

An alternate procedure for resynthesis of *Brassica juncea*

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ABSTRACT

Direct isolation of a digenomic species through hybridization of non parental digenomics, is proposed as an alternate source of genetic variability in *Brassica* amphiploids. The process involves synthesis of F₁ hybrid between two digenomics {e.g. *B.napus* (AACC) x *B. carinata* (BBCC) → F₁ (ABCC)}, chromosome doubling of the F₁ hybrid (e.g. ABCC→ AABBCCCC), selfing and selection for target non parental digenomic morphotype (e.g. *B.juncea*; AABB) in successive generations of selfing / sib-mating. There is a rapid chromosome loss on selfing the octaploid, with preferential elimination of genome in tetrasomic dose (e.g. CCCC). Raising of a large self population after colchipoideity and use of genome specific molecular markers restricts the process to 2-3 generations of selfing. Such derived resynthesized amphiploids are expected to be superior to those developed using conventional resynthesis route as these are based on intensely bred parents. Further, it is a one step procedure to channelize breeding gains of two cultivated digenomics into a third. In the present studies, diversity analysis based on polymorphism generated by SSR markers demonstrated the distinctness of derived resynthesized amphiploids as compared to natural digenomic and conventionally resynthesized amphiploids developed using parental monogenomic species. Besides their breeding value, derived resynthesized allopolyploids will provide useful insight into evolutionary processes as these bring together genomes with varied evolutionary history.

Key words: Indian mustard- amphiploidy-genetic diversity-evolution-digenomics

INTRODUCTION

Resynthesis of novel *B. juncea* (AABB, 2n=36) following hybridization between genetically diverse germplasm of progenitor species (*B. rapa* and *B. nigra*) is routinely used to augment genetic diversity in cultivated *B. juncea*. However, the resynthesized amphiploids have generally not been found very useful for normal crop improvement activities (Bansal et al.,2009). This may be due to associated genetic and/ or phenotypic instabilities, linkage drag and consequently poor breeding value. This may also be due to the fact that two of the three *Brassica* monogenomic progenitor species (*B.nigra* and *B.oleracea*) used for resynthesis of *Brassica* allopolyploids have not faced any significant human intervention for evolution as oilseed crop. All the three *Brassica* digenomics, in contrast, have been bred specifically as oilseed crops in different ecological niches. In this paper, we describe a new approach which allows a single step resynthesis of an amphiploid species following hybridization between the non-parental *Brassica* digenomic species. Successful application of the technique to derive *B. juncea* has been presented along with demonstration of molecular diversity of derived amphiploids.

MATERIAL AND METHODS

Diverse accessions of *B. napus* (inc. Australian accessions) and two genotypes of *B. carinata* were used for the studies. Immature buds on the plants of the parents of the specific combination were emasculated and immediately pollinated with fresh pollen of desired pollen parent to accomplish hybridization. The F₁ seeds were raised carefully in pots and the resultant F₁ (ABCC) seedlings were subjected to colchicine treatment to induce chromosome doubling. Seed set on polyploid sectors (AABBCCCC) was used to raise A₂ generations. *B. juncea* type plants were initially identified in A₃ generation. Putative digenomic plants showing high degree of pollen fertility and seed set were further selfed to raise A₄ generation. In the event of low fertility or inability to identify desired morphotype another cycle of selection was also carried out. The process of resynthesis of derived amphiploids has been depicted in Fig.1.

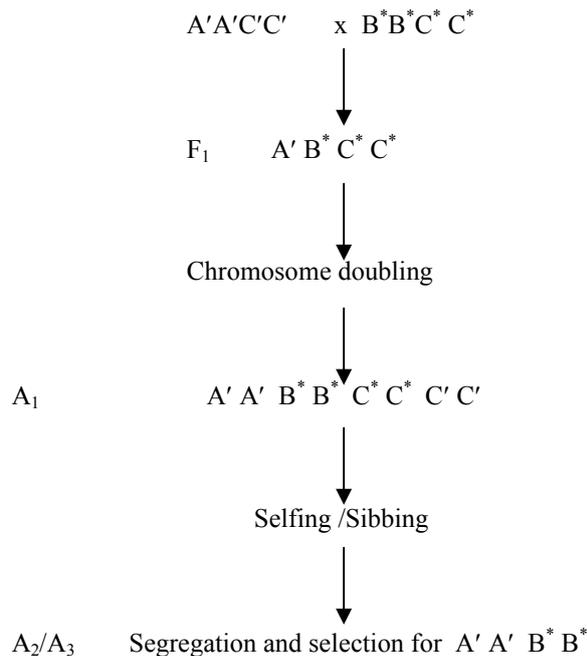


Fig.1: Hybridization scheme to develop non parental digenomic (*B. juncea*) species

