GENOME BIOLOGY OF THE CULTIVATED BRASSICA

Habib Ahmad, Shahida Hasnain** and Afzal Khan****

ABSTRACT

Studies on Brassica genomes and the genome relationship among various species were begun during second decade of the 20th century AD. The genome relationship was elaborated through chromosomal associations and their morphology. Genome analysis and preferential pairing were also utilized for characterization of the Brassica genomes. Molecular characterization technologies, supplemented with the conventional analytical tools, are nowadays successfully employed for understanding the infrastructure of Brassica genomes. This paper is an attempt to generalize the developments made in the genome biology of Brassica during the recent past.

Key Words: cytogenetics - breeding - genome biology - *Brassica*.

1. INTRODUCTION

Brassica, the type genus of family Brassicaceae, is the nearest related genus to the recently sequenced first angiospermic species: *Arabidopsis thaliana* [1]. In Pakistan, Brassica is represented by 8 species, out of which *B. tournefortii* Gouan. and *B. deflexa* Boiss. are reported only from the wild [2] and the remaining 6 species are cultivated as agricultural crops. Among the agriculturally important species of the genus *B. campestris* L. and *B. napus* L. are categorized as rapeseed [3], which are mainly cultivated for the production of rapeseed and canola oil. *Brassica juncea* Czern. & Coss. , *B. carinata* Burn. and *B. nigra* (L.) Koch. are placed in the mustard group [4]. Besides their major role in the production of mustard oil, they are globally known as a commercial spice [5]. *Brassica oleracea* the sixth agriculturally important species of the genus is known for its use as vegetable and fodder crops, under the common names of cabbage, cauliflower, broccoli, Brussels-sprout and marrowstem kale, etc.

After the successful demonstration of the classical hybrid Raphanobrassica [6] and the artificial re-synthesis of *Brassica napus* [8], during the first quarter the 20th Century AD, the scientists were encouraged to resolve the genome architecture and inter-genomic relationship of the Brassica species. Genome relationship among the species was elaborated either through the analysis of secondary association in the diploids [28,30], karyotyping of the somatic [29] or meiotic chromosomes [9,36] and genome analysis through preferential pairing in haploids [43,44] or amphihaploids [7 -10]. Hardships are still there in distinguishing individual chromosomes in various Brassica genomes [11]. However, the new era starting with the sequencing of *Arabidopsis thaliana* genome [1], associated with the recent development of molecular characterization techniques, will hopefully lead to elaborate the Brassica genomics. This communication attempts to generalize developments made in the genome biology, in its thorough historical perspectives

2. MATERIAL AND METHODS

Remarkable developments regarding the genome relationship and genomics in Brassica, during the 20th century are generalized here. The sequential developments are logically arranged into wide hybridization; inter-specific relationship among different species; intra-genomic characterization through karyotypic analysis; inter-genomic relationship through preferential pairing in haploids; and amphihaploids, and an overview of the genomics in *Brassica.* Reprints of the papers included in this communication were collected directly from the authors, their websites, journals or from the publishers. Books for the relevant information were borrowed from the libraries.

3. RESULTS AND DISCUSSION

3.1 Breeding Results

Upto the first quarter of the 20th Century AD, the *Brassica* species were considered as fixed taxonomic entities and the introgression and transgression of genes among various genomes practically seemed impossible. Sinskaia was probably the first who tried to cross Brassica species and supersede the long-lasting belief of the taxonomic boundaries regarding the crossibility of species [12]. The successful demonstration of Raphanobrassica [13], the first intergeneric fertile hybrid

* & ** Genetics Research Lab., Deptt. of Botany, Govt. Jehanzeb Postgraduate College, Saidu Sharif, Swat. ***Dept. of Botany, University of the Punjab, Lahore.

of *Raphanus sativus* and *Brassica oleracea* further strengthened the idea of genome manipulation through wide hybridization. Through the pairing analyses in *B. nigra* X *B. juncea* hybrids Morinaga [7] was able to provide a clue that the genus Brassica is a polyploid-complex, having both the elementary (diploid) and amphidiploid series of species. The diploid series comprises *B. campestris* (*B. rapa*), *B. nigra* and *B. oleracea,* represented by genomes AA, BB and CC, respectively. Whereas the amphidiploids originated through the intercrossing of the elementary species are represented by *B. carinata*, *B. nigra* and *B. napus* are represented by the genomes BBCC, AABB and AACC genomes, respectively. These studies were later verified by the artificial resynthesis of *B. napus* from *B. oleracea* X *B. campestris* [8]. It was, thus, concluded that all the species of the genus are monopyletic in origin and have been evolved from an obscure six chromosomal prototype [28], either through the process of secondary polyploidy [29] aneuploidy [36,43] or a combination of modified tertiary-and-compensatory trisomy [47]. This elaboration encouraged the breeders to integrate species diversity through wide hybridization for improved oilseed [16], vegetable [17,18], fodder [19] and forage *Brassica* crops [20,21]. All the developments were only possible after the primary elucidation of the basic architecture of the *Brassica* genomes, through the refined cytotechnological procedures, which still needs a lot of explanation [14,24].

