.0	120.0	4.0	230.0	0.210	40.7
	5	4	10.0	23.0	70.0	125.0	5.0	328.0	0.220	42.7
	6	1	19.0	23.0	69.0	110.0	4.0	295.0	0.200	42.8
	6	2	16.0	23.0	69.0	125.0	3.0	260.0	0.250	42.2
	6	3	15.0	23.0	70.0	120.0	4.0	314.0	0.190	40.9
	6	4	18.0	23.0	69.0	120.0	4.0	265.0	0.180	40.0
	7	1	18.0	23.0	69.0	120.0	4.0	343.0	0.260	42.3
	7	2	22.0	23.0	70.0	125.0	4.0	335.0	0.220	40.9
	7	3	15.0	23.0	69.0	125.0	5.0	257.0	0.200	41.4
	7	4	14.0	23.0	69.0	120.0	5.0	328.0	0.210	40.0
	8	1	11.0	23.0	69.0	123.0	5.0	316.0	0.240	41.3
	8	2	16.0	23.0	70.0	125.0	4.0	350.0	0.180	41.5
	8	3	9.0	23.0	70.0	120.0	5.0	252.0	0.250	42.0
	8	4	18.0	23.0	70.0	120.0	5.0	316.0	0.210	41.4
	9	1	24.0	23.0	69.0	125.0	4.0	295.0	0.200	42.1
	9	2	18.0	23.0	70.0	115.0	4.0	355.0	0.240	40.2
	9	3	13.0	23.0	69.0	120.0	5.0	232.0	0.180	41.2
	9	4	22.0	23.0	69.0	120.0	3.0	196.0	0.150	40.0
	10	1	19.0	23.0	70.0	115.0	5.0	302.0	0.200	40.9
	10	2	16.0	23.0	69.0	120.0	4.0	319.4	0.220	40.9
	10	3	6.0	23.0	70.0	125.0	5.0	212.0	0.200	42.2
	10	4	10.0	23.0	69.0	125.0	4.0	211.0	0.140	41.0
Winnipeg	C2-4	1	36.0	27.0	66.0	110.0	5.0	195.0	0.300	45.2
	C2-4	2	27.0	27.0	66.0	95.0	5.0	244.0	0.260	46.1
	C2-4	3	31.0	27.0	67.0	110.0	5.0	318.0	0.250	46.2
	C2-4	4	29.0	26.0	66.0	105.0	5.0	289.0	0.300	46.2
	C2-4	5	41.0	26.0	63.0	105.0	4.0	222.0	0.230	45.2
	C2-4	6	16.0	27.0	64.0	105.0	5.0	169.0	0.210	45.2

Appendix 7. (continued)

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Winnipeg	C2-8	1	29.0	26.0	66.0	100.0	5.0	276.0	0.320	45.0
	C2-8	2	31.0	26.0	66.0	95.0	4.0	320.0	0.360	47.3
	C2-8	3	21.0	16.0	56.0	110.0	5.0	268.0	0.230	47.4
	C2-8	4	20.0	27.0	67.0	100.0	5.0	249.0	0.250	45.6
	C2-8	5	30.0	26.0	66.0	105.0	5.0	206.0	0.270	46.4
	C2-8	6	25.0	24.0	64.0	105.0	5.0	186.0	0.230	47.0
	C2-12	1	23.0	28.0	64.0	100.0	5.0	247.0	0.270	43.7
	C2-12	2	21.0	30.0	66.0	90.0	5.0	226.0	0.270	45.6
	C2-12	3	16.0	24.0	60.0	110.0	5.0	221.0	0.250	47.2
	C2-12	4	13.0	19.0	60.0	100.0	4.0	176.0	0.270	45.8
	C2-12	5	19.0	27.0	63.0	110.0	5.0	216.0	0.240	47.7
	C2-12	6	20.0	29.0	66.0	105.0	5.0	153.0	0.240	46.8
	C2-22	1	19.0	17.0	56.0	100.0	5.0	282.0	0.310	45.2
	C2-22	2	25.0	24.0	63.0	115.0	5.0	270.0	0.300	45.2
	C2-22	3	31.0	26.0	66.0	105.0	5.0	263.0	0.310	46.7
	C2-22	4	27.0	23.0	64.0	100.0	5.0	234.0	0.250	47.7
	C2-22	5	33.0	27.0	66.0	95.0	5.0	259.0	0.260	47.6
	C2-22	6	17.0	24.0	60.0	105.0	5.0	222.0	0.300	45.9
	5	1	16.0	19.0	60.0	95.0	5.0	299.0	0.310	46.2
	5	2	19.0	15.0	56.0	95.0	4.0	273.0	0.320	45.9
	5	3	20.0	25.0	66.0	105.0	5.0	300.0	0.290	47.7
	5	4	14.0	16.0	56.0	100.0	5.0	268.0	0.270	46.8
	5	5	12.0	15.0	56.0	95.0	4.0	234.0	0.310	48.4
	5	6	10.0	15.0	56.0	100.0	4.0	208.0	0.300	46.5
	6	1	12.0	15.0	56.0	100.0	5.0	289.0	0.320	47.0
	6	2	14.0	15.0	56.0	100.0	5.0	285.0	0.340	46.3
	6	3	12.0	15.0	56.0	110.0	5.0	244.0	0.270	47.4
	6	4	13.0	15.0	56.0	100.0	5.0	253.0	0.320	47.2
	6	5	13.0	15.0	56.0	110.0	4.0	293.0	0.310	48.0
	6	6	13.0	15.0	56.0	100.0	4.0	200.0	0.250	46.8
	7	1	18.0	15.0	56.0	105.0	5.0	157.0	0.170	44.5
	7	2	24.0	22.0	63.0	95.0	4.0	305.0	0.340	46.9
	7	3	18.0	15.0	56.0	105.0	5.0	303.0	0.300	49.0
	7	4	19.0	15.0	56.0	105.0	5.0	259.0	0.320	46.9
	7	5	15.0	15.0	56.0	100.0	4.0	268.0	0.320	47.4
	7	6	19.0	15.0	56.0	105.0	5.0	188.0	0.240	47.3
	8	1	10.0	15.0	56.0	100.0	4.0	256.0	0.300	46.7
	8	2	19.0	19.0	60.0	100.0	5.0	304.0	0.300	47.2
	8	3	21.0	15.0	56.0	100.0	5.0	300.0	0.240	47.0
	8	4	16.0	19.0	60.0	100.0	5.0	238.0	0.240	47.4
	8	5	14.0	15.0	56.0	100.0	4.0	197.0	0.250	47.1
	8	6	13.0	15.0	56.0	100.0	4.0	192.0	0.260	44.1
	9	1	15.0	15.0	56.0	95.0	5.0	264.0	0.290	40.1
	9	2	13.0	15.0	56.0	100.0	4.0	225.0	0.280	47.6
	9	3	12.0	15.0	56.0	105.0	5.0	253.0	0.270	47.5
	9	4	23.0	20.0	60.0	100.0	5.0	235.0	0.290	46.8
	9	5	14.0	15.0	56.0	105.0	5.0	227.0	0.280	48.6
	9	6	9.0	15.0	56.0	105.0	5.0	209.0	0.280	47.2

