



# Allele segregation analysis of F<sub>1</sub> hybrids between independent *Brassica* allohexaploid lineages

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## Abstract

In the *Brassica* genus, we find both diploid species (one genome) and allotetraploid species (two different genomes) but no naturally occurring hexaploid species (three different genomes, AABBCC). Although hexaploids can be produced via human intervention, these neo-polyploids have quite unstable genomes and usually suffer from severe genome reshuffling. Whether these genome rearrangements continue in later generations and whether genomic arrangements follow similar, reproducible patterns between different lineages is still unknown. We crossed *Brassica* hexaploids resulting from different species combinations to produce five F<sub>1</sub> hybrids and analyzed the karyotypes of the parents and the F<sub>1</sub> hybrids, as well as allele segregation in a resulting test-cross population via molecular karyotyping using SNP array genotyping. Although some genomic regions were found to be more likely to be duplicated, deleted, or rearranged, a consensus pattern was not shared between genotypes. *Brassica* hexaploids had a high tolerance for fixed structural rearrangements, but which rearrangements occur and become fixed over many generations does not seem to show either strong reproducibility or to indicate selection for stability. On average, we observed 10 *de novo* chromosome rearrangements contributed almost equally from both parents to the F<sub>1</sub> hybrids. At the same time, the F<sub>1</sub> hybrid meiosis produced on average 8.6 new rearrangements. Hence, the increased heterozygosity in the F<sub>1</sub> hybrid did not significantly improve genome stability in our hexaploid hybrids and might have had the opposite effect. However, hybridization between lineages was readily achieved and may be exploited for future genetics and breeding purposes.

**Keywords** *Brassica* · Hexaploids · Interspecific hybridization · Karyotype rearrangement · Homeologous recombination

## Introduction

The *Brassica* genus belongs to the Brassicaceae family (Warwick et al. 2006) and includes many economically valuable crop species. The *Brassica* crops can be broadly classified into vegetable, oilseed, fodder, and condiment types. During the 1930s, the chromosome number and

genetic relationships between the cultivated *Brassica* species were established in what we know as the triangle of *U* (U 1935): the diploid species *B. rapa* (AA,  $n=10$ ), *B. nigra* (BB,  $n=8$ ) and *B. oleracea* (CC,  $n=9$ ) were defined as progenitors of the allotetraploid species *B. juncea* (AABB,  $n=18$ ), *B. napus* (AACC,  $n=19$ ), and *B. carinata* (BBCC,  $n=17$ ), which originated via pairwise spontaneous hybridization between these diploids. The *Brassica* vegetables, *B. oleracea* and *B. rapa*, are characterized by vast diversity in subspecies and varieties (Cheng et al. 2016). In these species, it is possible to find different domesticated morphotypes distinguishable by leaf types, inflorescence types or enlargement of roots or stems. In *Brassica* species it is also possible to find traits of agronomic interest, such as disease resistance (Chevre et al. 1996; Mei et al. 2011, 2013; Peng et al. 2014; Taylor et al. 2015; Fredua-Agyeman et al. 2019), abiotic stress tolerance (Gill et al. 2011; Hayat et al. 2011; Wilson et al. 2014; Irfan et al. 2014) and pod shatter

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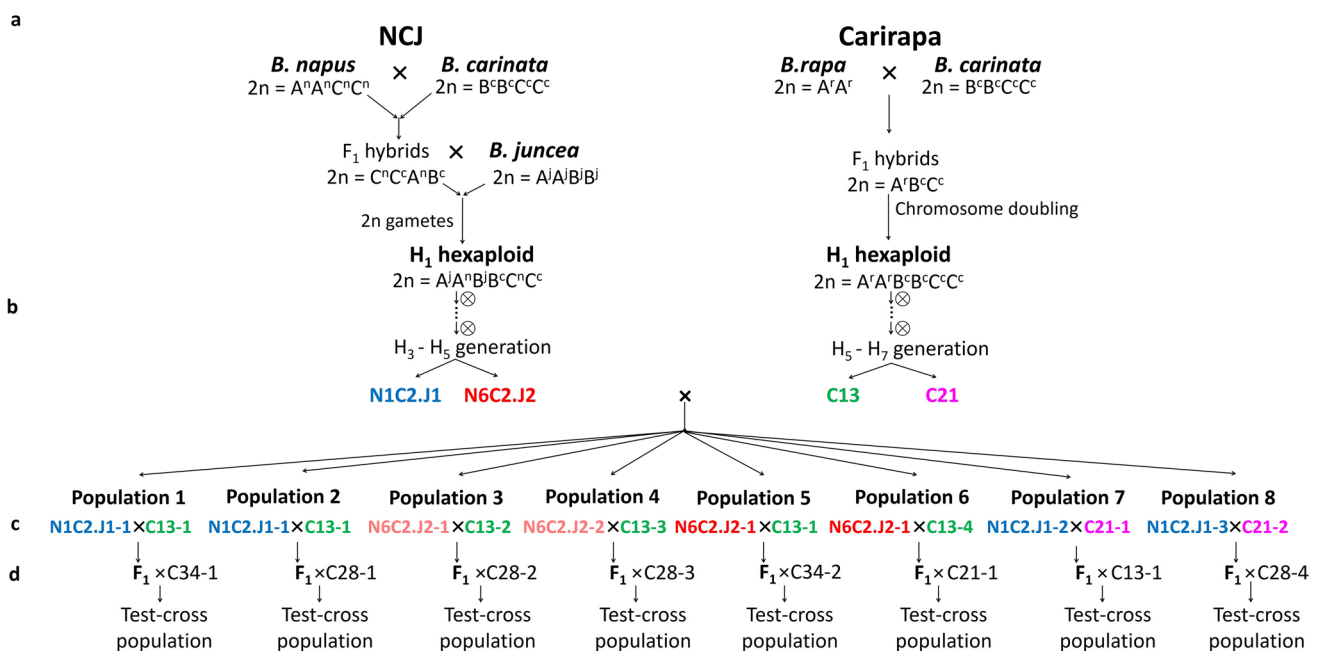
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resistance (Zhang et al. 2016), among other potential traits (reviewed in Katche et al. (2019)).

Commonly, in the *Brassica* genus, we find both diploid and tetraploid species but no naturally occurring hexaploid species ( $AABBCC = 2n = 6x = 54$ ). Despite this, it is possible to synthesize this hybrid via human intervention. The three most common cross combinations to produce an allohexaploid are: (i) *B. carinata* × *B. rapa* (Fig. 1a), (ii) *B. juncea* × *B. oleracea*, and (iii) *B. napus* × *B. nigra* (reviewed in (Gaebelein and Mason 2018)), that from now on will be referred to as “carirapa,” “junleracea,” and “naponigra” allohexaploid types (Zou et al. 2010). All of these hybrids are usually colchicine-treated to induce chromosome doubling following hybridization between a diploid and a tetraploid species. A more recent method to produce an allohexaploid is via two-step crossing and relies on unreduced gamete production: the hybridization between *B. napus* × *B. carinata* × *B. juncea* (Mason et al. 2012), referred to as “NCJ” allohexaploid types (Fig. 1a). Until now, many of the allohexaploid genotypes produced were solely used to cross to *B. napus*, either to introgress genetic diversity (Li et al. 2004, 2006; Jiang et al. 2007; Zou et al. 2010, 2018; Hu et al. 2019), or to transfer specific traits such as yellow seededness (Meng et al. 1998; Rahman 2001) or fungal disease resistance (Sjödín and Glimelius 1989). Despite this, a new allohexaploid hybrid has the potential to become a new species with the advantage of combining all the different

traits present in the six *U*'s triangle species, thus broadening the genetic resources available for breeders (Chen et al. 2011). In such an allohexaploid, it would also be possible to take advantage of “fixed heterosis” (Abel et al. 2005), where the heterosis is present between the subgenomes (A, B, and C) can be maintained in inbreeding lines. In addition, new phenotypes which are not present in the original parents may develop from the different crosses via novel mutations due to the hybridization event (Udall and Wendel 2006; Kaur et al. 2014).

