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Cytogenetic stability and genome size variations in newly developed derived *Brassica juncea* allopolyploid lines

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Abstract

Allopolyploids are normally resynthesized through interspecific hybridization and whole genome duplication. *Brassica* group has developed a new concept of derived digenomics, where an allopolyploid *Brassica* is synthesized through hybridization of two nonparental digenomic species. Genetic differences in allopolyploids result from recombination between the hybridized subgenomes or structural variation in cohabiting genomes which can significantly impact genetic constellations and consequently the phenotype. We analysed 62 *Brassica juncea* progenies, developed following hybridization between *B. napus* and *B. carinata*, for cytogenetic stability and genome size changes in S_5 and S_6 generations. In derived *B. juncea* genotypes, pollen grain stainability ranged from 65 to 95 percent. Meiotic analysis of the derived *B. juncea* lines conducted during S_5 and S_6 generations revealed standard 18II configuration for the majority of test genotypes. During S_5 generation, some genotypes revealed abnormal meiotic configurations. These genotypes were: DJ 1-1, DJ 22, DJ 25, DJ 115, DJ 116 (all 18II+1I), DJ 21 and DJ 19-2 (both 19II) and DJ 55 (17II). All these genotypes generally had monosomic or disomic addition of, possibly, one chromosome each from C genome. Anaphase I distribution was also aberrant in these genotypes. This perhaps caused impaired pollen fertility. One generation of selfing and selection for improved fertility, however, led to correction of meiotic aberrations in all these instances. All the derived *B. juncea* genotypes were crossed as female with natural *B. juncea* to identify random chromosome substitutions. All the consequent F_1 genotypes were cytologically analysed during meiosis1. Occurrence of 17II and 2I in the F_1 with natural euploid *B. juncea* was considered to be indicative of substitution for one chromosome pair. Disomic chromosome substitution was indicated for DJ 15, DJ 18, DJ 19-2, DJ 21, DJ 38, DJ 58, DJ 61, DJ 92, DJ 97, DJ 103 and DJ113. Molecular characterization helped to resolve these substitution lines into six distinct groups. Most of these substitution lines are expected to involve A/ C substitutions. Genome size variations were also observed. On the basis of population mean (over 62 derived *juncea* types), average genome size was 528.274 Mbp, representing a decline of almost 1.072 percent DNA during six generations of selfing following allopolyploidization. These studies confirmed the viability of the concept of developing derived amphiploids.

Keywords: *Chromosome substitution, Indian mustard, meiosis, polyploidy, resynthesis*

Introduction

Three diploid species, namely, *Brassica rapa* (AA, 2n = 20), *B. nigra* (BB, 2n = 16), *B. oleracea* (CC, 2n = 18) and their three natural amphidiploids species namely *B. napus* (AACC, 2n = 38), *B. juncea* (AABB, 2n = 36) and *B. carinata* (BBCC, 2n = 34) constitute crop Brassicas. These are cultivated across the globe as sources of vegetables, oils and condiments. Of these *B. napus* accounts for the maximum area. It contributes a bulk of vegetable edible oil production in Europe, Canada, Australia and China. *B. juncea* predominates in Indian subcontinent while *B. carinata* has areas of adoption in Africa. Severe genetic bottlenecks are also implicit in their origin