Appendix 8. Field measurements for parameters characterizing the DSC-3 C1 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Carman	C1-4	1	20.0	28.0	42.0	110.0	4.0	100.0	0.160	40.3
	C1-4	2	21.0	29.0	42.0	120.0	3.0	100.0	0.166	40.5
	C1-4	3	7.0	28.0	40.0	120.0	5.0	80.0	0.145	40.3
	C1-4	4	11.0	28.0	41.0	120.0	4.0	73.0	0.146	40.3
	C1-8	1	20.0	28.0	42.0	105.0	4.0	145.0	0.161	41.7
	C1-8	2	22.0	29.0	39.0	110.0	3.0	118.0	0.157	40.8
	C1-8	3	13.0	28.0	40.0	120.0	4.0	129.0	0.143	40.8
	C1-8	4	17.0	28.0	41.0	120.0	5.0	162.0	0.162	40.2
	C1-12	1	13.0	27.0	41.0	110.0	3.0	193.0	0.167	40.7
	C1-12	2	17.0	28.0	40.0	110.0	3.0	164.0	0.172	41.5
	C1-12	3	6.0	29.0	41.0	155.0	3.0	69.0	0.138	40.1
	C1-12	4	8.0	28.0	41.0	120.0	5.0	124.0	0.118	38.3
	C1-22	1	16.0	28.0	41.0	110.0	4.0	96.0	0.128	40.8
	C1-22	2	11.0	27.0	42.0	120.0	4.0	258.0	0.198	42.6
	C1-22	3	2.0	27.0	41.0	155.0	4.0	154.0	0.171	42.2
	C1-22	4	14.0	27.0	41.0	120.0	3.0	145.0	0.161	40.5
	5	1	15.0	22.0	47.0	100.0	3.0	326.0	0.159	40.0
	5	2	23.0	22.0	46.0	115.0	4.0	310.0	0.206	41.7
	5	3	9.0	23.0	47.0	120.0	5.0	349.0	0.183	41.6
	5	4	16.0	22.0	47.0	120.0	3.0	392.0	0.174	40.3
	6	1	15.0	22.0	47.0	115.0	3.0	398.0	0.209	42.6
	6	2	23.0	22.0	46.0	110.0	3.0	353.0	0.294	42.8
	6	3	18.0	22.0	47.0	155.0	4.0	43.0	0.019	41.7
	6	4	4.0	22.0	48.0	120.0	5.0	290.0	0.175	40.6
	7	1	10.0	22.0	47.0	110.0	4.0	432.0	0.210	40.9
	7	2	18.0	22.0	48.0	120.0	4.0	473.0	0.205	42.6
	7	3	12.0	22.0	47.0	115.0	3.0	318.0	0.167	40.0
	7	4	17.0	22.0	47.0	125.0	5.0	317.0	0.134	40.4
	8	1	19.0	22.0	47.0	115.0	4.0	509.0	0.261	42.6
	8	2	19.0	22.0	47.0	120.0	5.0	429.0	0.182	41.4
	8	3	18.0	22.0	46.0	120.0	5.0	358.0	0.183	42.0
	8	4	25.0	22.0	47.0	120.0	4.0	329.0	0.188	42.5
	9	1	19.0	22.0	47.0	120.0	3.0	443.0	0.210	42.3
	9	2	15.0	22.0	47.0	120.0	3.0	369.0	0.410	41.5
	9	3	15.0	22.0	46.0	120.0	5.0	406.0	0.180	41.5
	9	4	23.0	22.0	47.0	120.0	5.0	307.0	0.110	40.8
	10	1	27.0	22.0	48.0	115.0	3.0	452.0	0.215	41.7
	10	2	12.0	22.0	47.0	110.0	3.0	354.0	0.196	38.9
	10	3	13.0	22.0	46.0	120.0	5.0	371.0	0.164	40.2
	10	4	18.0	22.0	46.0	120.0	5.0	374.0	0.178	40.8
Winnipeg	C1-4	1	8.0	19.0	56.0	95.0	4.0	80.0	0.230	43.0
	C1-4	2	17.0	18.0	57.0	90.0	4.0	88.0	0.200	44.7
	C1-4	3	15.0	19.0	56.0	100.0	5.0	139.0	0.230	42.4
	C1-4	4	23.0	19.0	56.0	95.0	4.0	130.0	0.260	43.2
	C1-4	5	22.0	19.0	56.0	100.0	4.0	129.0	0.180	43.6
	C1-4	6	7.0	21.0	56.0	100.0	4.0	53.0	0.270	41.0

Appendix 8. (continued)

