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Evolution of Genomes and Genome Relationship among the Rapeseed and Mustard

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Abstract: Reports regarding the chromosomal architecture of *Brassica* genomes and genome relationship among various species of the genus appeared in the second quarter of the 20th century AD. In the second quarter genome relationship was elaborated through secondary associations, pachytene and somatic karyotypes. Genome analysis and preferential pairing were extensively utilized for characterizing *Brassica* genomes in the third quarter. In last quarter of the century molecular characterization supplemented the conventional analytical tools for understanding the infrastructure of *Brassica* genomes. Whether it is the breeding system analysis, chromosome morphology, meiotic associations or molecular characterization proves that *Brassica* is monophyletic origin and it descended from an unknown six chromosomal prototype. Scientific developments made in *Brassica* genetics, from genome analysis to genomics during the 20th century AD are generalized in this paper.

Key words: *Brassica*, preferential pairing, genome analysis, karyotyping, genomics

Introduction

Phylogenetically *Brassica* is the nearest related genera to the recently sequenced *Arabidopsis thaliana* (Anonymous, 2000). Eight species of *Brassica* are reported from Pakistan (Nasir and Ali, 1973), among which *B. tournefortii* and *B. deflexa* are reported only from the wild and the rest are cultivated as important agricultural crops. *Brassica rapa* and *B. napus* among the agriculturally important species are conventionally grouped as rapeseed (Khan and Munir, 1986), cultivated mainly for the extraction of rapeseed and canola oil. *Brassica juncea*, *B. carinata* and *B. nigra* are placed in the mustard group (Khan *et al*., 1987). Besides their major role in the production of mustard oil, they are also cultivated for their demand as commercial spices (Hemingway, 1976). *Brassica oleracea*, the 6th agriculturally important species is cultivated for its use as vegetable and fodder under the common names of cabbage, cauliflower, broccoli, brussels sprouts and marrowstem kale etc.

The successful demonstration of *Raphanobrassica* (Karpechenko, 1929) a classical intergeneric hybrid and the artificial resynthesis of *Brassica napus* (U, 1935) during the second quarter of the 20th

century inclined the scientists to resolve the genome constitution of *Brassica* species. Genome relationship among the species were primarily elaborated either through the analysis of secondary association during microsporogenesis of diploids (Alam, 1936; Catcheside, 1937) or somatic karyotyping (Richharia, 1937). Meiotic karyotyping (Robbelen, 1960; Prakash, 1973), preferential pairing in haploids and amphihaploids (Prakash, 1974; Armstrong and Keller, 1981; Robbelen, 1960; Attia and Robbelen, 1986) were later on used for in genome analysis. Though hardships are still there in distinguishing *Brassica* chromosomes (Hasterok and Maluszynska, 2000), developments in the field of genomics during the recent past, however proved very effective in producing successfully high yielding and pest resistant genetically engineered RR Canola (Phillipson, 2001). This review will provide an insight of the genome biology and other genetic developments of the in *Brassica*, especially the rapeseed and mustard.

Wide hybridization: Till the first quarter of the 20th century AD, *Brassica* species were considered as fixed taxonomic entities and the alien gene transfer among different genomes, practically seemed impossible. Sinskaia (1927) was the first who tried to cross *Brassica* species and succeeded to change the long lasting belief of the taxonomic boundaries regarding the crossability of *Brassica* species. Karpechenko (1928) successfully synthesized *Raphanobrassica*, the first intergeneric fertile hybrid of *Raphanus sativus* and *Brassica* oleracea. His demonstration further strengthened the idea of genome manipulation through wide hybridization. Besides their use in breeding system analysis the interspecific crosses were employed in different ways to elaborate their genome relationship also. Hence Morinaga (1934) analyzed meiotic pairing in *B. nigra* X *B. juncea* hybrids and concluded thereby that the genus *Brassica* is a polyploid complex having both the elementary (diploid) and amphidiploid genomes. *Brassica rapa*, *B. nigra* and *B. oleracea* are represented by genomes AA, BB and CC respectively are diploid. Whereas *B. carinata*, *B. nigra* and *B. napus* are represented by the genomes BBCC, AABB and AACC respectively, are amphidiploid, originated through the intercrossing of the elementary species. These studies were later on verified by the artificial resynthesis of *B. napus* from *B. oleracea* X *B. rapa* (U, 1935). It was later on verified that *Brassica* species are monophyletic in origin and have been evolved from an obscure six chromosomal prototype (Alam, 1936), either through the process of secondary polyploidy (Richharia, 1937) aneuploidy (Prakash, 1973; Prakash, 1974) or a combination of modified tertiary-and-compensatory trisomy (Armstrong and Keller, 1982). These elaboration encouraged breeders to integrate species diversity through wide hybridization for improved oilseed (Prakash, 1980), vegetable (Nishi, 1980; Lange *et al.*, 1989), fodder (Namai, 1971) and forage *Brassica* crops (Hosoda, 1950, 1953). All the developments were only possible after the primary elucidation of the basic architecture of the *Brassica* genomes through the refined cytotechnological procedures, still want a lot of explanation (Lan *et al.*, 2000; Soltis and Soltis, 1999).