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through only a few spontaneous hybridization events between fewer plants, representing at times only a fraction of the available diversity, of respective diploid progenitors that occurred millennia ago. In addition, Brassicas were first domesticated as vegetables and subsequently as edible oil crops (Prakash *et al*., 2010). Consequently digenomic Brassicas possess a narrow base. This inherently narrow genetic basis is now limiting their potential for improving seed and oil yield, especially through hybrid breeding. The potential advantageof heterosis in hybrid breeding programs depends on combining genetically distant gene pools, based on the assumption of a positive correlation between genetic distance (GD) and mid-parent heterosis (Falconer and Mackay, 1996). Different approaches have been used to extend genetic variation in the *Brassica* breeding pool with genetically distant material, thereby adding new alleles for agronomic traits. The broadening of the Canadian spring rapeseed gene pool was facilitated through introgression from winter rapeseed gene pool (Kebede *et al*., 2010). Qian *et al*., (2006, 2009) proposed the use of Chinese semi-winter lines as hybrid parents into the European winter oilseed rape gene pool. Becker *et al*., (1995) used molecular markers to analyze genetic distances between adapted cultivars and resynthesized *B. napus* lines and emphasized the application of resynthesis route for expanding the genetic basis of the existing *B. napus.* Increased seed yield have been reported after use of *B. napus* resynthesized lines as hybrid parents into spring rapeseed (Girke *et al*., 2001; Udall *et al*., 2004 and Seyis *et al*., 2006). To further increase genetic diversity, the concept of subgenome was introduced by Chinese scientists, with particular reference to *B. napus* (Maoteng *et al*., 2010). This concept sought to benefit from inherent variations in *Brassica* genomes. The A genome of *B. rapa* and C genome of *B. carinata* were shown to be diverse from A and C genome of *B. napus* (Li *et al*., 2006). A new nomenclature of *Brassica* genomes was also suggested, where the symbols of A^r, A^j and A^n represented the A genome in the *B. rapa*, *B. juncea* and *B. napus* respectively. B^b, B^j and B^c for the B genome of *B. nigra*, *B. juncea* and *B. carinata*, C^o, C^n and C^c for the C genome of *B. oleracea*, *B. napus* and *B. carinata*. Introgression of A genome of *B. rapa* and C genome of *B. carinata* into *B. napus* was expected to widen the genetic bases of *B. napus*. This partial introgression of subgenomic components from different *Brassica* species led to stronger heterosis (Zou *et al*., 2009). Despite continued enthusiasm, the resynthesized amphiploids have generally not been found very useful for standard crop improvement activities. Possibly due to associated genetic and/ or phenotypic instabilities, linkage drag and consequently poor breeding value. This may also be because two of the three *Brassica* monogenomic progenitor species (*B. nigra* nd *B. oleracea*) used for resynthesis of *Brassica* alkaloids did not face any significant human intervention for evolution as an oilseed crop. To overcome these problems, Banga and Kaur (2009) reported a procedure for direct isolation of a digenomic species through hybridization of non parental digenomics, as an alternate source of genetic variability in *Brassica* amphiploids. Present research was an attempt to develop the idea further by demonstrating genomic stability of newly evolved derived *B. juncea* genotypes.

Materials and Methods

To create *B. juncea* ($A^nA^nB^cB^c$), *B. napus* ($A^nA^nC^nC^n$) was hybridized as female with *B. carinata* ($B^cB^cC^cC^c$). Chromosome doubling was induced in the F_1 for the synthesis of octoploid ($A^nA^nC^nC^nB^cB^cC^cC^c$) plants. These were meiotically unstable, but some selfed seed could be obtained. The chromosomesin the tetrasomic dose ($C^cC^cC^nC^n$) had poor transmission frequency due to the multivalent formation, whilethose in the disomic dose (A^nA^n/ B^cB^c) largely formed bivalents. *B. juncea* ($A^nA^nB^cB^c$) type segregates could be scored easily due to their phenotype in A2/A3 generations. Normal euploid plants (2n=36) were advanced through single seed descent method.62 $S_{5/}$ S_6 derived *B. juncea* allopolyploid progenies ($A^nA^nB^cB^c$) were evaluated for cytogenetic stability, associated chromosomal changes and genome size variations.Experiments were conducted at the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India.

Cytogenetic analysis

Cytological studies were carried out to determine

the meiotic configuration of the sampled plants of each derived *B. juncea*. The flower buds were fixed around 7.00-9.30 a.m. in Carnoy's solution II (Ethanol: Chloroform: Acetic acid in a ratio of 6:3:1) to which one drop of the ferric acetate was added as a mordant (saturated solution of the ferric acetate prepared by adding ferric chloride in glacial acetic acid). After 48 hours, squash preparations were made in 2 per cent acetocarmine and observed under Olympus BX61 TRF microscope. At least, 20 PMC's with well spread metaphase-I/ diakinesis/anaphase-I were examined per plant for the determination of chromosome number and pairing behavior. In the case of an aberrant meiosis, 100 PMCs were analysed.

Nuclear DNA content

Standard flow cytometry method based on Dolezel and Bartos (2005) was used for estimating DNA content of the test samples. A reagent kit, PartecCyStain UV precise P, was used for nuclei extraction and DNA staining of nuclear DNA from plant tissues in order to determine genome size variations and ploidy level. Leaf samples were analyzed with PartecCyFlowPloidy Analyzer. *B. carinata* was used as a reference. DNA content of reference *B. carinata* sample was calculated based on a reference tomato genotype. Five plants per replication (a total of 10 plants) for each genotype were sampled for ploidy determination.