Location	Plant Id.	Replicate	EMG	DTF (d)	LOD (d)	LOD (cm)	HT (1-5)	YLD (g)	HI	OIL (%)
Winnipeg	C1-8	1	7.0	18.0	56.0	100.0	4.0	75.0	0.210	43.4
	C1-8	2	20.0	19.0	57.0	105.0	5.0	83.0	0.180	44.2
	C1-8	3	18.0	18.0	56.0	100.0	5.0	140.0	0.250	36.1
	C1-8	4	12.0	18.0	56.0	105.0	4.0	113.0	0.190	44.1
	C1-8	5	23.0	20.0	56.0	100.0	4.0	88.0	0.200	40.8
	C1-8	6	12.0	19.0	56.0	105.0	5.0	64.0	0.210	42.1
	C1-12	1	13.0	19.0	57.0	100.0	5.0	120.0	0.200	43.5
	C1-12	2	10.0	18.0	56.0	105.0	5.0	131.0	0.240	45.6
	C1-12	3	6.0	19.0	56.0	110.0	5.0	156.0	0.280	44.6
	C1-12	4	27.0	18.0	56.0	100.0	5.0	134.0	0.240	43.8
	C1-12	5	24.0	19.0	57.0	110.0	5.0	103.0	0.210	43.3
	C1-12	6	12.0	18.0	56.0	105.0	5.0	118.0	0.240	43.4
	C1-22	1	9.0	17.0	56.0	100.0	5.0	118.0	0.210	44.6
	C1-22	2	27.0	18.0	56.0	90.0	5.0	86.0	0.220	44.3
	C1-22	3	18.0	17.0	56.0	105.0	5.0	152.0	0.220	42.9
	C1-22	4	23.0	18.0	56.0	100.0	4.0	120.0	0.200	43.5
	C1-22	5	19.0	17.0	56.0	105.0	5.0	104.0	0.230	44.4
	C1-22	6	22.0	19.0	56.0	100.0	5.0	94.0	0.210	43.3
	5	1	21.0	19.0	60.0	95.0	5.0	345.0	0.280	47.1
	5	2	17.0	26.0	67.0	110.0	5.0	317.0	0.320	47.7
	5	3	23.0	15.0	56.0	105.0	5.0	247.0	0.290	47.2
	5	4	19.0	15.0	56.0	95.0	5.0	296.0	0.280	46.6
	5	5	8.0	19.0	60.0	100.0	5.0	387.0	0.320	45.8
	5	6	15.0	16.0	56.0	100.0	5.0	213.0	0.280	46.9
	6	1	9.0	15.0	56.0	100.0	4.0	287.0	0.260	47.1
	6	2	20.0	16.0	56.0	105.0	5.0	239.0	0.280	47.7
	6	3	14.0	16.0	56.0	105.0	4.0	458.0	0.330	46.2
	6	4	22.0	15.0	56.0	100.0	4.0	271.0	0.290	47.3
	6	5	15.0	19.0	60.0	100.0	5.0	304.0	0.280	47.0
	6	6	27.0	19.0	60.0	100.0	4.0	335.0	0.300	46.2
	7	1	21.0	26.0	67.0	100.0	4.0	329.0	0.330	47.8
	7	2	13.0	19.0	61.0	100.0	5.0	312.0	1.040	47.0
	7	3	14.0	19.0	60.0	105.0	5.0	409.0	0.310	46.1
	7	4	17.0	19.0	60.0	95.0	4.0	279.0	0.270	47.4
	7	5	11.0	16.0	57.0	105.0	5.0	290.0	0.240	46.4
	7	6	21.0	16.0	56.0	105.0	4.0	292.0	0.310	46.2
	8	1	23.0	15.0	56.0	95.0	5.0	305.0	0.310	47.0
	8	2	14.0	25.0	66.0	95.0	4.0	290.0	0.290	48.0
	8	3	19.0	16.0	56.0	110.0	5.0	285.0	2.850	46.7
	8	4	12.0	20.0	60.0	105.0	4.0	322.0	0.260	44.9
	8	5	19.0	16.0	56.0	100.0	4.0	379.0	0.300	45.1
	8	6	18.0	16.0	56.0	110.0	5.0	281.0	0.300	46.1
	9	1	28.0	26.0	67.0	95.0	4.0	421.0	0.320	43.4
	9	2	16.0	15.0	56.0	100.0	4.0	728.0	0.630	46.9
	9	3	21.0	23.0	64.0	105.0	5.0	468.0	0.310	45.2
	9	4	15.0	23.0	64.0	100.0	4.0	309.0	0.290	46.7
	9	5	21.0	15.0	56.0	105.0	5.0	318.0	0.280	45.2
	9	6	17.0	16.0	56.0	100.0	3.0	335.0	0.300	45.3

Appendix 9. Field measurements for parameters characterizing the DSC-3 C2 populations and the Reward checks (Plant Id. 5-10, Carman; 5-9, Winnipeg), 1996. Parameters are seedling emergence (EMER), days to flowering (DTF), days to maturity (DTM), height (HT), lodging (LOD), seed yield (YLD), harvest index (HI) and seed oil content (OIL).

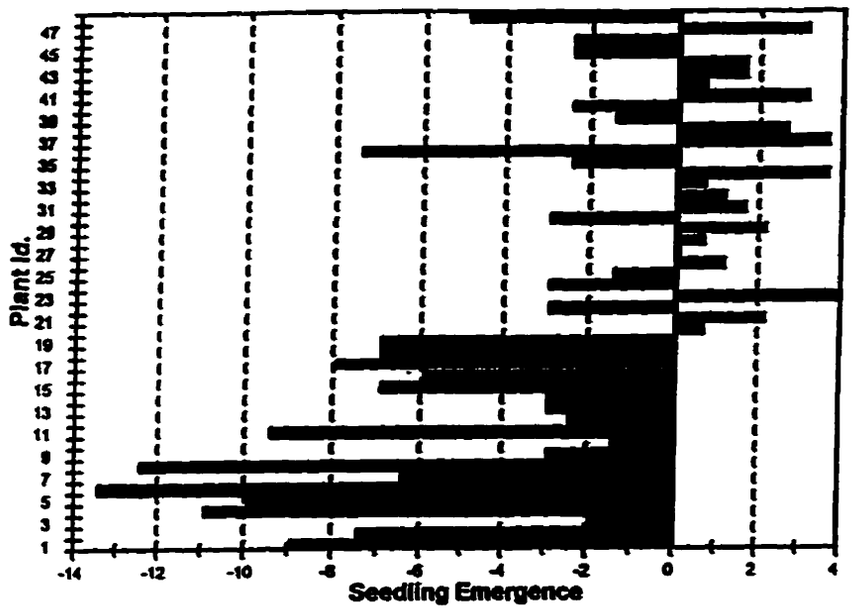
Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Carman	C2-4	1	20.0	28.0	68.0	115.0	4.0	544.0	0.240	40.0
	C2-4	2	15.0	27.0	68.0	120.0	5.0	190.0	0.170	40.0
	C2-4	3	19.0	27.0	69.0	115.0	4.0	362.0	0.210	42.2
	C2-8	1	22.0	29.0	69.0	120.0	4.0	132.0	0.150	41.2
	C2-8	2	14.0	29.0	69.0	120.0	5.0	101.0	0.110	40.0
	C2-8	3	9.0	28.0	68.0	120.0	3.0	161.0	0.140	39.1
	C2-12	1	25.0	28.0	69.0	120.0	4.0	151.0	0.160	40.8
	C2-12	2	15.0	28.0	68.0	115.0	5.0	151.0	0.140	40.7
	C2-12	3	11.0	28.0	68.0	115.0	5.0	94.0	0.120	40.0
	C2-22	1	16.0	25.0	69.0	120.0	5.0	284.0	0.200	40.9
	C2-22	2	17.0	27.0	68.0	115.0	5.0	167.0	0.140	40.7
	C2-22	3	11.0	27.0	69.0	125.0	5.0	178.0	0.130	41.5
	5	1	17.0	22.0	68.0	120.0	5.0	455.0	0.210	41.6
	5	2	8.0	22.0	68.0	120.0	5.0	746.0	0.440	42.7
	5	3	11.0	22.0	68.0	125.0	4.0	390.0	0.200	42.3
	6	1	17.0	22.0	68.0	120.0	4.0	423.0	0.210	41.1
	6	2	11.0	23.0	70.0	120.0	4.0	298.0	0.160	40.5
	6	3	10.0	23.0	69.0	115.0	4.0	300.0	0.140	41.1
	7	1	27.0	22.0	68.0	115.0	5.0	389.0	0.220	41.1
	7	3	21.0	22.0	68.0	120.0	5.0	393.0	0.180	41.0
	7	2	18.0	22.0	68.0	120.0	4.0	434.0	0.200	40.5
	8	1	14.0	22.0	68.0	120.0	5.0	412.0	0.190	41.2
	8	2	16.0	22.0	69.0	120.0	5.0	400.0	0.190	42.5
	8	3	20.0	22.0	68.0	115.0	5.0	315.0	0.210	41.1
	9	1	27.0	22.0	69.0	125.0	5.0	423.0	0.210	41.1
	9	2	19.0	22.0	69.0	120.0	5.0	333.0	0.150	42.3
	9	3	13.0	22.0	69.0	115.0	4.0	427.0	0.170	40.8
	10	1	13.0	22.0	68.0	120.0	3.0	436.0	0.210	42.7
	10	2	8.0	24.0	70.0	120.0	5.0	452.0	0.230	42.8
	10	3	9.0	22.0	69.0	120.0	5.0	364.6	0.190	42.5
Winnipeg	C2-4	1	20.0	27.0	66.0	100.0	4.0	172.0	0.290	45.4
	C2-4	2	22.0	19.0	56.0	95.0	4.0	140.0	0.280	42.6
	C2-4	3	21.0	17.0	56.0	105.0	5.0	170.0	0.230	43.7
	C2-4	4	17.0	23.0	60.0	105.0	5.0	110.0	0.220	44.5
	C2-4	5	13.0	19.0	56.0	100.0	5.0	152.0	0.220	43.9
	C2-4	6	23.0	19.0	56.0	100.0	5.0	137.0	0.250	43.7
	C2-8	1	14.0	27.0	66.0	100.0	5.0	108.0	0.220	44.3
	C2-8	2	15.0	23.0	63.0	100.0	5.0	122.0	0.240	45.4
	C2-8	3	20.0	21.0	60.0	105.0	5.0	141.0	0.220	44.3
	C2-8	4	12.0	27.0	66.0	100.0	5.0	255.0	0.510	46.1
	C2-8	5	22.0	16.0	56.0	100.0	5.0	116.0	0.230	45.0
	C2-8	6	27.0	20.0	56.0	105.0	5.0	102.0	0.260	45.4
	C2-12	1	14.0	27.0	63.0	95.0	5.0	148.0	0.250	43.8
	C2-12	2	19.0	26.0	64.0	100.0	5.0	122.0	0.220	46.0
	C2-12	3	10.0	16.0	56.0	100.0	4.0	96.0	0.190	43.9
	C2-12	4	19.0	16.0	56.0	95.0	4.0	91.0	0.170	43.0
	C2-12	5	20.0	23.0	60.0	105.0	5.0	94.0	0.210	45.0
	C2-12	6	20.0	18.0	56.0	100.0	5.0	103.0	0.230	44.3