3.2 Genome Relationship Among Species

Section 3.1 reveals that the cultivated Brassica may either be diploid or amphiploid in nature. The diploid or elementary species includes *B. nigra* (BB, 2n = 16), *B. oleracea* (CC, 2n= 18) and *Brassica campestris* (AA, 2n = 20). The amphidiploid species includes *B. carinata* (AABB, 2n= 34), *B. juncea* (BBCC, 2n=36) and *B. napus* (AACC, 2n = 38). Crossibility among elementary species supported by the genome behavior in dihaploids [7,8,36,41] also demonstrated that; *B. juncea* is an amphidiploid of *B. campestris* X *B. nigra, B. napus* is amphidiploid of *B. campestris* X *B. oleracea* and *B. carinata* is amphidiploid of *B. campestris* X *B. nigra. Arabidopsis thaliana,* a weedy species of Brassicaceae, is an ideal plant for genetics and molecular studies [22]. Comparative maps of *Arabidopsis thaliana* and *Brassica* are becoming popular [14] for understanding the Brassica genomes. Using the comparative maps of *Arabidopsis thaliana* and *Brassica nigra,* it was also supported [48] that the diploid Brassica species are descended from a hexaploid ancestor and that the *A. thaliana* genome is similar in structure and complexity to those of each of the hypothetical diploid progenitors of the proposed hexaploid. Thus, the *Brassica* lineage probably went through a replication after the divergence of die lineages leading to *A. thaliana* and *B. nigra*. Contrary to the hexaploid origin [48], variation in isozyme, chromosome numbers (n= 6-75) and their related systematic relationships of tribe Brassiceae [38] revealed that aneuploidy and chromosome fusion-splitting have played a more significant role in the evolution of higher basic chromosome numbers in *Brassica*. The detection of widespread isozyme duplication in the tribe is consistent with reports of extensive gene-duplication in the *Brassica* species, and suggests that the common ancestor of the tribe already had undergone polyploid event, i.e. complete genome duplication, prior to aneuploid divergence.

3.3 Chromosome Morphology

Chromosomes of *Brassica* species are very small and poorly differentiated. Their identification through the ordinary cytogenetic techniques is extremely difficult. Progress in molecular analysis of *Brassica* species can only be made after the proper cytogenetic maps of their chromosomes. For karyotyping, chromosome-specific markers can probably distinguish particular pairs of homologous chromosome [11]. Karyotypic investigations of the *Brassica* genomes started with the analyses of secondary association in diploids [27,28], which reveals the monopyletic origin of *Brassica* from an obscure 6 chromosomal prototype. The analysis of Somatic chromosomes, based upon the chromosomal length and centromeric position in [29], recognized six to seven types of chromosomes and was designated the genomic formula ABCDDEEFFF to *Brassica campestris* and ABBBCDDEF to *Brassica oleracea.* The meiotic associations [30,28,9] also confirmed the monopyletic six chromosomal prototypic origin of *Brassica,* and the genomic formulae thus derived were " AABCDDEFFF", "ABCDDEFF " and " ABBCCDEEF" for *Brassica campestris*, *Brassica nigra* and *Brassica oleracea,* respectively [9]. It was also concluded [29] that, all the elementary *Brassica* species i.e. *B. campestris*, *B. oleracea* and *B. nigra* are balanced secondary polyploids, diploidized with the passage of time, having precisely 1:1 chromosomal association and

1/1 disjunction patterns [31]. A number of efforts were made in the second half of the 20th Century AD to make standard karyotypes for different members of the polyploid series of the genus as well, but is still not established scientifically. The recent use of fluorescence *in situ* hybridization (FISH) and differential staining [11,32,33] has recognized markers for some of the chromosome pairs in genome A, B and C of *B. campestris, B. nigra* and *B. oleracea,* respectively, which will hopefully be helpful for successful karyotyping of the *Brassica* genomes. Comparative data on quantitative trait loci (QTLs) may also be helpful [15] in karyotypic analysis.