Molecular investigations

These studies were carried out using 60 A genome (Li *et al*., 2010) and 48 B genome chromosome specific SSR primers (Parkin, pers. commun.) to document genetic diversity in 11 *B. juncea* chromosome substitution lines. Automated high-throughput electrophoresis system (CaliperLab Chip GX version 3.0.618.0) was used to separate the PCR products.The data were scored as present "1" or absent "0" for a band at a particular position in the gel in reference to a base-pair ladder. Every band position was considered as a locus. DNA polymorphisms obtained following amplification of 108 BAC anchored SSRs allowed detection of genetic modifications in derived *B. juncea* lineages in comparison to the parents (*B. napus* and *B. carinata*) and diploid genome donor species. PAleontological Statistics (PAST) software Version 2.11 (Hammer *et al*., 2001) was used to conduct an analysis of molecular variation.

Results and discussion

Pollen grain stainability is usually considered as a reflection of stability of meiosis. In neo-polyploids, meiotic stability and hence pollen grain stainability was initially low. It tended to improve following few generations of selfing and selection. In the present context (Table1 and Fig. 1a-b), the pollen grain stainability in S_5 generation varied from 40 percent (DJ 15) to 90 percent, with relatively greater number of genotypes hovering around 90 percent pollen grain stainability. Genotypes with intermediate pollen grain stainability were: DJ 1, DJ 19-1, DJ 20, DJ 22 and DJ 55. The level of pollen grain stainability improved significantly following additional cycle of selfing. During S_6 generation, it ranged from 65 to 95 percent. Meiotic analysis of the derived *B. juncea* lines conducted during both the generations revealed typical 18II configuration for most of the test genotypes (Table 1 and Fig. 1c to e). During S_5 generation, abnormal meiotic configurations were detected in some genotypes. These genotypes were: DJ 1-1, DJ 22, DJ 25, DJ 115, DJ 116 (all 18II+1I), DJ 21 and DJ 19-2 (both 19II) and DJ 55 (17II). All these genotypes generally had monosomic or disomic addition of, possibly, one chromosome each from C genome (Table 2 and Fig. 2a to i). Anaphase I distribution was also aberrant. This perhaps caused impaired pollen fertility as discussed earlier. One genotype, DJ55, showed 17II during metaphase I. One generation of selfing and selection for improved fertility, however, led to correction of meiotic aberrations in all these instances. Restoration of 18II configuration for DJ 55 was surprising. Based on this, it is expected that DJ 55 showed 17 II not because it was nullisomic for a complete set of chromosomes, but it was rather a double monosomic for two separate but sympatric chromosomes. Interspecific hybridization of *Brassica* digenomics is known to cause random chromosome substitution between A and C genomes due to their very close homologies (Banga, 1988). In order to identify substitution lines, all the test derived *B. juncea* genotypes were crossed as female with natural *B. juncea*. Consequent F_1 combinations were cytologi-

Table 1**:** Pollen grain stainability and meiotic configurations in derived *B. juncea* genotypes

Genotypes	% Pollen Grain Stainability		Meiotic configurations		Meiotic configurations of F1 with *Brassica juncea*	
	2010-2011	2011-2012	Metaphase	Anaphase	Metaphase	Anaphase
DJ 1	60	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 1-1	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 1-2	80	92	18 II	18 I+18 I	18 II	18 I+18 I
DJ 2	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 6	85	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 9	70	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 14	75	70	18 II	18 I+18 I	18 II	18 I+18 I
DJ 15	40	65	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 17	80	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 18	80	80	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 19-1	60	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 19-2	60	75	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 20	50	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 21	80	80	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 22	50	78	18 II	18 I+18 I	18 II	18 I+18 I
DJ 24	90	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 25	80	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 26	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 27	85	92	18 II	18 I+18 I	18 II	18 I+18 I
DJ 28	85	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 30	80	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 31	90	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 38	90	90	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 49-1	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 55	80	85	18 II	18 I+18 I	18 II	18 I+18 I
DJ 57	55	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 58	70	80	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 59	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 60	90	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 61	70	80	18 II	18 I+18 I	17 II+ 2I	18 I+18 I
DJ 63	80	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 65	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 66	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 68	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 71	90	90	18 II	18 I+18 I	18 II	18 I+18 I
DJ 72	95	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 73	85	85	18 II	18 I+18 I	18 II	18 I+18 I
DJ 78	90	95	18 II	18 I+18 I	18 II	18 I+18 I
DJ 79	80	85	18 II	18 I+18 I	18 II	18 I+18 I
DJ 80	80	80	18 II	18 I+18 I	18 II	18 I+18 I
DJ 81	85	90	18 II	18 I+18 I	18 II	18 I+18 I