For meiotic studies young inflorescences were fixed in Carnoy's solution (ethanol: chloroform: acetic acid, 6:3:1). Anthers having pollen mother cells were squashed in 2% acetocarmine and viewed under the microscope for meiotic configurations. Male fertility status of the putative derived amphiploids was inferred from pollen grain stainability in 2% acetocarmine. For morphological assessments; derived genotypes (A_5) were evaluated along with natural or resynthesized digenomics and the parent species in a randomized block design with three replications. Normal cultural practices were followed throughout the growing season. Diversity analysis was carried out using variation in the DNA amplification generated through ISSR primers. Only public domain primer sequences were used for molecular studies using standard PCR protocols. A weighted neighbour-joining (NJ) analysis was performed on the dissimilarity matrix to determine clustering behaviour. Support for clustering was determined by a bootstrap procedure applied on ISSR bands. DARwin software was used for statistical analysis (Perrier *et al.* 2003). Derived genotypes (A_5), natural or resynthesized digenomics and the parent species were included for the molecular investigations.

RESULTS

Interspecific hybrids having *B.napus* as the female and *B.carinata* as male were successfully produced without any embryo rescue intervention. The F_1 hybrid plants exhibited intermediate phenotype and were semi sterile. Hybridity was confirmed through cytological and molecular studies. Meiotic analysis of F_1 hybrids ($ABCC;2n=37$) indicated the occurrence of quadrivalents/ trivalents in about 87 percent of the pollen mother cells. Application of colchicine (.2%) to the apical buds induced sectoral polyploidy .The amphiploid shoots (A_1) were identified by their deep green colour, 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/open pollinated seeds on amphiploid sectors. There was lot of morphological variation in A_2 generation. Plants resembling *B. juncea* phenotype were identified in each combination and subjected to the cytological investigations. Pollen stainability in *B. juncea* type A_2 plants was generally low (25-30%) and only few seeds were obtained following forced selfing. These were grown to raise A_3 generation. Both pollen and seed fertility improved significantly following one generation of selfing. The derived *B. juncea* plants were late in maturity than the parents. Plant height in the derived A_4 progenies was more than that observed in natural *B.juncea*. Seed yield of the derived plants was also low

as compared to that of the natural *B. juncea*. Derived *B. juncea* genotypes had intermediate erucic acid content, suggesting the involvement of parental *B. carinata* (high erucic acid) and *B. napus* (low erucic acid) in their heredity. Representative plant(s) of the derived *B. juncea* progenies in A₄ generation were evaluated for the extent of genetic dissimilarity with natural and resynthesized *B. juncea* (developed by hybridizing respective diploid species). These were subjected to the diversity analysis using variation in DNA amplification generated through ISSR primers. A total of 20 ISSR primers were evaluated, of which 18 were polymorphic and these amplified 206 loci. Huge variation was apparent as diversity analysis partitioned 96 genotypes into twenty loose groupings. Low bootstrap values of many of the groups indicated intra group heterogeneities. Natural and resynthesized *B. juncea* types were grouped closely. The alignments in the derived amphiploids reflected closeness to pedigree as the derived amphiploids sharing similar parentage tended to be grouped together.