Allohexaploid *Brassica* ( $AABBCC = 2n = 54$ ), as a new polyploid, has to overcome the major challenge of establishing regular meiosis (Pelé et al. 2018). The correct pairing of homologous chromosomes during meiosis is a key factor to ensure correct cross-over and chromosome segregation. In the case of this allohexaploid, meiosis is challenging due to the presence of three ancestrally homologous chromosome sets from different evolutionary lineages, known as homologous chromosomes (Ramsey and Schemske 2002). If nonhomologous pairing during meiosis occurs, it can lead to different chromosomal rearrangements, such as deletions, duplications and translocations (Udall et al. 2005), heavily affecting genome stability. In a synthetic trigonomic hybrid, we usually observe the A and the C genome are more likely to pair during meiosis, compared to the A-B or C-B homoeologs (Mason et al. 2010). In the case of natural allopolyploids, such a *B. napus*, meiosis progresses



**Fig. 1** Crossing scheme. The arrows indicate the direction of the crossing. **a** Crossings involved in the production of the NCJ and carirapa hexaploid lines. **b** Selfing and selection of the genotypes. **c** Crossings between NCJ and carirapa genotypes. For the NCJ geno-

type combination N6C2.J2, two different lineages were used for crossing, indicated by a different shade of red. The number after the genotype combination represents the plant number. **d** F<sub>1</sub> test-cross to another carirapa hexaploid

normally, although nonhomologous exchanges do still occur at lower frequency (Parkin et al. 1995; Udall et al. 2005; Higgins et al. 2018).

Many different types of cross-combinations have been tested to produce meiotically stable  $2n = AABBC$  allohexaploids (reviewed in Gaebelein and Mason (2018)). It has been shown that early generation ( $F_2$ ) carirapa hexaploids have low pollen viability and irregular configurations during meiosis (Howard 1942; Iwasa 1964). It has also been seen that fertility and number of  $2n = 54$  (putatively euploid) plants can increase by selection over successive generations (Tian et al. 2010; Zhou et al. 2016). Genotype combinations seem to play a great role in the success of the hexaploid, as many of the more stable and fertile hexaploids produced are derived from just a few lines (Tian et al. 2010). A specific *B. rapa* genotype (R01) was also reported to result in a meiotically stable carirapa allohexaploid (Gupta et al. 2016). Carirapa combinations that included this *B. rapa* genotype had a high frequency of bivalent formation during meiosis, progeny with the expected 54 chromosomes, and no major translocations observed (Gupta et al. 2016). Similarly, genotype-specific effects on fertility and meiotic stability have been observed in NCJ hexaploids (Mwathi et al. 2017).

In the present study we analyzed chromosome and allele segregation resulting from the meiosis of five different  $F_1$  hybrids produced between crosses of putatively stable *Brassica* hexaploids carirapa and NCJ. We hypothesized that the new hybrids might show improved meiotic stability (more regular chromosome segregation and fewer nonhomologous recombination events) compared to their parents. We also aimed to determine if chromosome rearrangements found at high frequency or in independent lineages of the parent hexaploid types (e.g., both in NCJ and carirapa) might be associated with improved meiotic stability, since all lineages underwent strong selective pressure for fertility.

## Materials and methods

### Plant material and crossing scheme

The most advanced genotypes available from the two NCJ parental genotype combinations, N1C2.J1 and N6C2.J2 (one and two lineages, respectively) (Mason et al. 2012; Mwathi et al. 2017) and as well as one lineage from each carirapa allohexaploid genotypes C13, C21 (Tian et al. 2010), were selected based on chromosome number and total seeds produced (Fig. 1b, Supplementary Table 1). In total, 12 plants were grown from the selected genotypes, with 6 plants per allohexaploid type: three plants N1C2.J1, two plants N6C2.J2 lineage one, one plant N6C2.J2 lineage two, four plants C13, and two plants C21 (Fig. 1c). Initially, two plants from each of hexaploid genotypes C13 (plants 2 and 3) and N6C2.

J2 (lineage one, plants 1 and 2) were grown and crossed under greenhouse conditions at Justus Liebig University, Giessen, Germany, while the rest of the plants were grown under field conditions at Huazhong Agricultural University, Wuhan, China. In the first round of crossings ( $F_1$  hybrid production), the NCJ hexaploid plants were used as a female parents, and the carirapa plants were used as the pollen donor to produce eight different populations (Fig. 1c). The cross was done by hand via emasculating flower buds and gently rubbing the anthers over the exposed stigma. The pollinated buds were then labeled and covered with a micropore-perforated bag to prevent contamination from other pollen sources.

The  $F_1$  hybrid seeds were collected and grown under field conditions at Huazhong Agricultural University, Wuhan, China. To be able to analyze the meiotic performance and allele segregation from this new  $F_1$  hybrid, a test-cross was carried out. Selection of the  $F_1$  hybrids was done based on qualitative phenotyping (e.g., high pollen production, relatively normal agronomic phenotype, and plants looked like true hybrids) for individual plants resulting from the crosses between the lines. A total of eight  $F_1$  hybrid plants were selected and test-crossed to another carirapa hexaploid (genotypes C21, C28, and C34) (Tian et al. 2010) (Fig. 1d). The test cross-progeny varied in size depending on the population, ranging from 14 to 22 individuals that were grown under field conditions at Huazhong Agricultural University, Wuhan, China.

### DNA extraction and SNP analysis

From each plant, a piece of leaf sample was collected, and DNA was extracted using the CTAB method (Doyle and Doyle 1990). For the following plants, the original leaf material was not available, but three different sibling plants were used instead:  $F_1$  hybrid population 3 and test-cross parent population 4. In the case of *B. carinata* and *B. rapa* parental lines of the carirapa hexaploids, we extracted DNA from two different plants to account for potential heterosis in the material. The DNA was then genotyped using the Illumina Infinium *Brassica* 90 K SNP array (Illumina, San Diego, CA, USA) following manufacturer's instructions. A total of 77,970 SNPs distributed across the A (23,482), B (25,822), C (26,731), and unplaced location (1877) *Brassica* subgenomes were obtained after applying the recommended cluster file for the A and C genomes (Clarke et al. 2016, Supplementary Data set Table 2) and by automated clustering in Genome Studio for the B genome. SNP positions for the A and C genomes were determined by the top hit (highest *e*-value) based on BLAST to the *B. napus* Darmor-*bzh* v. 8.1 reference genome (Bayer et al. 2017). For the B genome, we used the positions provided in the Illumina Infinium 90 K SNP array, which were based on the *B. nigra* Ni100 short

read reference genome assembly (Perumal et al. 2020). The SNP data was initially cleaned by removing non-specific alleles and SNPs with undetermined genomic locations.

The first step in the analysis was to carry out paternity testing to verify the genetic composition of the  $F_1$  hybrid between the NCJ and carirapa allohexaploid parents for each of the eight populations. For this, homozygous polymorphic alleles for each parent in the B subgenome were used as diagnostics. If the expected heterozygosity was observed in the  $F_1$  plant (e.g. NCJ allele AA × carirapa allele BB →  $F_1$  hybrid allele AB), it was considered a true  $F_1$  hybrid. The second step was to determine true segregating progeny between the  $F_1$  hybrid and the test-cross carirapa allohexaploid parent. To do this, the same approach was used by selecting homozygous polymorphic alleles in the B subgenome between the  $F_1$  and test-cross carirapa parent: if the segregating progeny had the expected heterozygous allele, they were considered true test-cross progeny. If the population were true hybrids in both analyzed cases, subsequent analyses were done.