cally analysed during meiosis I. The hypothesis was that the genotypes having chromosome substitutions will show 17II and 2I in the F_1 with natural euploid *B. juncea*. A perusal of Table 1 and Fig. 1f to i will confirm that chromosome substitution indeed took place as expected. It was indicated for DJ 15, DJ 18, DJ 19-2, DJ 21, DJ 38, DJ 58, DJ 61, DJ 92, DJ 97, DJ 103 and DJ 113. All the remaining genotypes had normal euploid configuration as they showed normal 18II in crosses with euploid *B. juncea*. Most of these substitution lines are expected to involve A/ C substitutions. In the absence of chromosome painting tools, it was difficult to identify the substitutions due to small size and lack of chromosome landmarks for individual *Brassica* chromosomes. To facilitate this, all the derived *B. juncea* lines having euploid chromosome number were crossed with one natural *B. juncea* line. This helped in the identification of 11 chromosome substitution lines, out of 62 evaluated. DNA polymorphism generated by 108 chromosome specific SSR markers also allowed us to speculate on the differences among substitution lines for the substituted chromosome(s). Diversity dendrogram

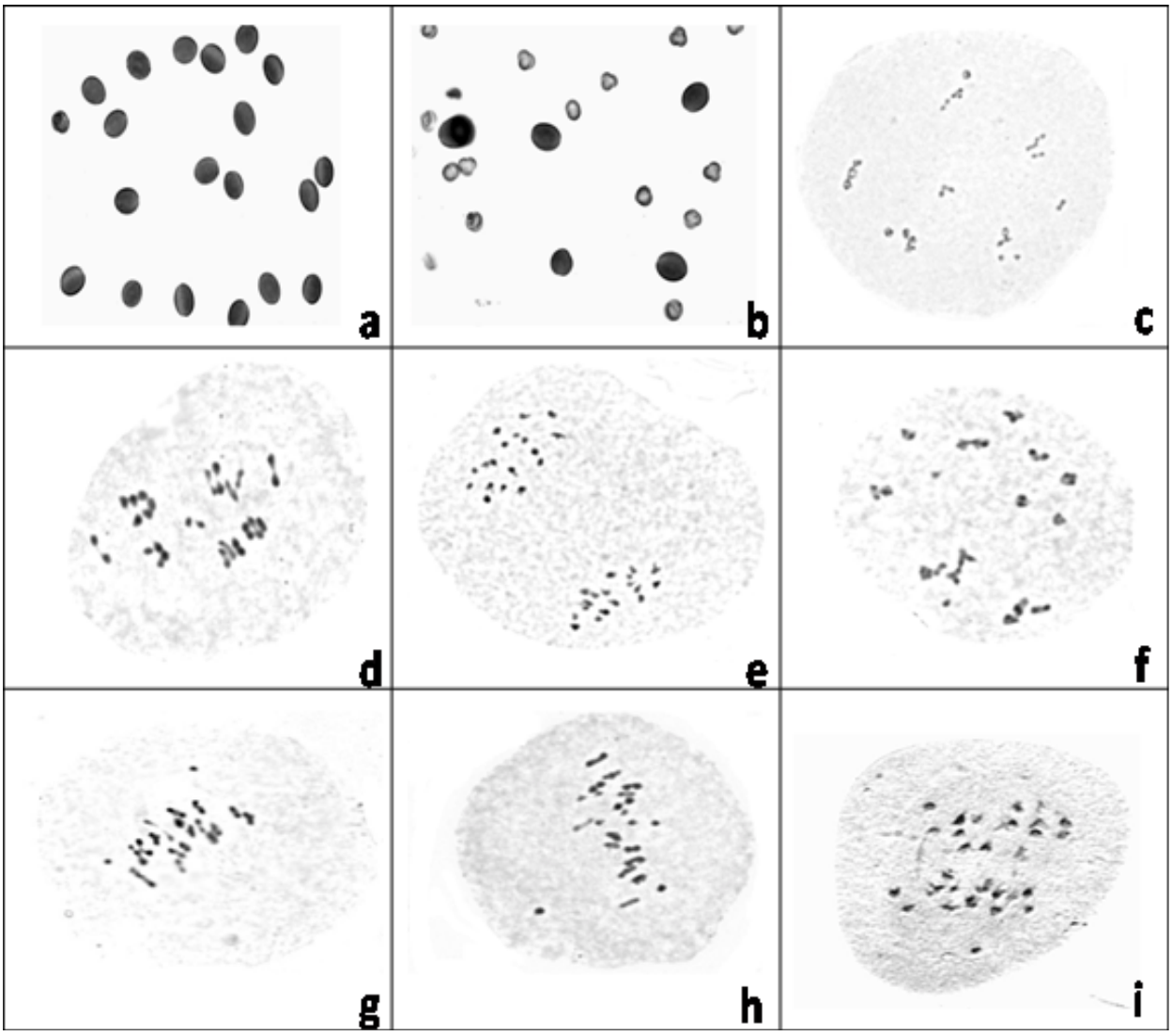

Fig. 1a to i: Meiotic configurations in derived *Brassica juncea* lines. (a) normal pollen grains stainability, (b) low pollen grains stainability, (c,d) 18 II, (e) 18-18 distribution at anaphase, (f) 17II+2I at diakinesis, (g,h) 17II+2I at metaphase, (i) 18-18 distribution at anaphase

