Molecular characterisation of microspore-derived progeny from the interspecific F_1 of *Brassica napus* × *B. carinata*

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Abstract

We report the molecular characterisation of microspore-derived progeny from an interspecific F_1 hybrid (ABCC genome) of a *Brassica napus* (AACC genome) × *B. carinata* (BBCC genome) cross using microsatellite markers with known genetic map locations on the *Brassica* A, B and C genomes. We observed unusual inheritance patterns of chromosomes from the A, B and C genomes with the majority of microspore-derived lines possessing chromosome complements similar to, but not the same as, the interspecific F_1. We discuss possible explanations for this phenomenon including the hypothesis that these F_1-like progeny are products of unreduced (2n) gametes, possibly through a mechanism of First Division Restitution.

Key words: Oilseed rape, Ethiopian mustard, doubled haploidy, unreduced gametes, microsatellite markers

Introduction

The unique genomic relationships among the six cultivated *Brassica* species are well known, having first been summarised in the Triangle of U more than 70 years ago (U, 1935). The three allotetraploid species (*B. juncea*, AABB; *B. napus*, AACC; and *B. carinata*, BBCC) were formed from pairwise combinations of the three diploid species (*B. rapa*, AA; *B. nigra*, BB; and *B. oleracea*). The allotetraploid species are thought to have formed on few occasions; these genetic bottlenecks served to restrict the extent of genetic diversity in the allotetraploid species compared to the diploid species. Oilseed rape (*B. napus*) underwent a second genetic bottleneck during the development of canola quality strains. Genetic diversity of Australian canola underwent a third genetic bottleneck because it was formed from just 18 introduction lines from Europe, Japan and Canada of which 11 introduction lines now contribute 98.7% of the pedigree composition of the core population (Cowling, 2007). Future genetic gains in canola depend on increasing the available genetic diversity in *B. napus*.

Interspecific crossing is a common approach for increasing allelic diversity in genetically moribund crop species. *B. napus* has been crossed successfully to all other Triangle of U species and also to many other related species. However, high infertility rates are generally observed, which are normally remedied by repeated backcrossing to the targeted crop species. A disadvantage of this approach is the loss of allelic diversity from the donor species during the backcrossing process, with an average of 50% reduction in donor species per backcross generation. Consequently, interspecific crossing in *Brassica* has generally been successful only for transferring major genes as these have a greater chance of persisting backcross progeny compared to polygenic traits. Most agronomically significant traits are polygenic in nature; therefore, a technique that could allow the transfer of large segments of donor species into a target crop species would be very useful for breeding purposes.

In a previous paper, we developed the theoretical basis for the application of microspore culture of interspecific F_1 to produce fertile progeny, using *B. napus* × *B. carinata* hybrids (ABCC genome) as a case study (Nelson et al. 2006). In that study, we developed a simple model of expected chromosome composition of gametes based on binomial distributions of A and B genome chromosomes (assuming no homoeologous interactions) and normal disomic inheritance of the shared C genome chromosomes (assuming normal pairing of the C-genome chromosomes of *B. napus* and *B. carinata*). In that paper, we presented results of estimated ploidy levels based on total DNA content measured by flow cytometry. Most microspore-derived progeny fell in the predicted 4*x* (tetraploid) distribution, with few in the 2*x* (diploid) and 8*x* (octoploid) distributions.

This current paper extends the analysis of these lines using microsatellite markers with known chromosomal locations, which indicated that segregation of A, B and C chromosomes was not as we expected.

Materials and Methods

Genetic materials: The genetic material used in this study was a set of 28 microspore-derived lines derived from microspores of a single F_1 plant of an interspecific cross between *B. napus* ("Trilogy") and *B. carinata* ("24.2"); for details of the interspecific crossing and microspore culture process, refer to Nelson et al. (2006). DNA from the parents, F_1 and microspore-derived lines was purified using a standard CTAB extraction for subsequent marker analysis.

Microsatellite markers: Microsatellite markers were obtained under material transfer agreement from the Agriculture and AgriFood Canada *Brassica* Microsatellite Consortium (A and C genome markers) and *Brassica juncea* Microsatellite Consortium (B genome markers). Marker locations in *B. napus* (A and C genome markers) and in *B. juncea* (B genome markers) were provided (personal communication, Andrew Sharpe and Derek Lydiate, AAFC Saskatoon Research Centre,

Canada). Microsatellite markers were amplified using 25 µL reactions with the final concentrations: 2 ng/µL DNA template, 1x PCR buffer (Promega), 0.04 U/µL Taq Polymerase, 2 mg/µl $MgCl_2$, 200 µM of each dNTP and 0.2 µM of each primer. PCR was performed using an Eppendorf Mastercycler with the following thermal cycling: initial denaturing of 5 minutes at 94 °C; followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 60 s at 72 °C; with final extension at 72 °C for 7 minutes (B genome markers) or 15 minutes (A and C genome markers). The 5′ nucleotide of the forward primers of the A and C genome markers were fluorescently labelled using 6FAM, PET, NED or VIC (Applied Biosystems, AB) and amplification products assayed on an AB3730xl capillary DNA sequencer (AB), and fragment analysis conducted using Genemapper software (AB). B genome marker amplification products were electrophoresed in TBE buffer using 2% agarose gels and were scored for presence or absence of amplification products.