Interspecific relationship: It is clear from the above discussion that the cultivated *Brassica* has two

types of genomes i.e. the diploid (elementary) and amphidiploid genomes. The elementary species includes *Brassica rapa* (AA, 2n=20), *B. nigra* (BB, 2n=16) and *B. oleracea* (CC, 2n=18). The amphidiploid species includes *B. carinata* (AABB, 2n=34), *B. juncea* (BBCC, 2n=36) and *B. napus* (AACC, 2n=38). These relationships among *Brassica* were elucidated with the artificial resynthesis of *B. juncea*, *B. carinata* and *B. napus* (Morinaga, 1934; U, 1935; Ramanujam and Srinivasachar, 1943; Prakash, 1973) through hybridizing *B. rapa-B. nigra*, *B. rapa-B. nigra* and *B. rapa-B. oleracea*, respectively. Comparative maps of *Arabidopsis thaliana*, an ideal plant for genetics and molecular studies (Meyerwitz and Somerville, 1994) and *Brassica* are becoming popular (Lan *et al.*, 2000) for understanding the *Brassica* phylogenetic relationships. The comparative maps of *Arabidopsis thaliana* and *Brassica nigra* (Lagercrantz, 1998) shows 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. Furthermore according to Lagercrantz (1998) the *Brassica* lineage probably went through a replication after the divergence of die lineages leading to *A. thaliana* and *B. nigra*. However the findings of hexaploid origin of *Brassica* was neither verified by its preferential (Ahmad, 2001), nor through its isozyme analysis (Warwick, 1999). Variation in isozymes, chromosome numbers and the related systematic relationships of tribe Brassiceae (Warwick, 1999) reveal that aneuploidy and segmental polyploidy have played a more significant role in the evolution of amphidiploid *Brassica*. Furthermore the widespread isozyme duplication in *Brassica* (Warwick, 1999) and the frequently occurring multivalents in the intergenomic haploids, colchiploids and species hybrids (Ahmad, 2001) determines extensive gene duplication resulting from the polyploidization of the common ancestor of the Brassiceae tribe, prior to the aneuploid divergence of the species.

Karyotypic overview: The *Brassica* chromosomes are very small and poorly differentiated. Their identification through the ordinary cytogenetic techniques is extremely difficult. Progress in molecular analysis of *Brassica* species still needs their proper karyotyping, the chromosome-specific markers for differentiating the particular homologous pair (Hasterok and Maluszynska, 2000) is an encouraging development in this direction.

Karyotypic investigations of the *Brassica* genomes started with the analysis of secondary association in diploids (Catcheside, 1934; Alam, 1936). The secondary pairing revealed the monophyletic origin of *Brassica* from an obscure 6 chromosomal prototype. Somatic karyotyping based upon the chromosomal length and centromeric position in (Richharia, 1937) also recognized six to seven types of chromosomes. Richharia (1937) designated the genomic formulae ABCDDEEFFF and ABBBCDDEF to *Brassica rapa* and *Brassica oleracea* respectively. Robbelen (1960) analyzed pachytene chromosomes of *Brassica rapa*, *B. nigra* and *B. oleracea* and allotted the genomic formulae AABCDDEFFF, ABCDDEFF and ABBCCDEEF respectively, to them. Besides meiotic associations in *Brassica* (Catcheside, 1937; Alam, 1936; Robbelen, 1960) also confirmed the findings of Morinaga (1934) and U (1935) regarding the monophyletic origin of *Brassica* from a six chromosomal obscure prototype.

A number of efforts were made in the second half of the 20th century AD for making standard karyotypes for different members of the polyploid series of the genus but is still not established scientifically. The use of fluorescence *in situ* hybridization (FISH) and differential staining (Hasterok and Maluszynska, 2000; Armstrong *et al.*, 1999; Fukui *et al.*, 1998) has recognized some markers for different chromosome pairs in genome A, B and C; of *B. rapa*, *B. nigra* and *B. oleracea* respectively. It will hopefully be helpful for the successful karyotyping of *Brassica* genomes. Comparative data on quantitative trait loci (QTLs) are also successfully used in karyotypic analysis of *Brassica* (Osborn *et al.*, 1997).