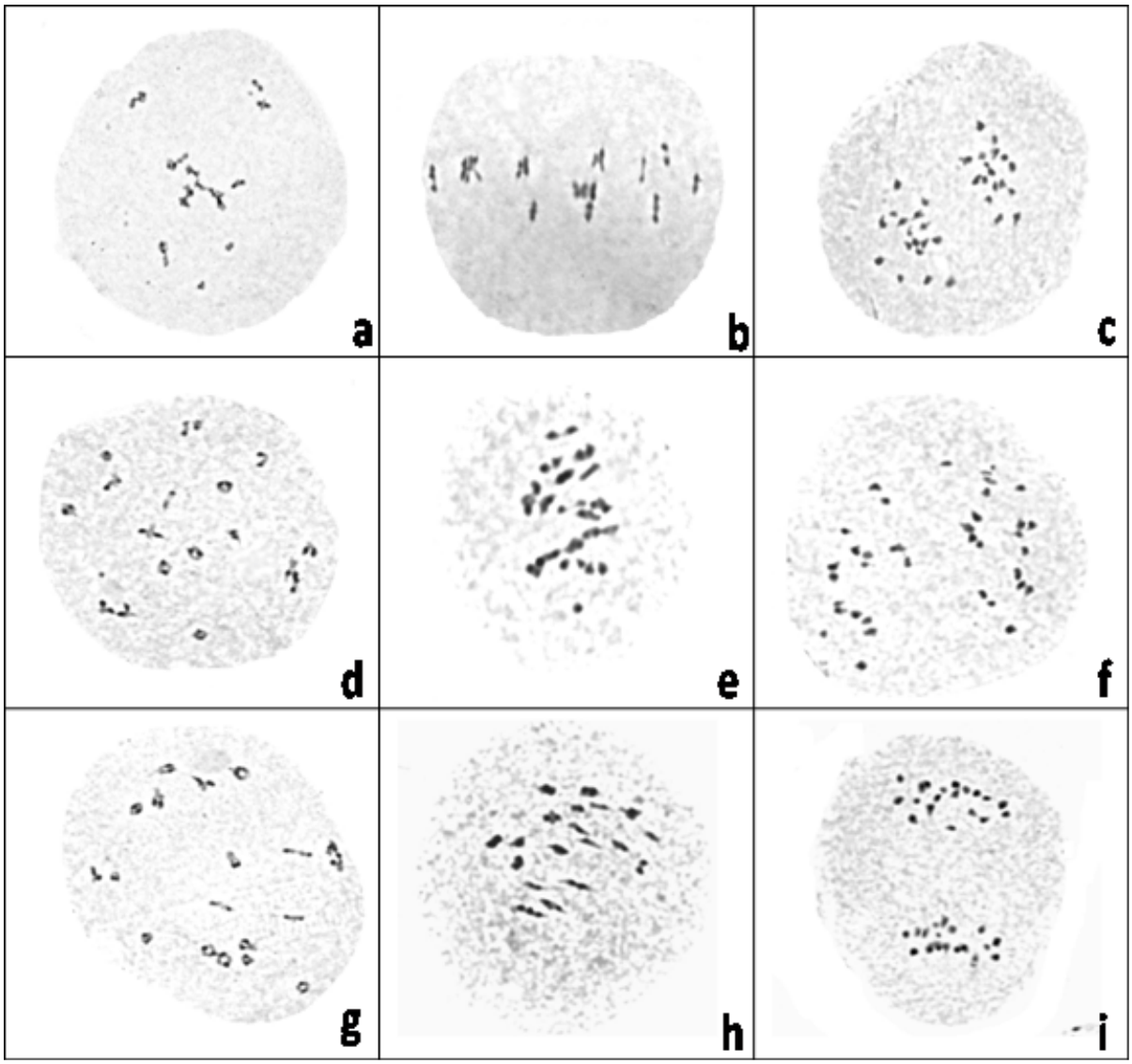

Fig. 2a to i: Aberrant meiotic studies in derived *Brassica juncea* lines, (a, b) 17II at diakinesis and metaphase, (c) 17-17 distribution at anaphase, (d, e) 18II+1I at diakinesis and metaphase, (f) 18I+19I distribution at anaphase, (g, h) 19II at diakinesis and metaphase, (i) 19I+19I distribution at anaphase.

Table 2: Derived *B. juncea* genotypes (S_5) showing aberrant meiotic configurations

Genotype	Metaphase	Anaphase
DJ 1-1	18 II + 1 I	18 I+18 I, 18 I +19 I
DJ 115	18 II + 1 I	18 I+18 I, 18 I +19 I
DJ 116	18 II + 1 I	18 I+18 I, 18 I +19 I
DJ 21	19 II	19 I+19 I
DJ 55	17 II	17 I+17 I
DJ 22	18 II + 1 I	18 I+18 I, 18 I +19 I
DJ 25	18 II + 1 I	18 I+18 I, 18 I +19 I
DJ 19-2	19 II	19 I+19 I