Appendix 9. (continued)

Location	Plant Id.	Replicate	EMG	DTF (d)	DTM (d)	HT (cm)	LOD (1-5)	YLD (g)	HI	OIL (%)
Winnipeg	C2-22	1	18.0	24.0	64.0	95.0	4.0	178.0	0.270	45.2
	C2-22	2	30.0	20.0	60.0	100.0	5.0	145.0	0.260	43.5
	C2-22	3	18.0	26.0	66.0	105.0	5.0	165.0	0.290	46.9
	C2-22	4	26.0	20.0	60.0	105.0	5.0	216.0	0.270	44.8
	C2-22	5	19.0	21.0	60.0	100.0	4.0	159.0	0.320	45.6
	C2-22	6	20.0	21.0	60.0	105.0	5.0	159.0	0.270	45.7
	5	1	24.0	23.0	64.0	95.0	4.0	383.0	0.310	47.6
	5	2	12.0	23.0	64.0	100.0	5.0	317.0	0.240	46.4
	5	3	10.0	19.0	60.0	100.0	5.0	304.0	0.250	46.8
	5	4	17.0	19.0	60.0	100.0	4.0	316.0	0.300	46.9
	5	5	17.0	15.0	56.0	100.0	4.0	346.0	0.300	46.7
	5	6	15.0	19.0	60.0	105.0	5.0	193.0	0.300	47.5
	6	1	19.0	23.0	64.0	100.0	4.0	302.0	0.300	47.6
	6	2	22.0	23.0	64.0	100.0	5.0	309.0	0.340	48.4
	6	3	12.0	15.0	56.0	105.0	5.0	349.0	0.290	48.6
	6	4	19.0	22.0	63.0	105.0	4.0	320.0	0.320	47.1
	6	5	17.0	19.0	60.0	100.0	4.0	319.0	0.300	47.7
	6	6	19.0	19.0	60.0	105.0	5.0	287.0	0.300	47.0
	7	1	27.0	22.0	64.0	100.0	3.0	308.0	0.290	46.7
	7	2	17.0	25.0	66.0	95.0	4.0	312.0	0.280	46.9
	7	3	14.0	19.0	60.0	105.0	5.0	363.0	0.300	45.8
	7	4	18.0	15.0	56.0	100.0	5.0	195.0	0.330	47.8
	7	5	24.0	22.0	63.0	100.0	4.0	256.0	0.280	46.9
	7	6	21.0	19.0	60.0	105.0	5.0	243.0	0.270	47.5
	8	1	12.0	23.0	64.0	100.0	5.0	306.0	0.280	47.7
	8	2	20.0	25.0	66.0	95.0	5.0	299.0	0.330	47.2
	8	3	25.0	19.0	60.0	100.0	5.0	311.0	0.310	47.7
	8	4	16.0	19.0	60.0	100.0	5.0	329.0	0.330	47.0
	8	5	12.0	15.0	56.0	95.0	5.0	329.0	0.300	46.5
	8	6	19.0	22.0	63.0	100.0	5.0	290.0	0.320	48.3
	9	1	20.0	25.0	66.0	100.0	4.0	303.0	0.290	47.3
	9	2	19.0	19.0	60.0	95.0	4.0	336.0	0.320	45.3
	9	3	19.0	26.0	67.0	100.0	4.0	460.0	0.350	46.1
	9	4	19.0	19.0	60.0	100.0	4.0	334.0	0.300	47.5
	9	5	17.0	15.0	56.0	105.0	4.0	256.0	0.240	46.4
	9	6	21.0	15.0	56.0	100.0	4.0	265.0	0.310	46.9