### Chromosome count and molecular karyotyping

To determine chromosome presence or absence, we used SNP and  $\text{Log}_2 R$  ratio data (Supplementary Data set Table 3). The absence of both chromosomes was seen across the entire chromosome, or most of it, as “no call” SNPs (NC). If the chromosome was present in the SNP data (at least one copy of the chromosome present), the  $\text{Log}_2 R$  ratio data was used to determine loss or gain of a chromosome copy by assessing the inheritance of the centromeric region of each chromosome (inheritance of the centromere was inferred to mean inheritance of that chromosome, since chromosome fragments cannot be transmitted without a centromere). Experimentally-derived values were used based on comparison to hybrid standards with known haploid and diploid chromosome complements.  $\text{Log}_2 R$  ratios between  $-0.5$  and  $-0.2$  were assumed to indicate one missing chromosome copy,  $\text{Log}_2 R$  ratios between  $0.2$  and  $0.5$  were assumed to indicate gain of an extra copy and  $\text{Log}_2 R$  ratios of more than  $0.5$  to indicate more than one extra chromosome copy gained. The approximate centromere locations for the A and C chromosomes were established according to the *B. napus* Darmor-*bzh* v8.1 reference genome (Bayer et al. 2017), based on remapping of previous genetic data (Mason et al. 2016). For molecular karyotyping, a similar approach was used. If no-call (NC) SNPs covering  $\geq 1$  megabases (Mb) in the chromosome was observed, it was categorized as both copies missing in that particular region of the chromosome. A similar size cut-off was used for the  $\text{Log}_2 R$  ratio values. Missing regions covering less than 1 Mb were not considered in the analysis due to SNP distribution and density constraints. If the duplication or deletion event was found

in two copies (on both homologous chromosome sets), we classified it as a “fixed” event since this must have resulted from previous meiosis, after which a self-pollination event allowed two gametes with the same rearrangement event to come together in the same plant. On the other hand, if a rearrangement was only present in one of the homologous chromosomes, it was considered a segregating rearrangement. If a rearrangement was not present in either of the parents but was detected in the next generation, it was classified as a *de novo* event. Putative translocations between the genomes were established based on initially scoring deletions/duplications based on the  $\text{Log}_2 R$  ratio values in combination with the already known primary homology relationships between the A, B, and C genomes (Chalhoub et al. 2014; Perumal et al. 2020) (Fig. 2, Supplementary Table 4). The final karyotype was plotted in RStudio using the R package chromDraw (Janečka and Lysak 2016), and posterior editing was done in GIMP ver. 2.10.20.

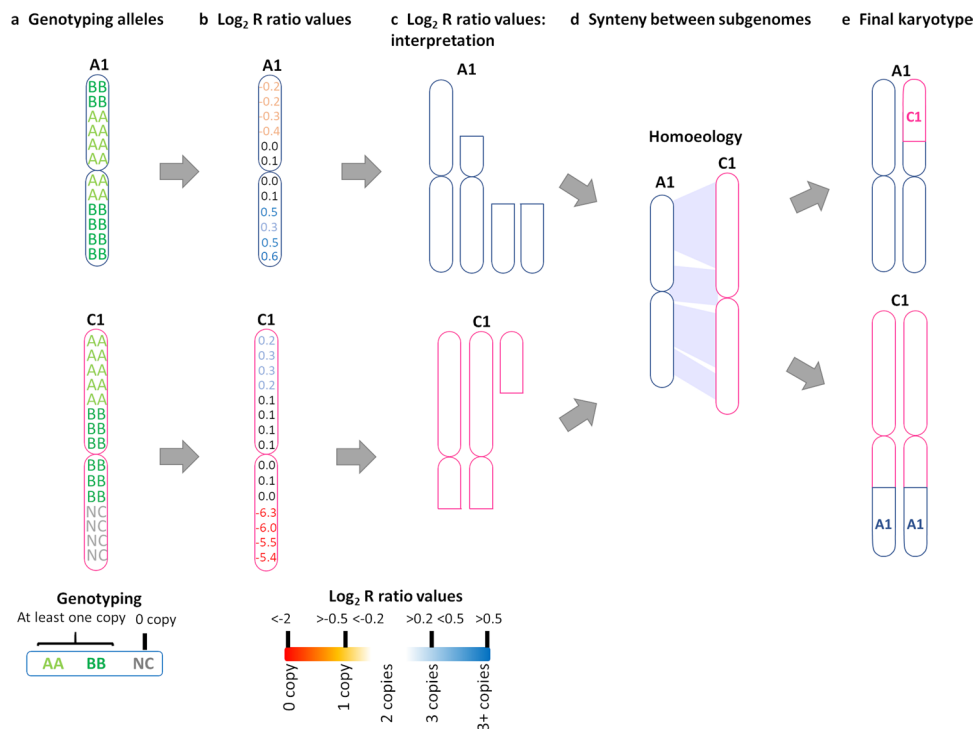
### Allele segregation in the $F_1$ hybrid

To analyze the allele segregation from the  $F_1$  hybrid, the alleles from each parent were analyzed independently. To analyze the NCJ parent, homozygous polymorphic alleles from the NCJ vs. carirapa parent 1/carirapa test-cross parent were filtered (NCJ allele: AA vs. carirapa parent 1/carirapa test-cross parent: BB), and vice versa for the carirapa parent. In each of the eight populations, the number of test-cross progeny ranged from 14 to 22 (Supplementary Table 1). The 1 : 1 AB:BB observed vs. expected allele segregation was tested using a  $X^2$  test with a significance level of  $p < 0.05$ .

## Results

### Preexisting fixed rearrangements in *Brassica* parental genotypes

*Brassica* genotypes used to produce the carirapa and NCJ allohexaploids were analyzed for fixed rearrangements in the genome. The parental genotypes used to produce the carirapa hexaploids, *B. carinata* accession “03,949,” and the *B. rapa* genotypes “Ankangzhong” and “WulitianYC” did not have any detectable fixed rearrangement events in their corresponding genomes. In the genotype “03949,” we did observe a segregating event at the top of B01, where a single copy was missing between 0 and 13.6 Mb. In the case of the genotypes used in the crossing of NCJ hexaploids, we only observed fixed events in the *B. napus* genotypes. In the case of the *B. napus* genotype “Surpass400\_024DH” (N1), we detected deletions in chromosomes A01 and A04, located at 2.2–4.7 Mb and 20.7–23.3 Mb, respectively. In the C genome of “Surpass400\_024DH,” we identified a



**Fig. 2** Example of the molecular karyotyping workflow. **a** Genotype for chromosomes A1 and C1 in *Brassica* hexaploids: AA and BB: homozygous for allele A or B, respectively. NC, no-call. The end of the C1 chromosome is missing. **b** Log<sub>2</sub> R ratio values: values obtained from the SNP chip. Experimentally derived values were used to establish the ranges for each type of copy number variation (details in the legend). **c** Log R ratio values interpretation. The top of chromosome A1 is present in a single copy, and the end of A1 has duplication. In the case of C1, there is duplication at the beginning of the chromosome and a deletion (both copies missing) at the

end of the chromosome. The extra copies do not have a centromere; as chromosome fragments are eliminated in mitosis without a centromere, these fragments must be located in a chromosome. **d** Synteny between subgenomes: *Brassica* homology (Chalhoub et al. 2014; Perumal et al. 2020). Homologous chromosomes can pair during meiosis, and translocations can occur. In this case, the fragment sizes and positions of the different copy number variation events suggest translocation events between A1 and C1 chromosomes. **e** Final drawing of the karyotype based on genotyping, Log<sub>2</sub> R ratio, and homology between the different subgenomes

deletion at the top of chromosome C01, located between 1.4 and 3.3 Mb. We also found two other deletion events located in chromosome C09, at positions 0–2 and 52.9–60.1 Mb, respectively. For the “Ag-Spectrum” genotype (N6), we observed a deletion on chromosome C02, located between 8–10.7 Mb.

### Chromosome inheritance and fixed karyotype changes in NCJ and carirapa allohexaploid types

From the available NCJ and carirapa collection, the most advanced and fertile genotype combinations were selected. From the NCJ type, the genotypes selected were N1C2.J1 and N6C2.J2, and from the carirapa type, C13, and C21. The carirapa plants were not fully homozygous, suggesting that pollen cross-contamination with other genotypes may have occurred during propagation in the field. In the NCJ-type hybrids, residual heterozygosity was present based on the method of producing these hybrids, but no cross-contamination was indicated by the presence of alleles other

than those from the parent genotypes. In total, six plants per allohexaploid type were grown and later on crossed to produce F<sub>1</sub> hybrids between the lines (Fig. 1c).