presented in Fig. 3, indicated six distinct groups. Thepossible groupings were DJ 19-2 and DJ 15; DJ 61 and DJ 113; DJ 92 and DJ 103; DJ 18 and DJ 38; DJ 58 and DJ 97. DJ 21 did not align with any other substitution line. It is expected that there was A/C substitution for seven different chromosomes. If confirmed, these lines constitute a very critical genetic resource that will allow us to evaluate breeding value of each substituted chromosome.The chances of A/ B substitution seem remote due to the procedure employed for generation of derived *B. juncea*. Further, random chromosome substitution occurs only in related chromosomes. While A and C are very close, A and B genome are highly diverged. Occurrence of stable meiosis with euploid chromosome number was testimony to the

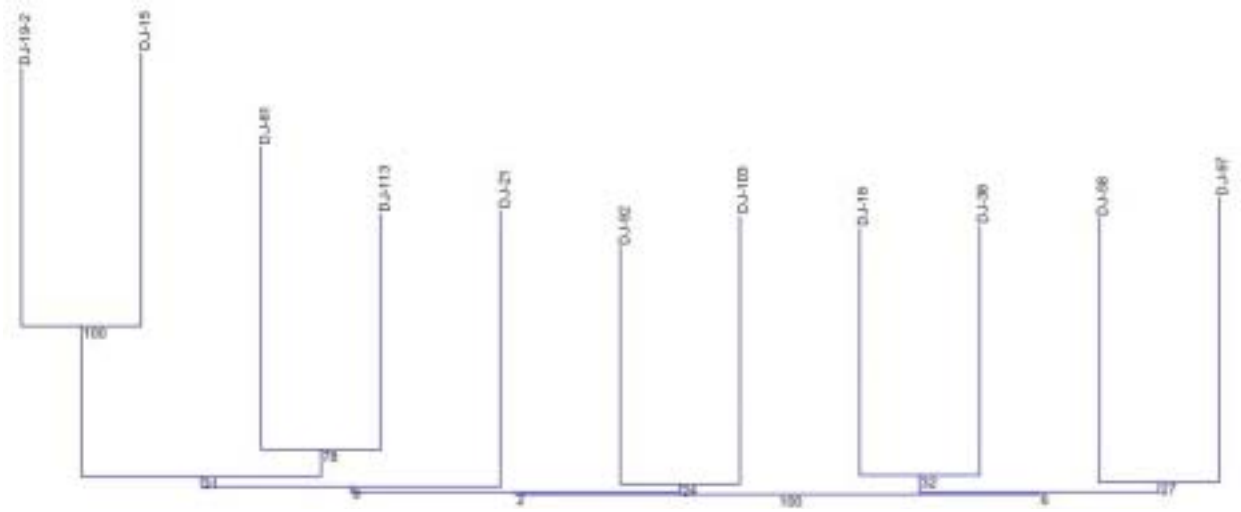

Fig. 3**:** Molecular characterization of chromosome substitution lines in *B. juncea*.