3.4 Genome Analysis of Brassica

The breeding-system-analyses, meiotic pairing and genome architecture, which leads to the systematic science of genome analysis [34], was extensively employed in Brassica {35] for the determination of phylogenetics in Brassica. Whether it is in the meiosis or mitosis, the homologous pairing is generally under the genetic control [45,46]. The analyses of homoeologous association provide sound base of the evolutionary relationships within the chromosomes of a particular genome and can also be employed for understanding the genome-relationship of the allied or distant taxonomic groups. In elucidating genomics through chromosomal pairing, the information got from homoeologous association is generally concealed with the role of genetic factors in meiotic pairing. In *Brassica* the presence or absence of genetic factors for suppressing the homoeologous pairing is still not established. Some of the renowned authorities on *Brassica* [29,49] reported that, in F1s the pairing-frequency among two homoeologous genomes enhances with the addition of a third, the apparently distant genome i.e genome B of *B. nigra*. They are all of the opinion that, no genetic factor for suppressing homoeologous pairing is present in *Brassica*. On the other hand, authorities like Prakash [43,50,51] suggest that, the existence of genetic factors for suppressing the homoeologous pairing exist in Brassica. It was proposed that low pairing among the chromosomes of genomes AB and BC was due to the genetic regulations of pairing-control [51] but the structural differentiation of chromosomes of *B. nigra* from that of *B. rapa* and *B. oleracea* [47] was the only reason for low pairing. These findings were further clarified by the predominant allosyndesis [37,52] among the chromosomes of genomes A and C than that of genome B. It was thus recognized that neither any genetic factor for suppressing pairing [52] or cytoplasmic factor for regulating pairing exists in *Brassica* [49]. Results of genome analysis in 19 hybrids among various genomes of *Brassica*, *Synapis*, *Eruca* and *Raphanus* concluded that, all the genomes were partially homologous; they were secondary polyploids and originated from a common prototypic genome. Though the idea of the origin of *Brassica* from a six-chromosomal obscure prototype [27,28,28] was widely verified by the scientists through the pachytene analysis [9], preferential pairing [10,36,37] and molecular studies [38], their genomic formulae, acceptable karyotypes and mode of origin is still unresolved [32,33,39] and need further elaboration.

3.5 Preferential Pairing During Meiosis

Preferential pairing is an important tool for understanding the chromosome homoeology intragenomically. It can also successfully be employed in determining the affinity among the chromosomes of various genomes and can also be used for determining the phylogenetic relationship of different taxons. In Brassica, studies on the genome elaboration started with the analyzing of the preferential pairing in alloploids.

The pioneer work for elucidating the genome relation among *Brassica* species was based upon the consistent occurrence of 8 IIs in the *B, juncea* x *B. nigra* hybrids [7]. These associations were attributed to the allosyndesis of 8 chromosomes, both from *B. nigra* and *B. juncea,* and the homologous genome with 8 chromosomes, both in *B. nigra* and *B. juncea,* was designated as “genome B”, whereas the remaining 10 unpaired chromosomes of *B. juncea*, were designated as “genome A” *B. chinensis* (2n=20) or *B. rapa* type. These findings laid the foundation for the genome elaboration in Brassica and were very soon confirmed by the artificial resynthesis of *B. napus* [8] from *B. campestris* and *B. oleracea*, *B. juncea* from *B. campestris* and *B. nigra* [41] and more recently through other means [9-11,33,24].

Preferential pairing in haploids has also unfolded interesting information regarding the genome relationship of various taxons, including *Brassica* [36,40,41]. The presence of two bivalents in the haploid PMC’s of *B. nigra* was thought to be [36] due to its origin from a six chromosomal prototype and the two chromosomes in

its 8-chromosomal gametic complement (X= 8) is due to the duplication of two original chromosomes of the progenitor genome. During the course of evolution, the synaptic ability of the duplicated chromosomes remained no more intact due to their being differentiated to a larger extent. But still these chromosomes retained some traces of genetic equivalence, resulting in allosyndetic associations in haploids. Thus, from the preferential pairing of haploids in *B. nigra* [36], *B. tournefortii* [43] and *B. rapa* [44], the genome relationship sketch of Morinaga [7] and the secondary association theorem of Catcheside [30] regarding the origin of these genomes i.e., they have been originated from a six chromosomal obscure prototype [9,28,30,42], were verified. Though the genome relationship sketch of Morinaga [7] is widely accepted up till now, the secondary association theorem is criticized by a number of scientists.