Genome analysis: Whether meiosis or mitosis, the homologous pairing is controlled genetically (Schulz-Schaefer, 1980; Sybenga, 1975). Partial pairing of homoeologous chromosomes is observed both among haploid and the amphihaploid genomes, during gametogenesis. It usually provides precise information regarding the evolutionary relationships of chromosomes within the genome and is therefore successfully employed in understanding the genome relationship among various taxonomic groups. Genome analysis (Stace, 1980) is the resolving of genome relationships through preferential pairing within the haploid or through partial pairing among the allied amphihaploid genomes.

In genome analysis 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 authorities (Richharia, 1937; Busso *et al.*, 1987) reported that in F1s (of genomes A and B) 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 reported that *Brassica* has no genetic factors for suppressing homoeologous pairing. On the other hand authorities like Harberd (1950), Prakash (1974) and Harberd (1976) through their series suggested the presence bear genetic factors for suppressing the homoeologous pairing *Brassica*. Harberd (1976) observed that low pairing among the chromosomes of genomes AB and BC was due to the genetic regulations of pairing control. But structural differentiation of chromosomes in *B. nigra*, both form *B. rapa* and *B. oleracea*, was the only reason for low pairing (Armstrong and Keller, 1982). The predominant allosyndesis among the chromosomes of genomes A and C than genome B (Attia *et al.*, 1987; Yang and Robbelen, 1994) further clarified

Table 1: Genome constitution of the cultivated *Brassica*

Groups	Chromosome number (n)	Genome involved	Species included	Remarks
I.	10	aa	*B. rapa* L., *B. pekinesis* Rupr., *B. rapa* L. *B. chinensis* L. *B. japonica* Sieb.	Elemental species I
II.	8	bb	*B. nigra* Koch.	Elemental species II
III.	9	cc	*B. oleracea* L., *B. alboglabra* Balley	Elemental species III
IV.	18	aabb	*B. juncea* Coss., *B. cernua* Hernsl.	Amphidiploid from I & II
V.	19	aacc	*B. napus* L., *B. napella* Chaix.	Amphidiploid from I & III
VI.	17	bbcc	*B. carinata* Braun.	Amphidiploid from II & III

Source: Morinaga (1934)

findings that no genetic factors for suppressing the homoeologous pairing were present in *Brassica*. It was thus recognized that neither any genetic factor for suppressing pairing (Yang and Robbelen, 1994) nor cytoplasmic factor for regulating pairing exists in *Brassica* (Busso *et al.*, 1987).

Primarily the work on genome analysis in *Brassica* started with the observation of consistently occurring 8 IIs in *B. juncea* x *B. nigra* hybrids (Morinaga, 1934). These associations were attributed due to the allosyndesis of 8 chromosomes both from *B. nigra* and *B. juncea* and this 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. Through his detailed observations Morinaga (1934) was able to classify the agriculturally important *Brassica* species into six cytogenetical groups as given in Table 1. These findings made the foundation for the genome analysis of *Brassica*. Results of the genome analysis among *Brassica* and its allied genomes in *Synapis*, *Eruca* and *Raphanus* concluded that all the genomes were partially homologous, they were secondary polyploids and originated from a common unidentified genome (Mizushima, 1950). The findings of Morinaga (1934) were soon confirmed by the artificial resynthesis of *B. napus* (U, 1935), *B. juncea* (Ramanujam and Srinivasachar, 1943) and other means (Robbelen, 1960; Soltis and Soltis, 1999; Hasterok and Maluszynska, 2000). The idea of the origin of *Brassica* from an unknown six-chromosomal prototype (Catcheside, 1934; Alam, 1936) is verified through pachytene karyotyping (Robbelen, 1960), chromosomal behavior at M1 (Attia and Robbelen, 1986; Prakash, 1973; Attia *et al.*, 1987) and molecular analysis of the species (Warwick, 1999). The acceptable genomic formulae, their karyotypes and mode of origin of various taxons is still unresolved (Armstrong *et al.*, 1999; Fukui *et al.*, 1998; Truco *et al.*, 1996).