Appendix 10. The mean performance of *B. rapa* cv. Reward DH lines (Id. 1 - 19) and donor checks (Id. 20 - 48) relative to the overall mean performance of the Reward donor checks for parameters: seedling emergence (a), days to flowering (b), days to maturity (c), height (d), lodging (e), harvest index (f) and seed oil content (g), 1996.

a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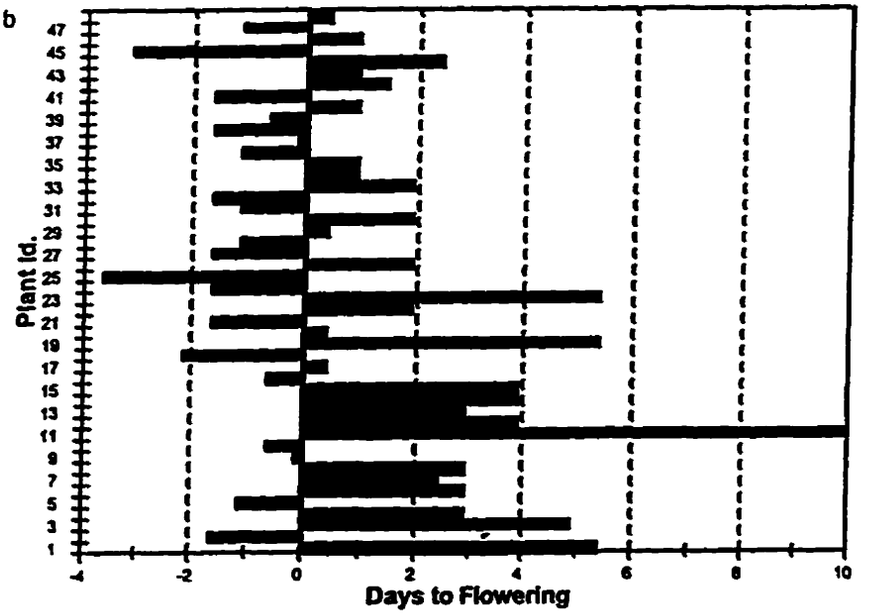
2
4
Seedling Emergence

b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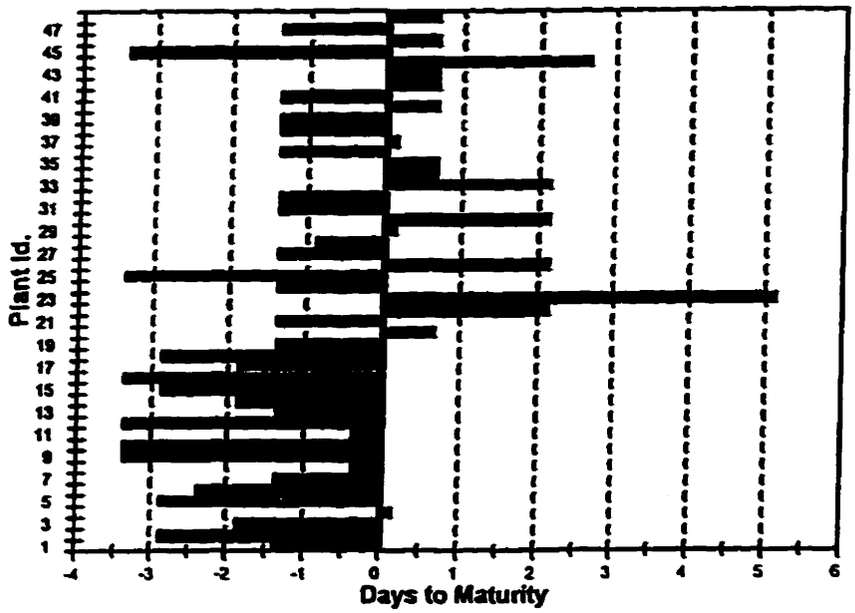
6
8
10
Days to Flowering

c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2
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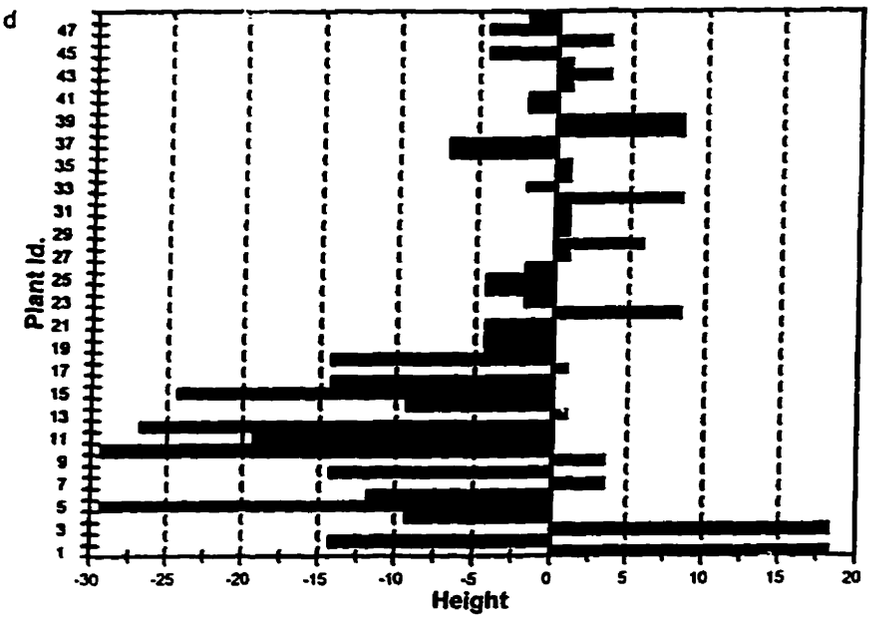
5
6

d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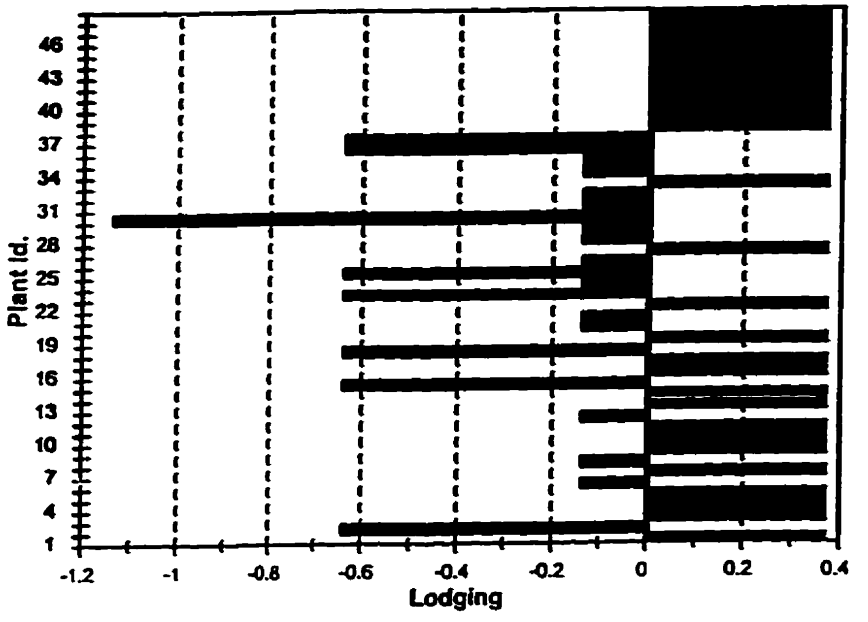
20