First, the relative chromosome number for the 12 allohexaploid parental plants was determined using SNP data and Log<sub>2</sub> R ratio values (Supplementary Table 1). For the “carirapa” type, the chromosome numbers were 51 (1 plant), 52 (2 plants), 53 (1 plant), and 54 (2 plants). In the case of the allohexaploid NCJ, the chromosome numbers observed were 50 (1 plant), 51 (1 plant), 52 (1 plant), 53 (1 plant), 54 (1 plant), and 55 (1 plant). The two carirapa plants with 54 chromosomes were aneuploids in terms of genome composition. In contrast, the NCJ plant with 54 chromosomes was an euploid individual (genotype combination N1C2.J1). Overall, in both hexaploid types, the chromosomes A05, A06, A08, A10, C03, C05, C07, B01, B02, B04, B06, and B08 showed no changes (Supplementary Fig. 1). Specifically, in the carirapa plants, no B chromosome changes were observed. Changes in B chromosomes were, however observed in the NCJ plants

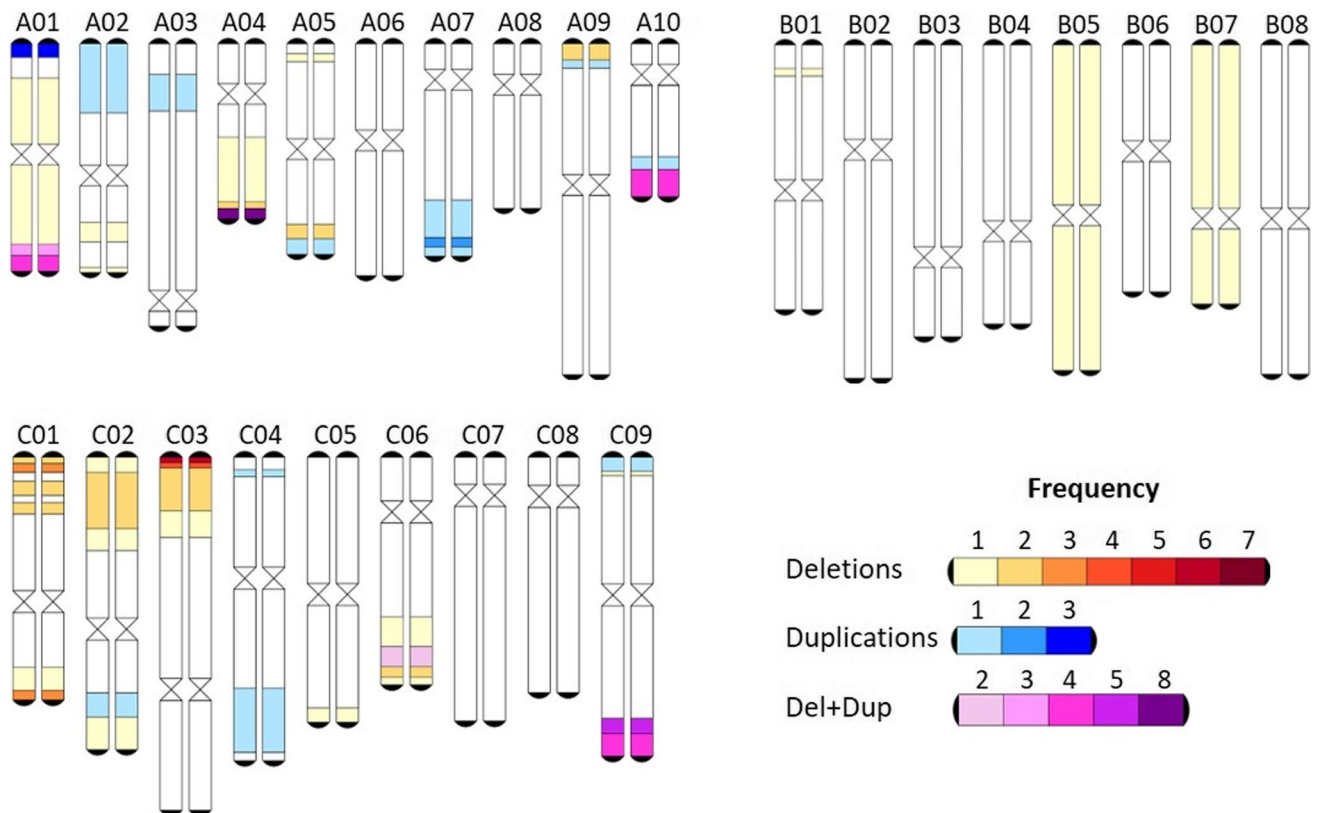


for chromosomes B03 (two plants), B05 (two plants), and B07 (one plant). For both allohexaploid types, most of the chromosome number changes were observed in the A01–A03, C01, and C02 chromosomes. Also, the chromosomes A01, B05, and B07 were both lost in one carirapa and two NCJ plants, respectively.

As the NCJ and carirapa allohexaploids have been independently selected by fertility (total seed number) through several generations, we analyzed the presence of fixed rearrangements (rearrangement present in both homologous chromosomes) in all the three genomes. To be able to have a better overview and frequency of all the fixed events, we combined them into one figure (Fig. 3). A single fixed event involving the B genome was detected putatively between B01/A04, where the B01 segment was lost (deletion) and replaced by the end of A04 segment (duplication) for this homologous region. The end of A04 (~ 3 Mb) was frequently lost, as was the case in seven out of 12 allohexaploid parents (four carirapa and three NCJ plants). However, this deletion event was already present in the *B. napus* cv. “Surpass400\_024DH” used in the cross and was inherited and fixed in the three NCJ plants analyzed. In the case of the carirapa plant C21-1 that had this deletion at the end of A04, the region was most likely replaced by the end of

chromosome C04, but evidence of this was inconclusive in the NCJ plants analyzed.

For chromosomes B05 and B07, both copies were missing in the plants belonging to the two independent N6C2.J2 lineages. Another region that was frequently lost was located at the top of chromosome C03, ranging from approximately 0–1.2 Mb in size: this region was missing in all four carirapa C13 parents (representing four plants of one lineage). The three plants from the genotype combination N1C2.J1 (one lineage) had in common a duplication/deletion event involving an extra copy of the top of chromosome A01 (~ 2 Mb) being putatively translocated into chromosome C01. The deletion of C01 was already present in the *Brassica napus* cv. “Surpass400\_024DH” (N1) used to produce the hexaploid, unlike the duplication of A01, which was a new rearrangement event. In some regions, we found an overlap of events (duplications and deletions) between the genotypes. The region at the end of chromosome A01 was duplicated in two carirapa C13 parents (one lineage), deleted in one carirapa parent (C21), and deleted in one NCJ parent (N6C2.J2). In the homologous region, there was no detectable duplication for any of the plants, hence it was more likely to be only a deletion event. Another region of overlap was found for



**Fig. 3** Karyotype of fixed events in *Brassica* allohexaploids NCJ (*B. napus* × *B. carinata* × *B. juncea*) and carirapa (*B. carinata* × *B. rapa*) type. The fixed partial or whole chromosome events are colored according to the legend for each of the three *Brassica* genomes

putative homologous translocations between chromosomes A10 and C09. The end of A10 had three duplication events (N1C2.J1) and one deletion event (C13), while C09 had one duplication (C13) and four deletions located at the end of C09 at ~53–60 Mb in three N1C2.J1 and at ~52–55 Mb for one C13 parent. For the NCJ plants from the genotype combination N1C2.J1, the putative translocation between C09/A10 was already present in the *B. napus* cv. “Surpass400\_024DH” and was inherited and fixed in the hexaploids. The rest of the events observed in the allohexaploids NCJ and carirapa were more parent-independent (occurred in only one plant). In total, we observed 50 fixed deletion events (32 carirapa, 18 NCJ) and 33 fixed duplication events (23 carirapa and 10 NCJ) involving the A, B, and C genomes of *Brassica* hexaploids. The fixed events observed in the carirapa hexaploids were not identified in either the *B. carinata* or the *B. rapa* accessions used as parents.

### Crossing NCJ and carirapa allohexaploids: F<sub>1</sub> hybrid and allele segregation

Seven out of eight F<sub>1</sub> hybrids were the result of a cross between carirapa and NCJ hexaploids, as expected (Table 1); one hybrid appeared to result from an outcross to an unknown paternal plant. The second step was to analyze the cross between the F<sub>1</sub> hybrid and the test-cross parent. To do this, the same approach as in the F<sub>1</sub> hybrid was used. In this case, populations 1 and 3 had only 7 and 5 individuals that corresponded to true test-cross progeny, and the remaining individuals resulted from unintended self-pollination to produce F<sub>2</sub> progeny seeds. The other

five populations were used to analyze allele segregation from the F<sub>1</sub> hybrid.