For example the genomic formulae of Richharia [29] are not in accordance with that of Catcheside [30] and Robbelen [9]. Ramanujam and Srinivasachar [41] reported 1II in haploids of *B. rapa* and was not in a position to conform the secondary pairing theorem. Similarly, in other studies [40,31] the scientists were not even able to confirm secondary association in *Brassica rapa,* on which the secondary pairing theorem [30,9] was based. However it was certainly generalized by most of these studies [45] that the aneuploid series in *Brassica* could have been derived from a combination of modified tertiary and compensating trisomics. Some recent studies of the linkage comparison the maps [39] in *B. rapa* (*B. rapa*), *B. nigra* and *B. oleracea* showed homologous regions shared by these species. This study also found intergenomic conserved regions, with the extensive reordering among the genomes and associated eighteen linkage groups (from all three species) on the basis of homologous segments, based on at least three common markers. Intragenomic homologous conservation was also observed for some of the chromosomes of A, B and C genomes and thus it was concluded that an ancestral genome was made up of at least five, and not more than seven chromosomes from the observed chromosomal inter-relationships. Moreover, some other findings also revealed that the diploid *Brassica* species have descended from a hexaploid [46] ancestor. It can be concluded from the discussion that, though a lot of work has been carried out on the genome architecture of *Brassica,* it is still unresolved and needs further investigations.

3.6 Genomics In Brassica

The introduction of PCR and other precise DNA technologies has revolutionized the fields of genetic finger- printing, isolation of genetic traits and molecular biosystematics. These technologies are also employed in the determination of genetic diversity at the intragenomic and intergenomic levels. RAPD analyses are successfully employed in detecting extensive intragenomic polymorphism [53], but its use at interspecific level is still limited and needs further refinements [54,55]. Mapping in *Brassica* however has been focused [54] mostly on *B. napus B. nigra*, *B. oleracea* and *B. rapa*, during the past 10 years. More recently, mapping has been expanded to include *B. juncea*. The maps produced in *Brassica* crops are based mainly on Fz progenies developed independently by various laboratories, which will require their integration for a more efficient use in future. The marker maps are being used to locate genes determining traits of economic interest, including quantitative trait-loci for utilization in applied genetics and breeding of the numerous *Brassica* crops. Another important application of the maps, which is quite active at the present time, is on the study of the structure, origin and evolution of the *Brassica* genomes. The *Arabidopsis* sequencing genome program puts the *Brassica* crops in an advantageous position because of the immediate application of this information.

Genetic linkage map of *Brassica juncea* on constructed RFLP detected by anonymous cDNA markers from *B. napus* [25] showed that sixty-two percent of the marker-loci were duplicated, and the majority of them were involved in interlinkage group duplications, illustrating that complex duplication and subsequent rearrangements have occurred in the species after allopolyploidy. Majority of the loci of genetic linkage map, consisting of 399 RFLP-defined loci of a cross between resynthesized *Brassica napus* and natural oilseed rape [23], however, exhibited disomic inheritance of parental alleles, demonstrating that *B. rapa* chromosomes were pairing exclusively with recognizable A-genome homologues in *B. napus* and that *B. oleracea* chromosomes were pairing similarly with the C-genome homologues. This behavior identified the 10 A genome and 9 C genome linkage groups of *B. napus* and demonstrated that the nuclear genomes of *B. napus*, *B. rapa*, and *B.*

oleracea have remained essentially unaltered since the formation of the amphidiploid species, *B. napus*. A range of unusual marker-patterns, which could be explained by aneuploidy and nonreciprocal translocations, were observed in the mapping population. These chromosome abnormalities were probably caused by associations between homoeologous chromosomes at meiosis in the resynthesized parent and the F-1 plant, leading to nondisjunction and homoeologous recombination.

Bulked segregant analysis for identifying RAPD markers linked to the restorer gene used in the Ogura radish cytoplasmic male sterility of rapeseed [26] revealed that the DNA polymorphisms generated by four of the primers were completely linked to the restorer gene. It was either associated with the fertility restorer or the sterility maintainer alleles. Pair-wise cross-hybridization demonstrated that the four polymorphic DNA fragments did not share any homology. Southern hybridization of labeled RAPD fragments on digested genomic DNA from the same three pairs of bulks revealed fragments specific to either the male sterile bulks or to the restored bulks and a few fragments common to all bulks, indicating that the amplified sequences are low copy. The four RAPD fragments that were completely linked to the restorer-locus were cloned and sequenced to develop sequence characterized amplified regions (SCARs). This will facilitate the construction of restorer lines used in breeding programs and is the first step towards map-based cloning of the fertility-restorer allele.

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