e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46
43
40
37
34
31
28
25
22
19
16
13
10
7
4
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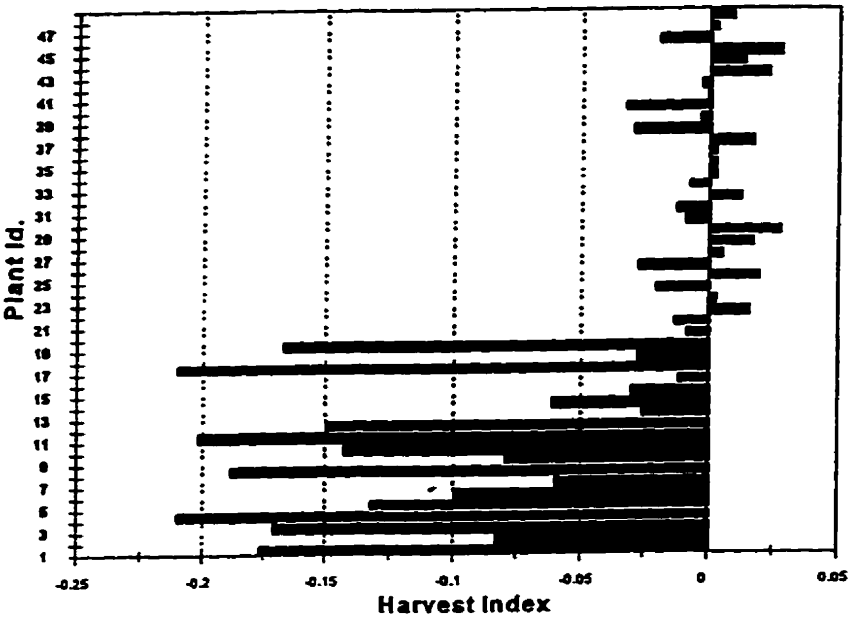
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
Lodging

f


Plant Id.
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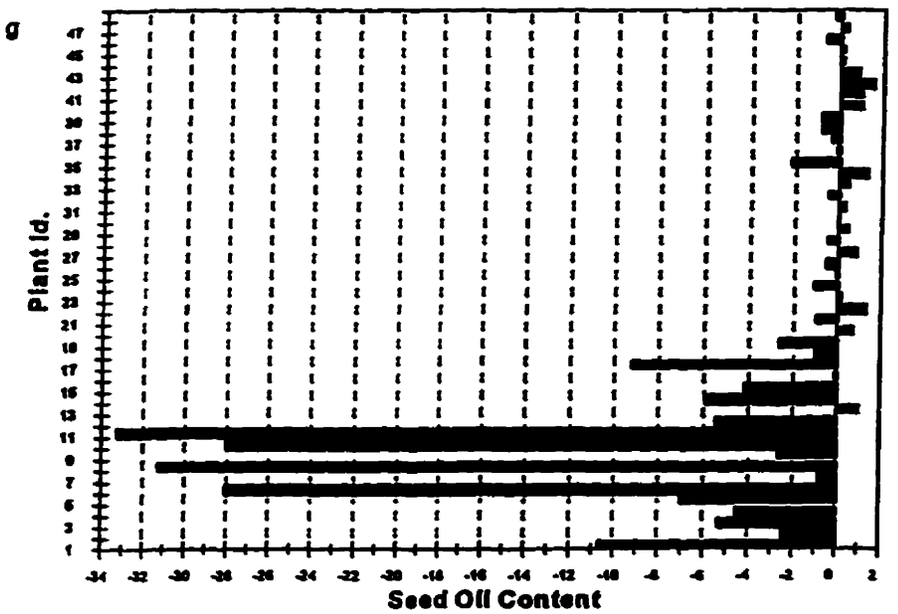
-0.2
-0.15
-0.1
-0.05
0
0.05
Harvest Index


Plant Id.
Seed Oil Content

Appendix 11. Data matrix of RAPD products for the DSC-3 donor population (D) and DH lines

Primer (UBC)	Marker size (kb)	D	DH lines 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
312	2.14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.82	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	0	1
	1.26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.76	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.69	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
	0.63	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	0
	0.56	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1
	0.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
318	2.32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.66	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
329	1.95	1	1	1	1	1	0	1	0	1	0	0	1	1	1	1	0	0	0	1	0	1	1	1
	1.86	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.74	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0	1	1
	1.66	1	0	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1
	1.48	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.32	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0	1	1	1	1
	1.26	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	1	1
	1.20	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	1
	1.15	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1	1	1	0
	0.96	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.83	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
337	1.74	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0
	1.62	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	1
	1.55	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	0	1	0	1	0	1
	1.35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	1.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	0.72	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
338	1.78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.48	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
	1.26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
	0.93	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.69	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	0.56	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1

Appendix 12. Data matrix of RAPD products for the Reward donor population (D) and DH lines

Primer (UBC)	Marker size (kb)	D	DH lines 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
312	2.51	0	0	0	1	1	0	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0
	2.24	0	0	1	1	1	0	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0
	2.00	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.90	1	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0
	1.74	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
	1.66	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	0	0	0	0	0	0	0
	1.55	1	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	1	1	1	0	1	1
	1.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
	1.20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.05	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1
	0.96	1	0	0	0	0	1	0	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	1	0
	0.91	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	0.83	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
	0.79	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.63	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1
318	2.40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.78	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.70	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.96	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.89	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.83	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	0	1	1	1	1
	0.72	0	0	1	0	0	1	0	0	1	1	1	1	1	0	0	1	0	1	0	0	1	0	1	0
329	1.86	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1
	1.70	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0
	1.62	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	1	1	1	1	1
	1.35	1	0	1	0	1	1	1	0	1	0	0	0	1	0	1	1	1	0	1	1	0	0	1	1
	1.26	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.10	1	1	0	0	0	1	0	1	1	1	0	1	1	0	0	0	0	0	1	0	1	0	0	1
	1.02	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1
	0.87	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.81	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.69	1	0	1	0	0	1	0	1	1	0	0	0	0	1	1	1	0	1	1	1	0	1	1	0
	0.65	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1
	0.48	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.44	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.36	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
337	1.86	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0
	1.62	1	0	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	1	0	0
	1.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.30	0	0	1	0	0	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1
	1.26	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.96	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1

Appendix 12. (continued)

Primer (UBC)	Marker size (kb)	D	DH lines 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
338	1.82	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1.51	0	0	1	1	0	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	1	1	0	1
	1.10	0	0	1	1	1	0	0	0	1	1	0	1	1	1	1	0	0	0	0	1	0	1	0	0
	1.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.91	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.83	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0

Appendix 13. Data matrix of RAPD products for the DSC-3 C1 and C2 populations with primers 329 and 338.