### Allele segregation per population

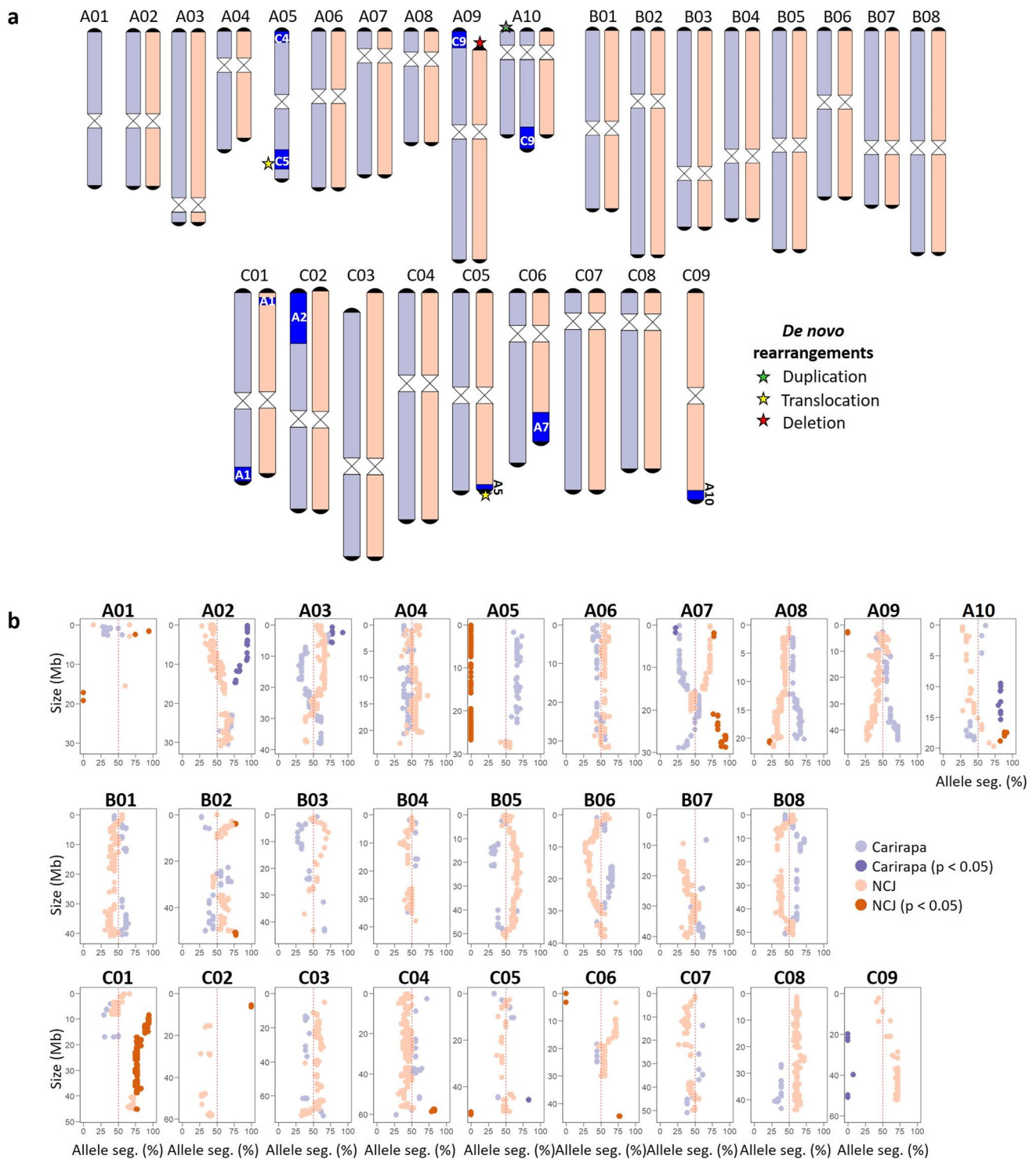
#### Population 2

The F<sub>1</sub> hybrid was the result of a combination between the genotypes N1C2.J1 × C13. This hybrid had 52 chromosomes distributed between the A genome (19), B genome (16), and C genome (17) (Fig. 4a). In the A genome, the chromosomes A01 and A05 were present in only a single copy, with just the chromosome from the carirapa parent present. Chromosome A01 was already a single chromosome in the NCJ parent, unlike A05, where both copies were present in the parent. The other chromosomes (A02–A09) were present in two copies, while chromosome A10 had at least one extra copy from the carirapa parent. In the A genome, putative nonreciprocal translocations between A05/C04, A05/C05, A09/C09, and A10/C09 were present in the chromosomes inherited from the carirapa parent. No translocations were observed in A-genome chromosomes from the NCJ parent. The bottom of chromosome A04 from NCJ was missing 2.5 Mb, corresponding to a known deletion present in the *B. napus* cv. “Surpass400\_024DH” used as a parent in the crossing. The top of chromosome A09 from the NCJ parent was missing ~3 Mb that corresponded partially to an extended deletion that was initially inherited from a deletion also present in the *B. napus* cv. “Surpass400\_024DH”. For the B genome, the F<sub>1</sub> hybrid contained the expected 16 chromosomes with no translocations or deleted regions detected. In the C genome, the chromosomes C01–C08 were correctly inherited from the carirapa parent. In the case of chromosome C09, just a portion (~5 Mb) from the end of the chromosome was present as a translocated region into chromosome A10. Part of the top of chromosome C03 (~7 Mb) was lost from the carirapa parent. The chromosomes C01–C09 were correctly inherited from the NCJ parent, despite the presence of only one copy of chromosome C02 in the parent. Putative translocations were observed in chromosomes coming from both parents. In the case of the carirapa parent chromosomes, the translocations present in the F<sub>1</sub> were C01/A01 and C02/A02, and in the NCJ parent chromosomes there were C01/A01, C05/A05, C06/A07, and C09/A10. From the above translocations, only two corresponded to putative *de novo* events identified in the F<sub>1</sub> hybrid.

Allele inheritance from the F<sub>1</sub> hybrid into the test-cross progeny was also analyzed (Fig. 4b). Segregation distortion was established if the observed allele segregation ratio was significantly different from the expected ( $X^2$  test,  $p < 0.05$ ). This test was performed independently for each of the parents of the F<sub>1</sub> hybrid. In population 2, 18 test-cross progeny individuals were used to assess allele segregation (for more

**Table 1** *Brassica* allohexaploid populations. Genotypes crossed between carirapa (C13 and C21, generations H5–H7) and NCJ (N1C2.J1 and N6C2.J2, generations H3–H5) allohexaploid types. Plant number is also specified after the genotype code. Test-cross progeny correspond to the total number of progeny that were determined to comprise true test-cross progeny between the F<sub>1</sub> and the test-cross parent. \*Populations removed from further analyses

Population	NCJ (female)	Carirapa (male)	Test-cross parent	Number of test-cross progeny
1*	N1C2.J1-1	C13-1	C34-1	7
2	N1C2.J1-1	C13-1	C28-1	18
3*	N6C2.J2-1	C13-2	C28-2	5
4	N6C2.J2-2	C13-3	C28-3	15
5	N6C2.J2-1	C13-1	C34-2	16
6	N6C2.J2-1	C13-4	C21-1	15
7*	N1C2.J1-2	C21-1	C13-1	Not true F <sub>1</sub>
8	N1C2.J1-3	C21-2	C28-4	18



**Fig. 4** **a** Molecular karyotype and **b** allele segregation for F<sub>1</sub> hybrid population 2. Chromosomes are colored based on hexaploid parent: carirapa in purple, NCJ in orange. Rearrangements are colored in blue. *De novo* translocations (present in the F<sub>1</sub> hybrid but not in the parents) are marked with a star in a different color depending on the

type (see legend). Chromosome sizes are represented in megabases (Mb). Expected segregation ratio of the alleles (50%) is marked with a red dotted line for each chromosome. Significant allele distortion ( $X^2$  test,  $p < 0.05$ ) is indicated with dark orange (NCJ) or dark purple (carirapa). Seg., segregation



details, see materials and methods). The allele segregation from the A01 and C02 chromosomes could not be established due to the lack of enough polymorphic alleles. The segregation distortion observed in the chromosomes A05 and C09 corresponded directly to the presence of only a single chromosome in the carirapa or NCJ parent, respectively. The allele segregation distortion observed in A07, A09, A10, and C05 corresponded to rearrangements present in the F<sub>1</sub> hybrid. Allele segregation distortion not directly explained by the karyotype of the F<sub>1</sub> parent was observed in chromosomes A02 (extension of a known duplication present in the F<sub>1</sub> hybrid, carirapa), A03 (although few markers were represented, carirapa), C01 (NCJ), C04 (NCJ), and B02 (NCJ). Potentially, these events correspond to *de novo* rearrangements produced during meiosis in the F<sub>1</sub>.

