

# Derived allopoloidy: an unexplored avenue for augmenting genetic variation in *Brassica* digenomics

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## Abstract

From empirical plant breeding perspective, allopoloidy is a genetic bottle neck. This is very much evident from generally a poor response to selection for yield in allopoloid *Brassica* species. Resynthesis of *Brassica* digenomics or homoeologous introgression from related digenomics has not helped greatly to enlarge the base of working germplasm in the past due to associated genetic and / or phenotypic instabilities besides poor breeding value. This is partly explained by the fact that two of the three *Brassica* monogenomic progenitor species (*B. nigra* and *B. oleracea*), used for resynthesis of *Brassica* allopoloids, have not faced any significant human intervention for evolution as oilseed crop. Derived allopoloidy, i.e. isolation of an amphiploid through hybridization of non parental amphiploids, is proposed as a way forward. The process involves development of F<sub>1</sub> hybrid between two digenomics {e.g. *B. juncea* × *B. napus* → F<sub>1</sub> (AABC)}, chromosome doubling of the F<sub>1</sub> hybrid (AABC → AAAABBCC), selfing and selection for target non parental digenomic (BBCC) in successive generations. There is a rapid chromosome loss with preferential elimination of tetrasomic genome (AAAA). Raising of a large self population following colchicoidy and use of genome specific molecular markers may restrict the process to 1-2 generations of selfing. Derived amphiploids are expected to be superior to resynthesized ones as these involve intensely bred parents. Further, it is a one step procedure to channelize breeding gains of two digenomics into a third. Using this strategy we could develop '0' *B. carinata* following hybridization between elite '0' / '00' *B. juncea* and *B. napus* types. Similarly, *B. juncea* was extracted by hybridizing *B. napus* and *B. carinata*. Besides the breeding significance, derived allopoloids will provide useful insight into evolutionary processes as these bring together genomes that have undergone varied genetic / epigenetic changes following their past tryst with natural allopoloidy, cohabiting with different genomic partner(s). Molecular diversity, cytogenetic stability and breeding value of the derived *B. carinata* and *B. juncea* will be discussed.

**Key words:** interspecific hybridization, *Brassica* digenomics species, derived *B. carinata*, genetic similarity.

## Introduction

Resynthesis of digenomics from their diploid progenitors has been profitably used to enhance the spectrum of variability for key economic traits in *B. napus* (Seyis *et al* 2003) and *B. juncea* (Srivastava *et al* 2004). Similar approach may not prove much useful in *B. carinata* as none of its two diploid progenitors has any history of human selection pressure for evolution as an oilseed crop. Therefore, newer options of variability enhancement merit a look in. These include selective introgression and /or whole genome transfer from the related amphiploid species like *B. napus* (AACC) and *B. juncea* (AABB). Such approaches may also help in capitalizing on gross structural evolutionary modifications that occurred in cohabiting genomes of *Brassica* digenomics during evolution (Song *et al* 1995).

## Material and methods

*B. napus* cv. MHO-18-1-184 ('00') and *B. juncea* cv. NJHO-3-25 were utilized in interspecific hybridization for resynthesis of *B. carinata*. Immature buds of plants of both the parents were emasculated and immediately pollinated with fresh pollen of each other to accomplish reciprocal cross combinations. Pollinated buds were bagged to prevent unwanted pollination. The F<sub>1</sub> seed was raised carefully in pots and the resultant F<sub>1</sub> plants (AABC) subjected to colchicine treatment to induce chromosome doubling. Seed set on polyploid sectors (AAAABBCC) was used to raise A2 generation. *B. carinata* type plants were identified in A3 generation. These plants were subjected to diversity analysis alongwith parental strains using variation in the DNA amplification generated through 32 decamer RAPD primers, as per the protocol described earlier (Bhaskar *et al* 2002). The polymorphism data from all the primers were used to estimate the similarity coefficients (NTSYS Pc version 2.02 e) on the basis of the number of shared amplified bands. Similarity was calculated with SIMQUAL function of NTSYS which computes a variety of similarity and dissimilarity coefficients (association coefficients) for qualitative/nominal data. The similarity matrix thus generated was used to generate a dendrogram.

## Results

*B. napus* × *B. juncea* hybrids were successfully produced without any embryo rescue intervention. The F<sub>1</sub> hybrid plants exhibited intermediate phenotype and were semi sterile. Hybridity was confirmed through cytological and molecular studies. Cytological studies revealed the expected somatic chromosome number 2n=37. The amphiploid shoots (A<sub>1</sub>) were identified by their deep green color, thick crinkled leaves, slower growth, bigger inflorescences and large flowers with well developed anthers carrying large pollen grains.