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12
329	1	4	2.51	1	1	1	1	1	-	-	-	-	-	-	-
			2.04	1	1	1	1	1	-	-	-	-	-	-	-
			1.74	1	1	1	1	1	-	-	-	-	-	-	-
			1.62	1	1	1	1	1	-	-	-	-	-	-	-
			1.45	1	1	1	1	1	-	-	-	-	-	-	-
			1.26	1	1	1	1	1	-	-	-	-	-	-	-
			1.15	1	1	1	1	1	-	-	-	-	-	-	-
			1.07	1	1	0	1	0	-	-	-	-	-	-	-
			1.02	1	1	1	1	1	-	-	-	-	-	-	-
			0.91	1	1	1	1	1	-	-	-	-	-	-	-
			0.76	1	1	1	1	1	-	-	-	-	-	-	-
			0.60	1	1	1	1	1	-	-	-	-	-	-	-
			0.47	1	1	1	1	1	-	-	-	-	-	-	-
			0.41	1	1	1	1	1	-	-	-	-	-	-	-
329	2	4	1.91	1	1	1	1	1	-	-	-	-	-	-	-
			1.70	1	1	1	1	1	-	-	-	-	-	-	-
			1.59	1	1	1	1	1	-	-	-	-	-	-	-
			1.35	1	1	1	1	1	-	-	-	-	-	-	-
			1.26	1	1	1	1	1	-	-	-	-	-	-	-
			1.20	1	1	1	1	1	-	-	-	-	-	-	-
			1.15	1	1	1	1	1	-	-	-	-	-	-	-
			1.10	1	1	0	1	1	-	-	-	-	-	-	-
			1.02	1	1	1	1	1	-	-	-	-	-	-	-
			0.91	1	1	1	1	1	-	-	-	-	-	-	-
			0.87	1	1	1	1	0	-	-	-	-	-	-	-
			0.72	1	1	1	1	1	-	-	-	-	-	-	-
			0.59	1	1	1	1	1	-	-	-	-	-	-	-
			0.53	1	1	1	1	1	-	-	-	-	-	-	-
			0.47	1	1	1	1	1	-	-	-	-	-	-	-
338	1	4	2.69	1	1	1	1	1	1	1	1	1	1	1	1
			2.34	1	1	1	1	1	1	1	1	1	1	1	1
			2.19	1	1	0	0	0	0	1	1	1	1	1	1
			2.04	1	1	1	1	1	1	1	1	1	1	1	1
			1.95	1	1	1	1	1	1	1	1	1	1	1	1
			1.82	1	1	1	1	1	1	1	1	1	1	1	1
			1.55	1	1	1	1	1	1	1	1	1	1	1	1
			1.26	1	1	1	1	1	1	1	1	1	1	1	1
			1.20	1	1	1	1	1	1	1	1	1	1	1	1
			1.15	1	1	1	1	1	1	1	1	1	1	1	1
			0.93	1	1	1	1	1	1	1	1	1	1	1	1
			0.91	1	1	1	1	1	1	1	1	1	1	1	1
			0.54	1	1	1	1	1	1	1	1	1	1	1	1
			0.46	1	1	1	1	1	1	1	1	1	1	1	1

Appendix 13. (continued)

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12
338	2	4	2.57	1	1	1	1	1	1	1	1	1	1	1	1
			2.04	1	1	1	1	1	1	0	1	1	1	0	1
			1.90	1	1	1	1	1	1	1	1	1	1	1	1
			1.45	1	1	1	1	1	1	1	1	1	1	1	1
			1.38	1	1	1	1	1	1	1	1	1	1	1	1
			1.29	1	1	1	1	1	1	1	1	1	1	1	1
			1.15	1	1	1	1	1	1	1	1	1	1	1	1
			1.07	1	1	1	1	1	1	1	1	1	1	1	1
			0.98	1	1	1	1	1	1	1	1	1	1	1	1
			0.91	1	1	1	1	1	1	1	1	1	1	1	1
			0.83	1	1	1	1	1	1	1	1	1	1	1	1
			0.68	1	1	1	1	1	1	1	1	1	1	1	1
			0.58	1	1	1	1	1	1	1	1	1	1	1	1

* The symbol ' - ' represents the absence of data for these plants.

Appendix 14. Data matrix of RAPD products for the Reward C1 and C2 populations with primers 329 and 338.

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
329	1	4	2.75	0	0	1	1	1	0	1	0	0	1	1	1	-	-	-
			2.14	0	1	1	1	1	1	1	1	0	1	1	1	-	-	-
			2.04	1	1	1	1	1	1	1	1	0	1	1	1	-	-	-
			1.82	1	1	1	1	1	1	1	1	1	0	0	1	-	-	-
			1.59	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.45	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.26	1	0	1	0	0	0	1	0	0	0	0	0	-	-	-
			1.15	0	1	0	1	1	1	1	1	1	1	1	1	-	-	-
			1.07	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.91	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.79	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.71	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.63	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.50	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
329	1	8	2.75	0	0	1	0	0	1	0	0	0	1	1	0	-	-	-
			2.14	1	1	1	1	0	1	0	1	1	1	1	1	-	-	-
			2.04	0	0	0	0	0	0	0	0	1	0	0	0	-	-	-
			1.82	1	1	1	1	1	1	1	1	0	1	1	1	-	-	-
			1.59	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.51	0	0	0	0	1	0	1	0	0	1	0	1	-	-	-
			1.45	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.26	1	1	1	1	0	0	0	0	0	1	1	1	-	-	-
			1.15	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.07	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.02	0	0	1	0	1	0	1	0	0	1	1	1	-	-	-
			0.91	1	1	1	1	0	0	0	1	1	1	1	0	-	-	-
			0.79	0	0	0	0	1	0	1	1	1	1	0	1	-	-	-
			0.71	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.63	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.50	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
329	1	12	2.40	0	0	0	0	1	1	1	0	0	0	0	0	-	-	-
			2.04	1	1	1	1	1	1	1	1	1	1	1	0	-	-	-
			1.82	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.74	1	1	1	0	1	1	1	1	1	1	1	1	-	-	-
			1.55	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.23	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.02	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.93	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.89	0	0	0	0	1	1	1	0	0	0	0	1	-	-	-
			0.83	1	1	1	0	0	1	0	0	0	1	0	0	-	-	-
			0.74	0	0	0	0	1	1	1	0	0	1	0	0	-	-	-
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.51	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.47	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-