Statistical analyses: Statistical analyses were performed using PRIMER 6 (Plymouth Routines In Multivariate Ecological Research) software (Primer-E, Plymouth, UK). A resemblance matrix was generated from the marker genotype data using Euclidean distances, from which a cluster analysis using group averages was performed. An ordination analysis was performed using a Multi-Dimensional Scaling (MDS) plot, which is analogous to a principle component analysis in this instance.

Results

Fifty-eight microsatellite markers detecting 21, 29 and 33 loci on A, B and C genome chromosomes were used to characterise 28 microspore-derived lines along with parental and F_1 controls. According to the simple binomial distribution model of meiotic products in the ABCC F_1 hybrid proposed by Nelson et al. (2006), the expected mean number of A and B genome chromosomes in the microspore-derived lines was 50% (that is, 294 A genome and 462 B genome marker alleles across 28 lines). The observed number of A and B genome alleles detected in 28 lines was 539 and 835, respectively. Therefore the model was rejected at $p < 0.001$ (χ^2 ($\nu = 1$) = 408.3 and 602.3, respectively). In the same model, C genome chromosomes from both *B. napus* and *B. carinata* parents were expected to pair and recombine in the F_1 and segregate equally in microspore-derived lines. Segregation of C genome marker alleles and recombination between marker loci on the same chromosomes was observed in only two lines (‘17’ and ‘18’); the other 26 lines possessed both parental alleles at the majority of C genome loci.

Based on the presence or absence of SSR marker alleles, the chromosome complements of all lines (including controls) were recorded as presence or absence of A, B and C genome chromosomes, and the resulting binary matrix was subjected to resemblance analysis (using dissimilarity based on Euclidean distances). Cluster analysis using this resemblance matrix showed that all microspore-derived lines were distinct from both parents, but that there were several clusters of microspore-derived lines that showed no SSR differences between individuals (Fig. 1). Ordination analysis using multidimensional scaling (MDS) confirmed that the majority of lines were similar, but not identical, to the F_1 individual (Fig. 2). The MDS analysis also showed that the most distinct microspore-derived lines were ‘17’ and ‘18’, the two lines mentioned above that appeared to have undergone normal meiotic pairing of C genome chromosomes.

