Genetic diversity of *Brassica carinata* with emphasis on the interspecific crossability with *B. rapa*

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With 3 figures and 1 table

Abstract

*Brassica carinata* (BBCC, 2n = 34) possesses many good agronomic characters and, to provide more genetic information about this species and utilize it further, 110 accessions of *B. carinata* were tested for genetic variation by using 233 amplified fragment length polymorphism markers, so that a dendrogram could be constructed. To combine the good traits of *B. carinata* and *B. rapa* (AA, 2n = 20) with those of *B. napus* (AACC, 2n = 38) which is the major rapeseed variety in China, interspecific crosses between these two species have been made which have resulted in 276 doubled hybrids (AABBCC). These hexaploids combine positive characters and can be used for crossing which have resulted in 276 doubled hybrids (AABBCC). These hexaploids combine positive characters and can be used for crossing with *B. rapa* and *B. carinata* as the female parents exhibited extensive differences in interspecific crossability and 20 high crossability accessions were identified. The Poisson Regression Model analysis result (P < 0.0255) indicated that such differences were due to the genotype of the accessions. Accessions with high crossability could promote gene flow within the genus *Brassica*.

Key words: *Brassica carinata* — *Brassica napus* — genetic variation — amplified fragment length polymorphism — interspecific cross — gene pool

Ethiopian mustard (also called Abyssinian Mustard or Abyssinian Cabbage *Brassica carinata*, BBCC, 2n = 34) possesses many desirable agronomic characteristics that are rare in the other *Brassica* oil crops: heat and drought tolerance, disease and pest resistance, and a yellow-seeded germplasm (Gugel et al. 1990, Bayeh and Gebre Medhin 1992, Yitbarek 1992). Its importance to breeders has increased because of rapid improvement in its seed quality (Getinet et al. 1994, Velasco et al. 2003, Teklewold and Becker 2005). As a result, its genetic diversity has prompted considerable interest among *Brassica* breeders. Alemayehu and Becker (2002) analysed 13 morphological and seed quality characteristics of 36 accessions of *B. carinata* and found extensive genetic variability. Warwick et al. (2006) used amplified fragment length polymorphism (AFLP) to evaluate the patterns and extent of genetic diversity of *B. carinata*. Exploring the genetic diversity of *B. carinata* may provide more information for plant breeders.

Interspecific hybridization is a useful way to transfer desirable traits from one species to another, and has enriched the gene pool of *Brassica* crops (Allard 1960, Prakash and Chopra 1998, Liu 2000). Over the past few decades, numerous efforts to widen the genetic basis of *B. napus* (AACC, 2n = 38) which is narrow when compared to *B. rapa* and *B. carinata* have been undertaken. In previous research, it was demonstrated that a hexaploid hybrid (AABBCC), derived from an interspecific cross between *B. carinata* and *B. rapa* (AA, 2n = 20), could be used as a bridge hybrid (Li et al. 2004). By crossing the hexaploid hybrid with *B. napus*, it was possible to create a pentaploid hybrid (AABBCC). This pentaploid hybrid was then used to create a variant of *B. napus* whose DNA comprised half the A genome of *B. rapa* and half the C genome of *B. carinata* (Li et al. 2004, 2006). The crossability between *B. carinata* and the three basic species of *Brassica* and *B. napus* is very low (Stebbins 1958). Several interspecific crosses involving *B. carinata* have been made, which have resulted in the transfer of some desirable traits from *B. carinata* to other *Brassica* species (Choudhary et al. 2000, Rahman 2001, Tonguc and Griffiths 2004). Therefore, exploring genotypes of *B. carinata* that have high interspecific crossability would be useful in order to promote gene flow within the genus *Brassica*.

This investigation has three objectives. First, to evaluate the genetic diversity of 110 accessions of *B. carinata*. Second, to create a new bridge germplasm (AABBCC) for transferring genetic material from *B. carinata* and *B. rapa* to *B. napus*. Thirdly, to screen genotypes of *B. carinata* for high crossability to *B. rapa*.