Appendix 14. (continued)

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
329	1	22	2.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.82	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.74	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.55	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.02	1	1	1	1	0	1	1	0	1	1	1	1	1	0	1
			0.93	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.89	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0
			0.83	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.74	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.51	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
329	2	4	2.75	0	0	1	1	0	0	1	0	1	1	0	0	-	-	-
			2.09	0	0	1	1	0	0	0	1	0	0	0	0	-	-	-
			2.00	1	1	0	1	1	1	1	1	1	1	1	1	-	-	-
			1.90	0	0	0	1	0	0	0	1	1	0	1	1	-	-	-
			1.66	1	1	1	1	1	1	1	1	1	0	0	1	-	-	-
			1.51	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.29	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.12	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.76	1	1	1	0	1	1	1	1	1	1	1	1	-	-	-
			0.72	1	1	1	0	1	1	1	1	0	0	1	1	-	-	-
			0.68	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.58	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.53	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.48	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
329	2	8	2.75	0	1	0	1	1	1	1	0	1	0	0	1	-	-	-
			2.09	0	1	1	0	1	0	0	1	0	0	1	1	-	-	-
			2.00	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.90	0	0	0	1	0	0	0	0	0	0	0	0	-	-	-
			1.66	1	1	1	0	1	1	1	1	1	1	1	1	-	-	-
			1.59	0	0	0	1	0	0	0	0	0	0	0	0	-	-	-
			1.51	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.29	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.12	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.76	0	1	0	0	0	1	1	0	0	0	1	1	-	-	-
			0.72	1	0	1	0	0	0	0	1	1	1	1	0	-	-	-
			0.68	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.58	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.53	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.48	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.44	0	1	1	0	1	1	1	1	0	0	0	0	-	-	-

Appendix 14. (continued)

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
329	2	12	2.82	0	0	0	0	1	0	0	1	1	1	0	0	-	-	-
			2.30	1	1	0	0	0	0	1	1	1	1	1	0	-	-	-
			2.14	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			2.00	1	1	1	1	1	1	0	0	0	1	1	1	-	-	-
			1.70	1	1	0	1	0	1	1	1	1	1	0	1	-	-	-
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.23	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.00	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.91	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.81	0	1	1	1	1	1	1	1	1	0	0	1	-	-	-
			0.65	0	0	0	0	1	0	0	0	0	1	0	1	-	-	-
			0.56	0	1	1	1	0	1	1	0	0	0	0	0	-	-	-
			0.46	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.41	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.33	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.30	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.27	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
329	2	22	2.14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			2.00	0	1	0	0	1	0	0	1	1	0	1	1	0	1	1
			1.70	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1
			1.38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			1.00	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.91	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
			0.81	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0
			0.65	1	0	1	1	0	1	0	1	1	1	1	1	0	1	1
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
			0.27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
338	1	4	2.34	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			2.00	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.62	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.55	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.45	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.29	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.15	1	0	1	1	1	1	0	1	1	1	1	1	-	-	-
			1.05	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.96	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.91	1	1	1	1	0	0	1	1	1	1	1	1	-	-	-
			0.68	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-

Appendix 14. (continued)

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
338	1	8	2.34	0	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			2.00	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.62	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.55	1	1	0	0	1	1	1	-	-	-	-	-	-	-	-
			1.45	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.29	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.15	1	1	1	1	1	1	0	-	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.96	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.91	1	0	0	0	0	0	0	-	-	-	-	-	-	-	-
			0.68	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.56	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
338	1	12	2.34	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			2.00	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.62	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.55	0	0	1	1	1	0	-	-	-	-	-	-	-	-	-
			1.45	0	0	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.29	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.15	1	1	0	1	1	1	-	-	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.96	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.91	1	1	0	0	1	1	-	-	-	-	-	-	-	-	-
			0.68	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.56	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
338	1	22	2.34	0	1	0	1	1	1	0	1	-	-	-	-	-	-	-
			2.19	0	0	0	0	0	0	1	0	-	-	-	-	-	-	-
			2.04	0	0	0	1	1	1	0	1	-	-	-	-	-	-	-
			1.82	1	1	1	0	0	0	1	1	-	-	-	-	-	-	-
			1.59	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.45	1	1	1	1	1	1	0	1	-	-	-	-	-	-	-
			1.35	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.00	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.91	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.81	1	1	1	0	0	0	1	1	-	-	-	-	-	-	-
			0.79	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.76	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.63	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.54	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.46	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-

Appendix 14. (continued)

Primer (UBC)	Composite Generation	Population Size	Marker size (kb)	Single plants 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
338	2	4	2.51	1	1	1	1	1	1	1	0	1	1	1	1	-	-	-
			1.82	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.59	1	1	1	1	1	0	1	1	0	0	1	1	-	-	-
			1.45	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.23	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			1.05	1	0	1	0	1	1	1	1	1	1	1	1	-	-	-
			0.98	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.89	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.83	1	0	0	0	0	0	1	0	0	1	0	1	-	-	-
			0.66	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
			0.56	1	1	1	1	1	1	1	1	1	1	1	1	-	-	-
338	2	8	2.51	0	1	0	1	0	1	0	-	-	-	-	-	-	-	-
			1.82	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.59	1	0	0	1	1	1	1	-	-	-	-	-	-	-	-
			1.45	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			1.23	1	1	1	1	1	1	0	-	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.98	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.89	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.83	0	0	0	0	0	1	1	-	-	-	-	-	-	-	-
			0.66	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
			0.56	1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
338	2	12	2.51	1	1	1	0	0	1	-	-	-	-	-	-	-	-	-
			1.82	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.59	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.45	1	1	1	0	0	0	-	-	-	-	-	-	-	-	-
			1.23	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.98	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.89	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.83	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-
			0.66	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
			0.56	1	1	1	1	1	1	-	-	-	-	-	-	-	-	-
338	2	22	2.14	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.70	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.55	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.38	0	0	0	0	1	1	0	0	-	-	-	-	-	-	-
			1.29	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			1.15	0	1	1	0	1	1	1	1	-	-	-	-	-	-	-
			1.05	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.98	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.91	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.85	1	0	0	1	0	1	1	0	-	-	-	-	-	-	-
			0.78	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.58	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
			0.50	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-

* The symbol ' -' represents the absence of data for these plants.