soundness of the concept of derived *B. juncea* as well as the inherent genomic differences between A and B genomes. Studies of resynthesized allopolyploids in *Brassica* and Wheat have shown that the polyploidization leads to rapid and extensive genome reorganizations (Song *et al*., 1995). As discussed earlier, these changes can be consequences of either of rapid and nonrandom elimination of particular low-copy DNA sequences, or other types of genomic modifications (Ozkan *et al*., 2001; Shaked *et al*., 2001). In the Wheat (*Aegilops-Triticum*) group, studies (Pegington and Rees 1970) have shown that the genome size of tetraploid and hexaploid Wheat was additive when they were compared to parental species. Contradictions have also been reported (Boykoet al., 1984); a decrease in DNA content was observed following synthesis of triticale (X Tritisecale Wittmack), a 9 percent reduction in genome size for octoploid triticale and as many as a 28%–30% decrease for hexaploid triticale were observed. No similar studies have been reported in Brassicas. We studied 62 newly synthesized derived *B. juncea* allopolyploids to address this question. Flow cytometry was used as it provides a fast and accurate way to look at differences in genome size as a consequence of the genome differentiation. For this study 1C nuclear DNA content was estimated from 10 plants of each test accession, five from each replication. Detailed data are presented in Table 3. There appeared to be significant within the progeny, and between progeny variations, 18 progenies did not show within the progeny variation. Compared to the reference DNA content (1.118) in the natural *B. juncea* cv. PBR210, the DNA content varied between 88.034 to 109.925 when reflected as percentage of the check. Apparently, DNA changes following the polyploidization were not unidirectional, rather there was upward as well as a downward shift in the genome size. DJ 19-2 (587Mbp) had the maximum genome size followed by DJ 6 (555Mbp). DJ 79 (470Mbp) had the least genome size. Of these, only DJ 19-2, possibly, carried A/C chromosome

Table 3: Nuclear 1C DNA amounts (pg) of newly synthesized amphiploids

Genotype	DNA as percent of standard	Minimum DNA content in pg	Maximum DNA content in Pg	Range	Mean 1c DNA content in Pg	Observed genome size in Mbp
DJ 1	104.454	1.133	1.152	0.019	1.141±0.005	558
DJ 1-1	102.705	1.104	1.126	0.022	1.122±0.004	548
DJ 1-2	97.184	1.061	1.061	0.000	1.061±0.000	519
DJ 2	98.092	1.026	1.095	0.069	1.071±0.015	524
DJ 6	103.983	1.095	1.298	0.203	1.135±0.041	555
DJ 9	97.183	1.058	1.075	0.017	1.061±0.003	519
DJ 14	95.886	1.047	1.047	0.000	1.047±0.000	512
DJ 15	100.872	1.089	1.110	0.021	1.102±0.001	539
DJ 17	97.676	1.065	1.068	0.003	1.067±0.005	522
DJ 18	97.862	1.054	1.075	0.020	1.069±0.004	523
DJ 19-1	100.377	1.092	1.113	0.021	1.096±0.004	536
DJ 19-2	109.925	1.176	1.217	0.041	1.200±0.008	587
DJ 20	98.844	1.071	1.092	0.021	1.079±0.003	528
DJ 21	102.569	1.101	1.145	0.044	1.120±0.010	548
DJ 22	101.887	1.113	1.113	0.000	1.113±0.000	544
DJ 24	100.270	1.095	1.095	0.000	1.095±0.000	535
DJ 25	97.804	1.068	1.068	0.000	1.068±0.000	522
DJ 26	103.495	1.118	1.158	0.040	1.130±0.008	553
DJ 27	100.270	1.095	1.095	0.000	1.095±0.000	535
DJ 28	100.377	1.092	1.113	0.021	1.096±0.004	536
DJ 30	100.220	1.087	1.106	0.019	1.094±0.005	535
DJ 31	97.197	1.026	1.081	0.055	1.061±0.010	519
DJ 38	94.186	1.001	1.089	0.088	1.029±0.017	503
DJ 49-1	101.256	1.068	1.068	0.000	1.106±0.010	541
DJ 55	91.754	0.989	1.005	0.016	1.002±0.003	490
DJ 57	100.270	1.095	1.095	0.000	1.095±0.000	535
DJ 58	101.132	1.092	1.113	0.021	1.104±0.005	540
DJ 59	97.939	1.051	1.083	0.032	1.069±0.005	523
DJ 60	101.887	1.113	1.113	0.000	1.113±0.000	544
DJ 61	100.000	1.092	1.092	0.000	1.092±0.000	534
DJ 63	100.000	1.092	1.092	0.000	1.092±0.000	534
DJ 65	97.484	1.065	1.065	0.000	1.065±0.000	521
DJ 66	96.896	1.043	1.068	0.025	1.058±0.006	517
DJ 68	98.703	1.078	1.078	0.000	1.078±0.000	527
DJ 71	98.513	1.068	1.089	0.021	1.076±0.005	526
DJ 72	97.804	1.068	1.068	0.000	1.068±0.001	522
DJ 73	99.889	1.089	1.092	0.003	1.091±0.001	533
DJ 78	95.119	1.026	1.047	0.021	1.039±0.005	508
DJ 79	88.034	0.915	1.018	0.102	0.961±0.017	470
DJ 80	96.439	1.042	1.045	0.004	1.053±0.010	515
DJ 81	98.954	1.068	1.089	0.021	1.081±0.005	528
DJ 86	98.105	1.058	1.075	0.017	1.071±0.003	524
DJ 89	95.047	1.038	1.038	0.000	1.038±0.000	508