#### Population 4

The F<sub>1</sub> hybrid resulting from the combination between NCJ N6C2.J2 × carirapa C13 had 51 chromosomes distributed between the A (21), B (15), and C (15) genomes (Supplementary Fig. 2a). In the F<sub>1</sub> hybrid, chromosomes A02 and A07 had at least one extra copy from the carirapa and from the NCJ parent, respectively. These chromosomes were also doubled in the corresponding allohexaploid parent. Chromosome A04 was present as a single copy in the F<sub>1</sub> hybrid, inherited only from the NCJ parent. In the carirapa parent, chromosome A04 was present as a single copy, and it was not inherited from the F<sub>1</sub>. In F<sub>1</sub>, the chromosome A05 inherited from the carirapa parent had a small region missing (1.6 Mb) located at chromosome position ~24 Mb, and no potential candidate translocation corresponding to this region was identified. In the B genome, the F<sub>1</sub> hybrid had only one B07 chromosome (carirapa origin). Chromosome B07 was completely absent in the NCJ parent. In the B03 chromosome of the F<sub>1</sub> hybrid (NCJ origin), a duplicated region was identified, but it was not possible to determine the position in the genome of this extra copy (colored gray, see Supplementary Fig. 2a).

In the C genome, chromosomes C01, C02, and C06 were present in single copies (NCJ). The chromosomes C01 and C02 were also present as a single copy in the carirapa parent, unlike C06, which was present as two copies in the carirapa parent and as a single copy in the NCJ parent. In the A genome, putative translocations between homoeologs A01/C01, A02/C02, A07/C07, and A09/C08 were observed. In the B genome just one putative translocation between B01/A04 (NCJ) was observed. In the C genome, we had putative translocations involving C02/A02, C04/A04, C05/B01, and C07/B02.

In the analysis of the allele segregation, most significant distortions were explained by the translocations described above (Supplementary Fig. 2b). The exceptions were present

in chromosomes A02 (carirapa), A07 (carirapa), A10 (carirapa), C06 (NCJ), B01 (carirapa), and B02 (NCJ). In addition, the single B07 chromosome in the F<sub>1</sub> hybrid was expected to be present in 50% of the test-cross population, but was only present in 18% of the individuals (3 out of 17).

#### Population 5

The F<sub>1</sub> hybrid (N6C2.J2 × C13) had 54 chromosomes distributed between the A (21), B (15), and C (18) genomes (Supplementary Fig. 3a). Chromosomes with at least one extra copy were present for A04 (NCJ) and C03 (NCJ). Chromosome A04 was already doubled in the NCJ parent, unlike chromosome C03, which resulted from a new chromosome duplication event. Single copies were observed for chromosomes B05 (carirapa) and C09 (NCJ). In the case of B05, both copies were missing in the NCJ parent. Chromosome C09 was present as a single copy in the carirapa parent, and did not get inherited into the F<sub>1</sub> hybrid. In the chromosomes from NCJ, putative translocations between A01/C01, A03/C03, A04/C04, B01/A05, C03/A03, and C08/A09 were found. In the case of chromosomes coming from the carirapa parent, we observed translocations involving the chromosomes A02/C02, A05/C04, A09/C09, C01/A01, C02/A10, and C03/A03 (Supplementary Fig. 3a).

In the population segregating for alleles from the F<sub>1</sub> hybrid, most of the allelic distortion was explained by CNV events in the parent F<sub>1</sub> (Supplementary Fig. 3b). The exceptions to this (putatively novel CNV events) were located in A02 (carirapa), A08 (NCJ and carirapa), B03 (NCJ), B04 (NCJ), B07 (NCJ), C02 (NCJ), C04 (NCJ and carirapa), and C08 (NCJ). Chromosome C09, as mentioned before, had just one copy in the F<sub>1</sub> hybrid, and it was present in fewer test-cross individuals than expected (25% presence vs. 50% expected). In the case of B05 as a single chromosome, only a few polymorphic alleles were present with which to make a proper comparison, but it also seemed to be present less often than expected (25% presence vs. 50% expected).

#### Population 6

The F<sub>1</sub> hybrid in this population was a cross between N6C2.J2 × C13 and had 51 chromosomes distributed between the A (19), B (15), and C (17) genomes (Supplementary Fig. 4a). Single-copy chromosomes were A04, B05, and C08. Chromosome B05 was already missing both copies in the NCJ parent. In the case of A04 and C08, both copies were present in the corresponding carirapa and NCJ parents, respectively. No extra chromosomes were observed. Putative translocations between A01/C01 and A09/C08 were detected in NCJ chromosomes. In the case of the carirapa chromosomes, observed possible translocations were located between A09/C09-C08, C01/A01, and C02/A02.

When analyzing the allele segregation for alleles from the F<sub>1</sub> hybrid (Supplementary Fig. 4b), most of the distortion was explained by rearrangement events, with the exception of the following: end of A02 (carirapa), A03 (carirapa and NCJ), end of chromosome A04 (NCJ), A09 (carirapa), C01 (NCJ), C04 (NCJ), and C09 (NCJ). The A09 chromosome was a special case, as it was present in two copies in the F<sub>1</sub> hybrid, but in the test-cross population, the A09 of carirapa origin was present at a higher frequency than expected (expected 50%, observed 86.6%). Interestingly, this chromosome had two translocations involving chromosomes C9 and C8. The single chromosomes A04 and C08 segregated as expected in the population, unlike B05, which was present in only two out of the 15 test-cross population individuals and not in half of them, as expected. No other changes were observed in the B genome.

### Population 8

The cross between N1C2.J1 (euploid,  $2n = 54$ ) × C21 gave rise to an F<sub>1</sub> hybrid with 52 chromosomes distributed between the A (19), B (16), and C (17) genomes (Supplementary Fig. 5a). Single chromosomes were observed for A03 and C01. Both chromosomes were present as two copies in the corresponding NCJ and carirapa parents. No chromosomes were doubled. In the NCJ chromosomes, we observed potential translocations between A02/C02, C01/A01, C06/A07, and C09/A10. Chromosomes A04 and A09 inherited from the NCJ parent had a deletion at the bottom and at the top of the chromosome, respectively. These deletions were already present in the parents, and we did not observe a duplicated homologous region that could have replaced this fragment in the F<sub>1</sub> hybrid. In the chromosomes from the carirapa parent, we observed putative translocations between A01/C01, C02/A02, C03/A03, and C09/A09.

In the allele segregation from the F<sub>1</sub> hybrid (Supplementary Fig. 5b), most of the events were explained by the rearrangements, with the exception of areas on chromosomes A03 (NCJ), A06 (carirapa), B02 (NCJ), B06 (NCJ), B08 (NCJ and carirapa), C08 (NCJ), and C09 (NCJ).