DISCUSSION

B. juncea, which shows very high level of variability, is known to have evolved many times in the sympatric areas of diploid progenitor species. Extents of its racial diversity and apparent morphotype variation have allowed its emergence as a crop species of significant economic importance. Intensive selection practiced by early humans and the crop breeders in recent past has led to a high level of genetic erosion. A simple procedure for developing newer forms of *Brassica* digenomics has been suggested to provide alternate sources of genetic diversity. Since all the three major cultivated *Brassica* species are natural amphiploids, these can benefit from variability enhancement through creation of non-parental amphiploid following hybridization between digenomic species. Selection for *B. juncea* (A' A' B* B') type variants using morphological and molecular screen (microsatellite markers) was successful as early as A₂ / A₃ generations. The octaploid (A' A' B* B* C* C* C' C') was meiotically unstable. This was expected as combinations having more than 2n=40 chromosome number are generally unstable and show varied chromosome number in the segregating generations in *Brassicac*s. Further the chromosomes belonging to the genome present in tetrasomic dose in a synthetic octaploid tend to be eliminated more rapidly as a consequence of multivalency during the process of meiotic division. Relatively normal bivalent formation is expected for the genomes in disomic dose. This approach of augmenting genetic diversity theoretically possess greater advantage of utilizing intensively bred elite digenomics in contrast to resynthesis route where excepting A genome species (*B. rapa*), none of the B (*B. nigra*) and C (*B. oleracea*) genome parental species have been exposed to the human selection pressure for their evolution as oil forms. Another advantage of this approach is the utilization of gross structural modifications that had occurred in cohabiting genomes of *Brassica* digenomics during evolution (Song et al. 1995), with the genome of forcing or male diploid progenitor modified to a greater degree than the genome of cytoplasm donor species. Thus B-genome of *B. juncea* (AABB) is distinct from B genome of *B. carinata* as well as B genome of *B. nigra*. Similarly, A genome of *B. napus* is distinct from A genome of *B. juncea*. Bringing together modified genomes via hybridization of the amphiploid species is further expected to result in different type of variability than that is available in primary gene pools of the natural *Brassica* amphiploids or through resynthesis route utilizing diploid donors. The process of genetic enrichment as attempted through derived amphiploidy during present investigations allowed bringing into cohabitation two complete genomes extracted from two related digenomic species.

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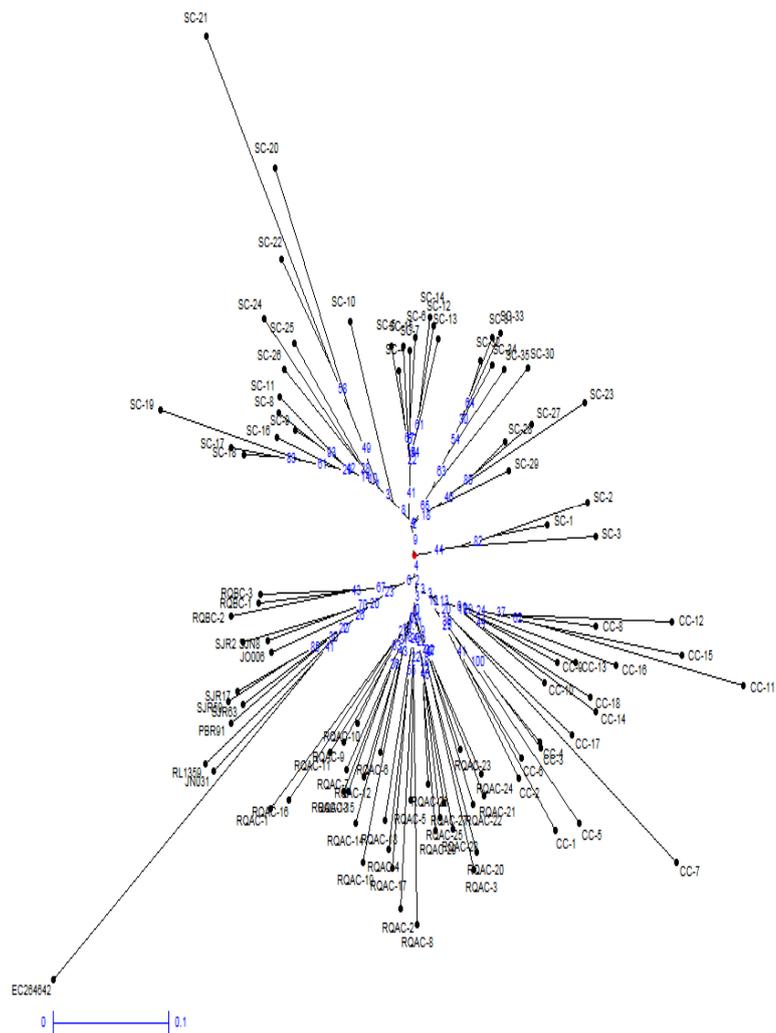


Fig 2: Tree showing molecular diversity in derived *Brassica juncea* genotypes