DJ 91	98.829	1.079	1.079	0.000	1.079±0.000	528
DJ 92	98.454	1.051	1.110	0.059	1.075±0.011	526
DJ 97	102.785	1.115	1.133	0.018	1.122±0.004	549
DJ 98	100.281	1.089	1.104	0.015	1.095±0.004	535
DJ 99	97.736	1.051	1.092	0.041	1.067±0.010	522
DJ 101	100.576	1.084	1.120	0.036	1.098±0.007	537
DJ 103	96.061	1.022	1.061	0.039	1.049±0.012	513
DJ 107	97.804	1.068	1.068	0.000	1.068±0.000	522
DJ 108	98.491	1.051	1.092	0.041	1.076±0.010	526
DJ 110	101.132	1.092	1.113	0.021	1.104±0.005	540
DJ 113	96.081	1.030	1.081	0.051	1.049±0.008	513
DJ 115	102.264	1.113	1.133	0.021	1.117±0.004	546
DJ 116	99.683	1.075	1.092	0.017	1.089±0.003	532
DJ 123	95.981	1.042	1.058	0.016	1.048±0.004	513
DJ 124	98.413	1.075	1.075	0.000	1.075±0.000	526
DJ 124-1	97.804	1.068	1.068	0.000	1.068±0.000	522
DJ 125	97.544	1.052	1.071	0.019	1.065±0.003	521
DJ 126	98.529	1.075	1.078	0.003	1.076±0.001	526
DJ 127	96.604	1.051	1.071	0.021	1.055±0.004	516
PBR210	100	1.118	1.118	0.000	1.118±0.000	534
RLC-1	99.6	1.0838	1.092	0.003	1.088±0.002	532

substitutions. We can not, however, be categorical to state whether this elimination or addition was due to hybridity or allopolyploidization. The behavior of genome size changes followed a similar trend in the designated substitution lines. On the basis of population mean (over 62 derived *juncea* types), average genome size was 528.274 Mbp, representing a decline of almost 1.072 percent DNA during six generations of selfing following allopolyploidization. Based on flow cytometric study we can not say if all of the parental genomes were affected equally. Similar studies in Wheat (Ozkan *et al*., 2001; Shaked *et al*., 2001) have shown that a limited set of loci could undergo rapid elimination in newly synthesized allopolyploids from the Wheat (*Aegilops-Triticum*) group. Our studies clearly showed the efficacy of derived amphiploidy for resynthesis of new allotetraploids. New concept also provides the possibility of identifying novel genetic stocks as chromosome substitution and addition lines. Such cytogenetic stocks are currently not available in Brassica oilseeds.

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