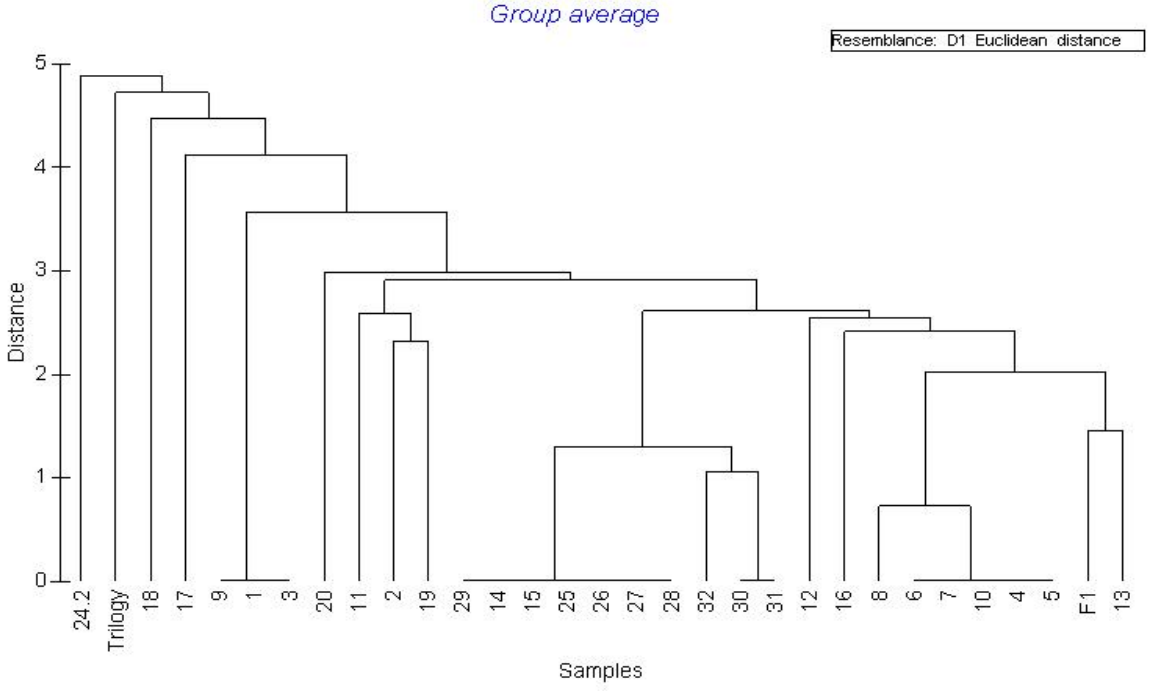

Fig. 1: Dendrogram of a set of 28 experimental progeny derived from microspores of a *Brassica napus* (“Trilogy”) × *B. carinata* (“24.2”) F_1 interspecific hybrid based on presence or absence of chromosomes as detected by microsatellite markers distributed among all A, B and C genome chromosomes.

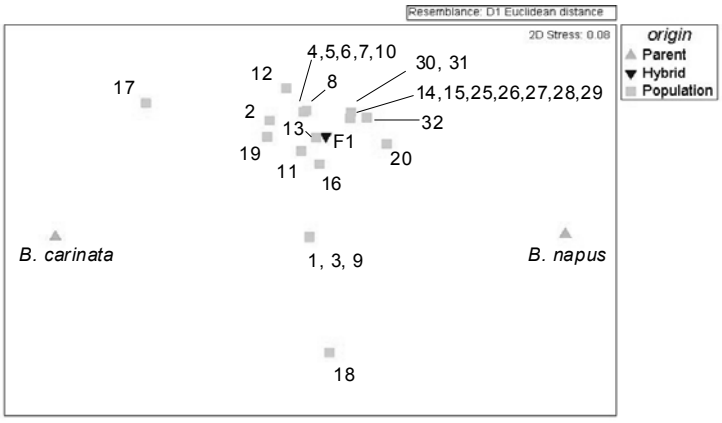

Fig. 2: Multi-Dimensional Scaling plot for a set of 28 experimental progeny derived from microspores of a *Brassica napus* ("Trilogy") × *B. carinata* ("24.2") F_1 interspecific hybrid based on presence or absence of chromosomes as detected by microsatellite markers distributed among all A, B and C genome chromosomes. The 2-D stress level of 0.08 indicates that this two dimensional scaling represents the complex interactions well.

Discussion

This work is unique in applying microspore culture from interspecific hybrids of *B. napus* × *B. carinata* to derive fertile interspecific progeny. We aimed to isolate microspore-derived embryos that had a viable chromosome complement after meiosis in the interspecific hybrid. If successful, this approach would be an efficient method to overcome infertility barriers commonly encountered when the F_1 plant undergoes meiosis and selfing. The marker analysis reported here revealed three surprising results. Firstly, there was a much larger representation of A and B genome chromosomes as was expected assuming non-interaction between univalent chromosomes: in brief, most individuals possessed most A and B chromosomes. Secondly, only 2 out of 28 microspore-derived lines showed the expected pairing and recombination of C genome chromosomes from each parental species (*B. napus*, AACC genome; *B. carinata*, BBCC genome), which was expected given the apparently unrearranged C genomes in U's Triangle species. The remaining 26 lines each possessed both parental C genome alleles at most (but not all) marker loci. Thirdly, there appeared to be four sets (of between 2 and 7 individuals) that had identical marker allele genotypes, consistent with being clonal in nature.

It is interesting to speculate on the cause of the unusual chromosome complements observed in the microspore-derived progeny in this experiment. Two possible explanations for the large proportion of lines showing near-F_1 chromosome complements are: 1) progeny may have been inadvertently derived from somatic rather than gametic tissue of the interspecific hybrid; or 2) most progeny were derived from unreduced (2n) gametes possibly arising through First Division Restitution. The first explanation is not convincing because little or no somatic tissue was observed in the isolated microspore culture, nor was there any indication of an intermediate callus phase before embryogenesis; this explanation would also require the loss of several chromosomes to explain the chromosome complements of the progeny. The second explanation, that of unreduced (2n) gametes, is more convincing as this phenomenon is known to occur at low frequencies even in naturally occurring populations of the Brassicaceae (Mackay and Low, 1975, Veilleux, 1985). It is reasonable to assume that such abnormalities would be more common in interspecific progeny; indeed there is evidence that this is the case in previous studies (Heyn, 1977; Eenink, 1975). A possible mechanism leading to unreduced gametes is First Division Restitution, which is the failure of the first meiotic division to separate homologous chromosomes after pairing resulting in all, or the majority of, the chromosomes remaining on one side of the division plate (Bretagnolle and Thompson, 1995). The mechanism of First Division Restitution provides a cohesive explanation for the presence of both parental C genome chromosomes and loss of chromosomes in most of the experimental individuals. Follow-up experiments will investigate the meiotic process in interspecific F_1 hybrids to test this hypothesis.

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