Preferential pairing in haploids and amphidihaploids: As it is clear from the preceding section, preferential pairing is important for understanding the chromosome homoeology and it is successfully employed in determining the affinity among chromosomes of various genomes and phylogenetic relationships of *Brassica* species. Preferential pairing in haploids has also unfolded interesting information regarding the genome relationship of various taxons including *Brassica* (Ramanujam and Srinivasachar, 1943; Thompson, 1956; Prakash, 1973). The presence of two bivalents in the haploid *B. nigra* were thought to be 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 two original chromosomes of the progenitor genome (Prakash, 1973). During the course of evolution the synaptic ability of the duplicated chromosomes remained no more intact. But still these chromosomes retained some traces of genetic equivalence resulting in allosyndetic associations in haploids. Thus from the preferential pairing in haploids of *B. nigra* (Prakash, 1973), *B. tournefortii* (Prakash, 1974) and *B. rapa* (Armstrong and Keller, 1981) the genome relationship sketch of Morinaga (Morinaga, 1934) and the secondary association theorem of Catcheside (1937) regarding the origin of these genomes, was verified. The origin of *Brassica* from a six chromosomal

obscure prototype (Robbelen, 1960; Alam, 1936; Catcheside, 1937; Venkateswarlu and Kamala, 1971). Though the genome relationship of Morinaga (Morinaga, 1934) is widely accepted, the secondary association theorem is criticized by a number of scientists. For example the genomic formula of Richharia (1937) is not in accordance with that of Catcheside (1937) and Robbelen (1960). Ramanujam and Srinivasachar (1943) reported 1II in haploids of *B. rapa* and was not in a position to conform the secondary pairing theorem. Similarly in other studies (Thompson, 1956; Ahmad and Khan, 1994) the scientists were not even able to confirm the secondary association in *Brassica rapa*, on which the secondary pairing theorem (Catcheside, 1937; Robbelen, 1960) was based. However it was certainly generalized by most of these studies (Schulz-Schaefer, 1980) 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 (Truco *et al.*, 1996) in *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 been descended from a hexaploid (Sybenga, 1975) 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.

Overview of genomics: The introduction of DNA analytical technologies particularly RAPD and RFLP 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 intra and intergenomic levels. RAPD analysis are successfully employed in detecting extensive intragenomic polymorphism (Rabbani *et al.*, 1998) but its use at interspecific level is still limited (Quiros, 1998; Thormann, 1994). During the last 10 years mapping in *Brassica* has however been focused on *B. napus*, *B. nigra*, *B. oleracea* and *B. rapa* (Quiros, 1998). More recently, mapping has been expanded to include *B. juncea*. The maps produced in *Brassica* crops are mainly based 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 is the study of structure, origin and evolution of the *Brassica* genomes. Arabidopsis sequencing program puts the *Brassica* crops in an advantageous position because of the immediate application of these information in its genomics.

Genetic linkage map of *Brassica juncea* on the cDNA markers of *B. napus* (Cheung *et al.*, 1997)

showed that 62% of the marker loci were duplicated and majority of them were involved in interlinkage group duplications. The study illustrates that complex duplication and subsequent rearrangements have been occurred in the species after allopolyploidy. Parkin *et al.* (1995) noticed that majority of the loci of genetic linkage map of a cross between resynthesized *Brassica napus* and natural oilseed rape exhibited disomic inheritance of parental alleles. The study demonstrated that the chromosomes of genomes A and C were pairing exclusively with their recognizable homologues of *B. rapa* and *B. oleracea* chromosomes in *B. napus* crosses. This behavior identified 10 and 9 linkage groups of the genome A and C types, respectively in *B. napus*. Moreover it was also concluded from the studies that the nuclear genomes of *B. napus*, *B. rapa*, and *B. oleracea* have remained essentially unaltered since the formation of 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.

It can be concluded from the above discussion that *Brassica* species are monophyletic in origin and could sexually be employed at any ploidy level for genetic introgression or alien genetic transfer. Furthermore the diploid species are phylogenetically more close to the amphidiploids than their diploid relatives. The aneuploidy and segmental polyploidy has played important role in its genome evolution. The chromosomes of genome A (irrespective of its source) have retained more chromosomal homology as compared with their homology to the chromosomes of genomes B and C. In alloploid condition genome A prefer to form autosyndetic IIs with its homologous genome or its chromosomes will remain as univalents in the absence of genome A. Some of the chromosomes of genome A also has homoeology with the chromosomes of genomes C and B, which give rise to the formation of allosyndetic multivalents. No one among the genomes B nor C have got any genetic role in affecting the homoeologous pairing of genome A from different backgrounds.

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