### De novo rearrangements and inheritance in the F<sub>1</sub> hybrids

Overall, 50 new rearrangement events (22 duplications and 28 deletions) were observed in the F<sub>1</sub> hybrids. Out of these events, 28% (6 duplications and 8 deletions) were triggered by a previous event nearby or overlapping the chromosomal location of the new event already present in the hexaploid parent. On average, there were 10 new events per population, affecting mostly the A (52%) and C genome (44%). Both parents contributed almost equally to the *de novo* rearrangements observed in the F<sub>1</sub> hybrids, with the exception of

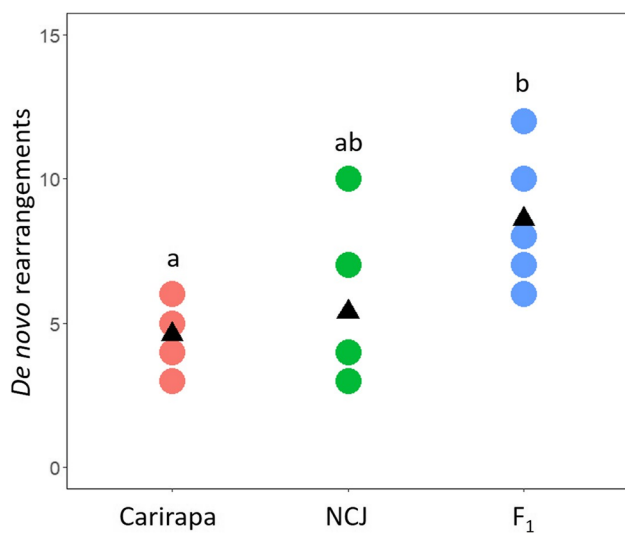
population 5, where the NCJ parent contributed to 10 rearrangements compared to 4 coming from the carirapa parent. As mentioned before, the least affected genome was the B, where just two events in two populations were observed. In the A genome, the chromosome most affected by rearrangements was A09, with a total of 8 events (2 duplications and 6 deletions). In the case of the C genome, the chromosome with more *de novo* rearrangements was C02, with six events (2 duplications and 4 deletions). We also observed nine *de novo* events involving whole chromosomes, where six chromosomes were lost, and three were present in an extra copy in the F<sub>1</sub> hybrids.

In the F<sub>1</sub> hybrids, we also observed that some of the rearrangement events present in the parental hexaploid plants were either inherited in the same size or reduced in size due to cross-overs. In total, 72 rearrangements were inherited from the hexaploid parental plants, with an average of 14.4 rearrangement events per F<sub>1</sub> hybrid. Out of the 72 rearrangements, 8.3% had a reduction in size due to a cross-over. Most of the events inherited from the parents corresponded to deletions (66.7%), with seven events involving the loss of a chromosome, affecting mostly the C genome (27 events).

When analyzing the putatively *de novo* events produced by the F<sub>1</sub> hybrids, we observed a total of 43 events (8.6 events on average per population). Out of these events in the F<sub>1</sub> hybrids, 25 events were potential duplications and 18 deletions. Interestingly, many of these events also affected the B genome (9 events total, 8 from NCJ and 1 from carirapa origin, respectively). Overall, more events were produced by one meiosis in the F<sub>1</sub> hybrid than by meioses coming from the grandparents, although the difference was only significant between the carirapa parent and the F<sub>1</sub>s (Fig. 5, one way-ANOVA,  $p < 0.05$ ).

### Discussion

From our study, it is clear that the new F<sub>1</sub> hybrids produced by the cross between carirapa and NCJ hexaploid lineages were able to tolerate the inheritance of different chromosome rearrangements, despite the putative impact of these on meiosis. Initially, we hypothesized that meiosis might be more stable in the F<sub>1</sub> compared to the parents, as heterozygosity has been linked to crossover frequency in some species (Valenzuela et al. 2012; Rowan et al. 2019), but we observed the opposite. We observed an increase in rearrangements in the F<sub>1</sub> hybrid, which was significant compared to the carirapa parent (Fig. 5). An overall comparison may suggest that the more heterozygous the material, the higher the number of rearrangements that were produced, although further studies are needed to confirm this and to eliminate the confounding effect of the different genotypes. We also found more potential new rearrangements in the B genome



**Fig. 5** *De novo* rearrangements produced by meiosis in *Brassica* hexaploids carirapa (derived from *B. carinata* crossed with *B. rapa*), NCJ (derived from crosses between *B. napus*, *B. carinata*, and *B. juncea*) and their F<sub>1</sub> hybrids. Per group, there are 5 different meiosis events representing individual gametes produced from the parents and the F<sub>1</sub> (red: carirapa, green: NCJ and the F<sub>1</sub> hybrid between them: blue). The number of *de novo* rearrangements (occurring during this specific meiosis and not inherited from a previous meiotic event in the lineage) for each of these five meiotic events for the two parents and their hybrid is indicated as a dot. The average per group is drawn with a black triangle. Significant differences are indicated with letters (Tukey's HSD,  $p < 0.05$ ): groups represented with a different letter (a, b) are significantly different, while groups that have a letter in common are not significantly different (a, ab and b, ab)

of the F<sub>1</sub> hybrids, mostly affecting alleles inherited from the NCJ parent.

From our analysis, it was also clear that *Brassica* NCJ and carirapa allohexaploids can also accumulate and tolerate multiple structural rearrangements over generations (Fig. 3). This occurred even under selective pressure for improved fertility (number of total seeds produced), which hypothetically would be expected to select against aneuploid chromosome complements. This is in contrast to observations of *Brassica napus* synthetic lines in later generations (S<sub>1:11</sub>), where fertility is majorly reduced in aneuploid lines compared to euploid lines (Xiong et al. 2011). Chromosome loss similarly affected fertility in a *Brassica* hexaploid mapping population from carirapa origin, where 30 out of 51 plants were infertile if missing one chromosome (Yang et al. 2018). However, Mason et al. (2014) also found no major effects of chromosome rearrangement on fertility in an NCJ allohexaploid-derived F<sub>2</sub> population.

The expected chromosome number from the hybrids analyzed in the present study was AABBC = 2n = 54, but this was rarely observed (Supplementary Table 1). In the case of the carirapa plants, two of them had 54 chromosomes but were not euploid. The only NCJ plant

with a euploid 2n = 54 chromosome complement failed to transmit a copy of chromosome A03 to the resulting F<sub>1</sub> hybrid, indicating that meiosis was still unstable in this plant. Our results may suggest that meiosis in these hybrids is still highly unstable but that recurrent selection for fertile, viable plants is also acting to eliminate many of these rearrangements so that plants move from euploid to aneuploid and back again, accumulating minor chromosome rearrangements along the way. Aneuploidy and nonhomologous recombination in polyploids, and particularly synthetic polyploids, are not rare events and may, in some cases, even be beneficial due to the potential for creating novel phenotypic variation (reviewed in Schiessl et al. (2019)).

Nonhomologous recombination events reflected known chromosome relationships between the A, B, and C genomes, as expected. Most translocations were observed between homologous chromosome pairs, as was previously observed in both natural and synthetic *B. napus* (Chalhoub et al. 2014; Samans et al. 2018) and in interspecific *Brassica* hybrids of various types as well as earlier generation NCJ allohexaploid populations (Mason et al. 2014; Gaebelein et al. 2019). For instance, greater changes were observed for homologous chromosomes A01/C01 and A02/C02, in accordance with results found in other synthetic *B. napus* lines (Gaeta et al. 2007; Xiong et al. 2011) and natural *B. napus* (Higgins et al. 2018). The B genome was the least affected by fixed genomic rearrangements (Fig. 3), and all events observed in this genome were present only in the NCJ allohexaploid types. A possible explanation of why the B genome was more affected in the NCJ lines compared to the carirapa lines is the different method used to generate these hybrid types. The NCJ lines were produced using a two-step crossing method, where initially, *B. napus* was crossed to *B. carinata* to form hybrids with genome compositions of 2n = CCAB = 36 (Mason et al. 2012). In these hybrids, the C genomes are present as homologous chromosomes, but the A and B genomes are haploid and occasionally pair nonhomologously with each other or with the C genome (Mason et al. 2010). As well, the preferential loss of the B genome over the A and C genome has been observed in other allohexaploid hybrids (Zhou et al. 2016). In the F<sub>1</sub> hybrids, we observed very few rearrangements involving the B genome. In the F<sub>1</sub> hybrid parent of population 4 (Supplementary Fig. 2a), there were two events involving the introgression of B fragments into the C genome, with a carirapa origin. These events were already present as segregating events in the carirapa parent. Based on homology between the *Brassica* genomes (Perumal et al. 2020), we identified the most likely position for these introgressions in the C genome. These events were very rare in all of the hexaploids analyzed, but may offer potential for introgression of chromosome fragments from the B genome carrying

useful agronomic traits into the A or C genome for *Brassica napus* (rapeseed) crop improvement.