Materials and Methods

**Plant materials:** One hundred and seven accessions of *Brassica carinata*, mainly of Ethiopian origin, were obtained from the Centre for Genetic Resources, Wageningen, the Netherlands (CGN; Supplementary Table 1). An additional three accessions (88-216845, 80-139, 88-219790) of *B. carinata* were obtained from Germany (Supplementary Table 1). Twenty-nine accessions of *B. rapa* were collected, mainly from various locations in China and grown for at least four generations in Wuhan, China (Supplementary Table 2).

**Field trial:** All accessions were sown in the field station of the Huazhong Agricultural University, Wuhan in September 2003. Each accession was planted in three rows with 10 plants per row. To ensure pollination by *B. rapa* throughout the flowering period of *B. carinata*, the seeds of *B. rapa* were sown 43 days and 55 days after sowing the accessions of *B. carinata*, respectively. When the plants flowered in March 2004, buds from three or four plants of each *B. carinata* accession were picked and randomly fertilized with pollen of *B. rapa*. At harvest, the crossability of different parents was analysed. In 2005, four accessions (CGN03941, CGN03949, CGN03995 and CGN04004)
of *B. carinata* with high interspecific crossability and one accession (CGN03931) with low crossability to *B. rapa* were selected for crossing with *B. rapa* to assess the environmental effect on the interspecific crossability. To make the triploid interspecific hybrids fertile, the putative hybrids were treated with 0.1% (w/v) colchicine.

**AFLP procedure:** DNA samples were prepared from young leaves of five plants of each accession of *B. carinata* to estimate the genetic variation amongst accessions. DNA was extracted using a modified Cetyltrimethyl Ammonium Bromide (CTAB) procedure (Doyle and Doyle 1990), concentration and purity were measured.
by a Beckman spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA). AFLP-PCR was performed according to the method described by Vos et al. (1995), with minor modifications. Genomic DNA (200 ng) was restricted with EcoRI (2 U/200 ng DNA) and MseI (2 U/200 ng DNA) for 5 h. After ligation for 10 h (1 U ligase/200 ng DNA), the preamplification was carried out in a Perkin-Elmer 9600 thermocycler (Foster City, CA, USA). The pre-PCR products were diluted 1 : 20 with deionized water and used for a second PCR with selective primers. The PCR products were separated on a 6% polyacrylamide gel followed by silver staining.

Data analysis: To facilitate scoring across gels, a GeneRuler™ 50 bp AFLP DNA ladder (MBI Fermentas, GmbH, Germany) was used. Polymorphic fragments between 50 and 400 bp in size were scored as either present (1) or absent (0). DNA markers were analysed by the Unweighted Pair Group Method with Arithmetic mean. A dendrogram was constructed by software NT SYS-PC 2.1 (Rohlf 2002) using SM coefficients (Sokal and Michener 1958). The SM coefficient was calculated with the formula:

\[ SM_{ij} = \frac{\left( n_{10} + n_{01} \right)}{\left( n_{11} + n_{01} + n_{00} \right)} \]

where \( n_{ij} \) was the number of bands in common between accessions \( i \) and \( j \), \( n_{10} \) was the number of bands present in \( i \) and absent in \( j \), \( n_{00} \) was the number of bands absent in \( i \) and present in \( j \), and \( n_{01} \) was the number of bands both absent in \( i \) and \( j \).

Bootstrap analysis was performed with the Phylogenetic Analysis, using Parsimony (PAUP) software (Swofford 1999) to evaluate the robustness of the nodes of the dendrogram.

Crossability analysis: The interspecific crossability index (ICI) between \( B. \) carinata and \( B. \) rapa was defined as the number of hybrid plants obtained from 100 cross-pollinated buds, an ICI value bigger than 20 being considered an accession with high crossability. After the ICI was determined, the Poisson Regression Model (PRM) in the GENMOD procedure of the Statistical Analysis System (SAS Institute 1999, Park 2005) was used to compare the differences in ICI for each female parent, where the female parents were included as factors and likelihood ratio tests were used to determine the appropriate model.

Results

Genetic variation amongst accessions of \( B. \) carinata

Morphological and agronomic characteristics varied amongst the \( B. \) carinata accessions. The colour of young stems or leaves differed, especially along the main leaf vein. Some plants showed purple colouring which made them easily distinguishable from the green plants. Bolting and flowering times of the accessions ranged between 90 and 212 days. Some of the accessions were seriously damaged by frost when the plants were bolting in the early winter.