The  $A_2$  generation was developed from the selfed seed of amphiploid sectors. There was lot of morphological variation in morphology of  $A_2$  plants. Two plants resembling *B. carinata* morphotype were selected and subjected to the cytological investigations. The PMCs of these  $A_2$  plants revealed the chromosome number to be 38 and 40 with mean bivalent frequency of 13.3. Multivalents were observed in majority of the cells. Out of the two *B. carinata* type  $A_2$  plants, one plant was completely male sterile and did not set seed on selfing. The pollen grain stainability of the second plant was also low (25-30%) and only 20 seeds were obtained on selfing. These were grown to raise  $A_3$  generation. Three  $A_3$  plants which were almost like *B. carinata* were identified and characterized. One plant (NJ 1) was completely male sterile. Remaining two (NJ 2 and NJ 3) showed partial pollen grain stainability. Both of them had  $2n=34$ . The derived *B. carinata* plants were almost 20 days late in maturity than the parents. Plant height in the derived plants (NJ 1 and NJ 2) was intermediate between the two parents. Seed yield of the derived plants was also low and most of the selfed seed set was shriveled.

The fatty acid profile of the seed set on both the plants was of parental type having '0' erucic acid. Apparently, the derived *B. carinata* inherited alleles for low erucic acid located on B and C genomes from donor *B. juncea* and *B. napus*, respectively. All the three plants had higher glucosinolates content like *B. juncea* parent.

Derived *B. carinata* types were evaluated for extent of genetic similarity with natural *B. carinata* cv. PC 5. These along with PC 5 and two parents were subjected to natural diversity analysis using variation in DNA amplification generated through 32 decamer primers. Clustering allowed partitioning the six genotypes into three groups. The three derived genotypes and natural *B. carinata* cv. PC5 were clustered at approximately 80 per cent similarity level. Among the derived genotypes NJ 2 and NJ 3 showed 86 per cent similarity whereas NJ 1, NJ 2 and NJ 3 were clustered at approximately 82 per cent similarity level. The two parental strains formed separate groups at a similarity level of 68 per cent (*B. napus*) and 63 per cent (*B. juncea*) with derived lines. The diversity analysis revealed that the derived *B. carinata* genotypes were closer to but distinct from natural *B. carinata* which clearly demonstrated the possibility of using *B. napus* × *B. juncea* crosses as a distinct gene pool for *B. carinata* improvement.

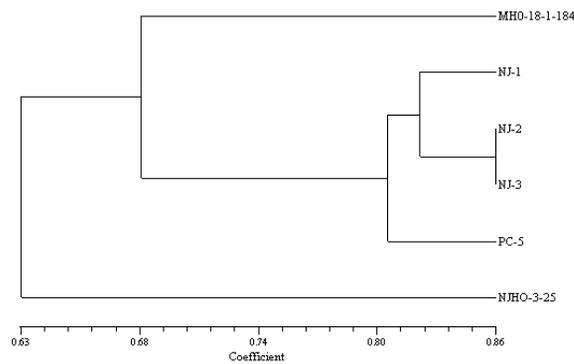


Fig 1: Dendrogram showing molecular diversity in 3 lines of derived *B. carinata*, 2 parental lines and one line of natural *B. carinata*.

## Discussions

Amphiploidy is a dynamic evolutionary force that involves natural synthesis through a large number of hybridization events encompassing diverse diploid progenitors. Interbreeding among these *de novo* amphiploids and the consequent release of recombinational variability allows the natural/artificial selection forces to act and carry forward the process of evolution. Crop *brassic*as constitute an excellent model to study the amphiploid evolution. Both *B. napus* and *B. juncea*, which show very high level of variability, are known to have evolved many times in the sympatric areas of diploid progenitor species. Extent of their geographic spread, racial diversity and apparent morphotype variation have allowed their emergence as crop species of significant economic importance. *B. carinata*, on the other hand, shows only a limited genetic variability, poor geographic reach and low economic value. This has been attributed to its narrow genetic base as a consequence of evolution from fewer number of hybridization events sampling a very low range of variability in diploid progenitors. The process of its genetic enrichment was attempted through derived amphiploidy which allowed bringing into cohabitation intact *Brassica carinata* genomes (BBCC) from non parental amphiploid species. That the attempts were largely successful was apparent from demonstrated similarity with the natural *B. carinata* at morphological and molecular level. Besides huge evolutionary significance of this material, derived *B. carinata* types (NJ 2, NJ 3) provide a new germplasm source for '0' erucic acid in *B. carinata*.

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