In the different F<sub>1</sub> hybrids analyzed, we observed *de novo* rearrangements involving different chromosomes depending on the population. In some cases, these *de novo* rearrangements occurred close to regions where rearrangements were observed in the parental plants. As an example, in population 8, we observed an increase in the size of the deletion at the top of chromosome C03. This deletion was already present in the carirapa parent, but had a size of ~6.6 Mb, compared to 16.3 Mb in the F<sub>1</sub> hybrid (Supplementary Fig. 5). This suggests an increase in size for some of the rearrangements, although it was not the rule, as other translocations were directly inherited from the parent without size change. However, already-present translocations may lead to additional irregular pairing during meiosis, as recombinant chromosomes enforce close proximity between homologous pairs in translocation heterozygotes, facilitating the production of additional crossover events (Udall et al. 2005), or it might be that those regions in general are more prone to recombine than others (De Muyt et al. 2009). Mwathi et al. (2019) also observed this effect of preexisting translocations in doubled-haploid NCJ allohexaploid lines, but due to the extreme instability of this population, *de novo* translocation events were found to be much more common (2.2 events per plant on average) than events triggered by preexisting rearrangements (0.8 events per plant on average). In our case, we observed on average 10 *de novo* rearrangements in the F<sub>1</sub> hybrids, and out of these, 2.8 events on average were proximal to or co-located with a preexisting event. The difference observed between the two experiments might be attributed to the fact that, in our case, the analyzed plants underwent more rounds of meiosis (H3–H7) compared to just two rounds for the Mwathi lines (H<sub>2</sub>) (Mwathi et al. 2019).

Although no fixed pattern of selection was observed across all the allohexaploid genotypes, several events notably seemed to occur in multiple independent lineages or to be preferentially selected for. This was observed in 3 plants belonging to the N1C2.J1 lineage, where the beginning of chromosome A01 was doubled and putatively translocated into C01 (Fig. 3). The region translocated between A01/C01 has a length of ~2 Mb, and contains the meiosis gene *Cell Division Cycle 20* (*CDC20*). The gene *CDC20* has been found to be crucial in *Arabidopsis thaliana* meiosis, although it might have a different function in *Brassica* (Niu et al. 2015).

The end of chromosome A04 was deleted (~1.1–2.5 Mb missing) in seven plants belonging to three independent lineages (two carirapa and one NCJ, Fig. 3). In the case of the NCJ plants, the deletion of this region was already present in the parental *B. napus* cv. “Surpass400\_024DH” and in the progenitor line “surpass” (Higgins et al. 2018). No doubling in the corresponding homologous region in C04

was observed for any of these lineages. Although no known meiosis genes fall in this region, the meiosis gene *ASYNAPTIC4* (*ASY4*) is located 2 Mb upstream of the deleted region of A04. In *Arabidopsis thaliana*, this protein is necessary to complete synapsis, cross-over formation, and normal localization of interacting proteins ASY1 and ASY3 (Chambon et al. 2018). Interestingly, the homozygous presence of an *ASY3* allele with a tandem duplication region has been associated with more stable autotetraploid *Arabidopsis lyrata* lines, as it causes a reduction in multivalent formation (Seear et al. 2020). The homologous region in C04 has recently been associated with fertility in synthetic *B. napus*, where a deletion of the last 1.5 Mb of chromosome C04 reduced seed number (Ferreira de Carvalho et al. 2021). However, this deletion was not associated with a reduction in the number of nonhomologous recombination events (Ferreira de Carvalho et al. 2021), suggesting that this effect may only be related to selection for fertility and not to increased stabilization of the genome. The top of chromosome C03 (~1.2–2.3 Mb) was also missing in four plants from the same carirapa lineage (C13, Fig. 3), most likely as the result of an event fixed very early on in this lineage, although the four plants differed slightly in the size of the deletion. This A03–C03 region has previously been identified in hexaploid lines as a translocation QTL associated with total seed number in NCJ hexaploids, where the top of chromosome C03 was replaced by a copy of A03 (Gaebelein et al. 2019). Within this region, we find a potential meiotic candidate gene called *BRCA2*. In *Arabidopsis*, the protein BRCA2 interacts in vitro with DMC1 (Dray et al. 2006) and plays a crucial role in homologous recombination (Seeliger et al. 2012).

Five populations from different crosses were analyzed for bias in allele segregation and chromosome inheritance. The B genome was least affected overall, although it was preferentially lost if present as single chromosomes (B05 and B07 in populations 4, 5, and 6). A similar case was observed for the single C09 chromosome (population 5, Supplementary Fig. 3a), which was also present less often than expected in one test-cross population. However, this was not an effect seen for all chromosomes present in a single copy: the majority (A03, A04, A05, C01, C02, C06, C08, and C09 in the different populations) were inherited as expected in the progeny (50%). Interestingly, in population 6, there was segregation distortion towards retention of chromosome A09 from the carirapa allohexaploid parent, although both chromosomes were present in the hybrid (Supplementary Fig. 4b). This chromosome inherited from the carirapa parent had putative translocations between chromosomes A09/C09 and A09/C08. Additionally, the chromosome A09 from carirapa had a translocated C09 region of 1.7 Mb, containing the meiosis gene *X-ray induced 1* (*XRI1*). This gene has been shown to be involved in meiosis and post-meiotic stages of male gametes development in plants (Dean et al. 2009). In



this F<sub>1</sub> hybrid, we also just had one C08 chromosome (NCJ), and potentially the retention of an extra A09 occurred as a trisomy compensation of the missing homologous region. Compensatory trisomy of chromosomes has been previously described in resynthesized *B. napus* (e.g., trisomy of C05 chromosome and single copy A05) (Xiong et al. 2011). The gain of extra chromosomes to compensate the loss of a homologous chromosome can help to prevent further chromosomal instability by providing a partner for the single chromosome (e.g., monosomic-trisomic substitutions) and by preventing the deleterious effects of aneuploidy and changes in gene balance (Xiong et al. 2011).

In the test-cross populations 5 and 6, the end of chromosome A08 had an allele segregation distortion toward the allele inherited from the carirapa parent (Supplementary Fig. 3b and 4b). This region was ~ 1.3 Mb in size and contained the meiosis genes *ADA2b* and *RAD51D*. Both populations shared the same carirapa and NCJ genotype combination (C13 and N6C2.J2). In rice, *RAD51D* has been shown to be associated with the prevention of non-homologous recombination during meiosis (Zhang et al. 2020). The preferential inheritance of an allele putatively related to increased meiotic stability from *B. rapa* over an allele from *B. napus* is unexpected, as *B. napus* is an established allopolyploid species with good meiotic control. However, at least one *B. rapa* genotype has previously been identified to contain allelic variation relevant for meiotic stability in allohexaploids with *B. carinata* (Gupta et al. 2016). Further confirmation is needed to support this observation.

In test-cross population 4, allele inheritance distortion was observed at the end of A07, where alleles from the carirapa parent were preferentially inherited over alleles from the NCJ allohexaploids (Supplementary Fig. 2b). The region has a size of ~ 5.6 Mb and has also been previously associated with total seed number in an NCJ population (Gaebelein et al. 2019), although it is hard to point to a meiosis candidate gene as several can be found within this interval.

## Conclusions

Our results show that *Brassica carirapa* and NCJ allohexaploids are highly tolerant of chromosome duplication and deletion events. No reproducible patterns of karyotype change were observed, although certain rearrangement events were present in more than one genotype. At the same time, no strong evidence of subgenome dominance was observed, although fewer rearrangements were found in the B subgenomes, as expected due to the lower degree of homology between the B compared to the A/C subgenomes (Perumal et al. 2020). Although genomic stability has not yet been achieved, the new allohexaploid material could

be intercrossed between lineages without major effects on meiosis. This suggests the opportunity to use this material in rapeseed breeding programs, with special interest in the different translocations observed. At the same time, intercrossing between lineages offers great potential to increase genetic diversity in our material and may in future lead to allelic combinations that might be selected for to provide meiotic stability and fertility in a new *Brassica* allohexaploid crop.

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**Author contribution** DQM carried out the experimental analyses, prepared the results figures and tables, and drafted the manuscript. ASM and JZ conceptualized the experiment. JZ and JM generated the experimental material. JZ managed all plants in the field and glasshouse, including selection, crossing and self-pollination, and DNA extraction. WSZ managed the crossing, self-pollination, harvest in the field, and DNA extraction. ASM supervised DQM, ASM, JB, and JZ revised the manuscript. JB managed the generation of SNP array data for the experimental population.

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**Data availability** All data associated with this manuscript is available in the Supplementary Data files.

## Declarations

**Competing interests** The authors declare no competing interests.

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