Two-hundred and thirty-three AFLP markers were generated from the 110 accessions of \( B. \) carinata with 36 primer pairs, of which 11 produced more than seven polymorphic bands (data not shown). The genetic similarity coefficients varied from 0.46 (CGN0391–CGN0399) to 0.88 (CGN0393–CGN0400 and CGN04032–CGN04036) and a dendrogram based on them was obtained (Fig. 1). Bootstrap analysis showed that only four of 109 nodes in the dendrogram had values higher than 0.5 (data not shown).

There were no significant relationships between accessions originating from the same region that could indicate gene flow between accessions from Ethiopia.

Interspecific crossability

In total, 409 interspecific combinations were made. Eventually, 13 000 seeds were obtained from 40,000 cross-pollinated buds involving 375 combinations. As in most cases \( B. \) carinata and \( B. \) rapa could be distinguished easily by their morphology, it was not difficult to identify true hybrid plants. Few of the hybrids required verification by molecular marker analysis and chromosome counting because they resembled their maternal parents. Eventually, more than 5000 hybrid plants were generated from 326 interspecific combinations (Supplementary Table 1). However, colchicine treatment doubled the number of chromosomes in only 23.3% of the hybrid plants, which resulted in 276 combinations of hexaploid hybrid plants.

The ICI of accessions of \( B. \) carinata and \( B. \) rapa differed considerably (Fig. 2). Some accessions of \( B. \) carinata yielded more than 80 hybrid seeds per 100 interspecific pollinated buds, whereas some produced none. A set of data selected from the random interspecific combinations met the requirement of PRM where the male parent, of \( B. \) rapa, ‘Baijian 13’, was crossed to nine female parents each involving three or four individual plants. The Value/DF of Deviance and Pearson chi-square were 0.21 and 0.16, respectively, which indicated that PRM was fitting the data set for the analysis. The PRM analysis result (\( \chi^2 = 17.48, df = 8, P < 0.0255 \)) showed that the ICI for each female parent was statistically significant, indicating that such differences were caused by the genotype of the accessions.

![Fig. 2: The interspecific crossability index of Brassica carinata and Brassica rapa. The accession number (x-axis) corresponds to the numbers listed for Brassica carinata in Supplementary Table 1 and Brassica rapa in Supplementary Table 2](image-url)

![Fig. 3: The distribution of 104 accessions of Ethiopian origin of Brassica carinata. The y-axis and the x-axis denote the latitude and longitude, respectively. The solid triangles denote the 20 accessions with high crossability to Brassica rapa, of which 16 were located in the southeast region of the Great Rift Valley (broken line)](image-url)
Table 1: Results from crossing five *Brassica carinata* accessions with *B. rapa* in the year 2005

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Accession name</th>
<th>Number of crossing combinations</th>
<th>Number of pollinated buds</th>
<th>Number of true hybrid plants</th>
<th>ICI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGN03941</td>
<td>Gommenzer (Amh.)</td>
<td>18</td>
<td>1089</td>
<td>874</td>
<td>80.3 (40)</td>
</tr>
<tr>
<td>CGN03949</td>
<td>Gommenzer (Amh.)</td>
<td>28</td>
<td>1617</td>
<td>1081</td>
<td>66.9 (28)</td>
</tr>
<tr>
<td>CGN03995</td>
<td>Gommenzer (Amh.)</td>
<td>62</td>
<td>2540</td>
<td>2506</td>
<td>98.7 (80)</td>
</tr>
<tr>
<td>CGN04004</td>
<td>Gommenzer (Amh.)</td>
<td>13</td>
<td>848</td>
<td>535</td>
<td>63.1 (47)</td>
</tr>
<tr>
<td>CGN03931</td>
<td>Gommenzer (Amh.)</td>
<td>20</td>
<td>1272</td>
<td>93</td>
<td>7.3 (6)</td>
</tr>
</tbody>
</table>

1The number in brackets is the interspecific crossability index (ICI) value from 2004.

Twenty accessions with ICI values above 20 were selected (Supplementary Table 1). Of these, 16 originated from the south-east region of the Great Rift Valley, Ethiopia. Furthermore, 13 of these accessions were geographically located between 850–920N and 3950–4205E (Fig. 3). The ICI data of 2004 and 2005 (Table 1) matched well, with a high correlation coefficient (r = 0.88), indicating that the basis of the variation of its interspecific crossability was mainly determined genetically.

**Discussion**

The genetic diversity detected in this trial is wider than that previously reported by Warwick et al. (2006) and may be due to a larger sample (110 accessions). This diversity could be useful for breeders when utilizing genotypes from different genetic subclusters in their breeding programmes.

The low support value of the bootstrap analysis may be partly due to the wide range of genetic diversity within most of the open-pollinated accessions. This is supported by observations on morphology and by molecular marker analysis. Low support values can arise when a relatively small number of polymorphic bands and dominant markers that may exhibit homoplasies are used for constructing the dendrogram (Koopman et al. 2001). Homoplasies in AFLP data sets can be caused either by scoring non-sequential identical fragments of equal length or bands that represent codominant loci as dominant loci. Nevertheless, genetic variability studies have shown consistency between AFLP-based phenetic dendrograms and pedigree or phylogenetic data, which suggests that homoplasies in AFLP data are relatively rare (Hill et al. 1996, Lombard et al. 2000, Seefelder et al. 2000, Koopman et al. 2001).

Previous studies on the cytogenetics and molecular genetics of Brassicaceae species have revealed significant differences between the A-, B- and C-genomes from the three diploid species and the three amphidiploid species (McGrath and Quiros 1990). A synthetic rapeseed (*B. napus*, AAC, 2n = 20) generated by combining half of the A-genome of *B. rapa* (AA) and half of the C-genome of *B. carinata* (BBC) is very different from the original *B. napus* and exhibits strong heterosis when crossed to the wild-type *B. napus* (Li et al. 2006). Bridge hexaploids (AABBCC) combine the positive characteristics of *B. rapa* and *B. carinata* and are essential for broadening the genetic variation of *B. napus*.

Interspecific reproductive isolation is the main mechanism for speciation and specific maintenance (Griffiths et al. 2002). However, genetic leaks in the isolation system have occasionally enabled interspecific gene flow and promoted the evolution of species. Incompatibility with related species in some genotypes of wheat and tomato have been overcome when the varieties are used as female parents (Snape et al. 1979, Sharma and Gill 1983, Poysa 1990). Similar findings have been reported in Brassicaceae species (Ripley and Beversdorf 2003). Earlier reports highlight the importance of the maternal genotype in this species (Meng et al. 1992, Meng and Lu 1993, Liu and Meng 1995). Choudhary et al. (2000) reported that the crossability between *B. carinata* and *B. rapa* is very low, especially when the latter is used as the female parent. In a previous report, two cultivars of *B. rapa* with a high interspecific crossability involving three accessions of *B. carinata* and nine cultivars of *B. rapa* were selected. It was not possible to identify a cultivar of *B. carinata* with good crossability due to the limited number of accessions being evaluated (Meng et al. 1992). In the present experiment, highly crossable accessions were selected and these lines could be used as bridge materials for transferring the desirable characteristic to other *Brassica* species and vice versa. In contrast to most other interspecific combinations where cross-pollinated siliques were developed between 15 and 20 days after pollination, the compatible interspecific combinations usually produced fully developed seeds within each cross-pollinated silique. This implies that accessions of *B. carinata* allow foreign pollen to germinate on their stigmas and enable pollen tubes to penetrate their styles. However, only cultivars with a high crossability have the mechanism(s) to enable the hybrid embryos to develop fully. Further studies on the genetic basis of interspecific crossability, to increase our understanding of the mechanism, will be undertaken.

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**Supplementary Materials**

The following material is available online at http://www.blackwell-synergy.com:

**Table 1:** Accessions of *B. carinata* obtained from the Netherlands and Germany and their crossability with *B. rapa*

**Table 2:** Accessions of *B. rapa* obtained from China and Sweden and their crossability with *B. carinata*