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ORIGINAL ARTICLE

Segregation for fertility and meiotic stability in novel *Brassica* allohexaploids

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Abstract

Key message **Allohexaploid *Brassica* populations reveal ongoing segregation for fertility, while genotype influences fertility and meiotic stability**.

Abstract Creation of a new *Brassica* allohexaploid species is of interest for the development of a crop type with increased heterosis and adaptability. At present, no naturally occurring, meiotically stable *Brassica* allohexaploid exists, with little data available on chromosome behaviour and meiotic control in allohexaploid germplasm. In this study, 100 plants from the cross *B. carinata*×*B. rapa* (A2 allohexaploid population) and 69 plants from the cross (*B. napus*×*B. carinata*)×*B. juncea* (H2 allohexaploid population) were assessed for fertility and meiotic behaviour. Estimated pollen viability, self-pollinated seed set, number of seeds on the main shoot, number of pods on the main shoot, seeds per ten pods and plant height were measured for both the A2 and H2 populations and for a set of reference control cultivars. The H2 population had high segregation for pollen viability and meiotic stability, while the A2 population was characterised by low pollen fertility and a high level of chromosome loss. Both populations were taller, but had lower average fertility trait values than the control cultivar samples. The study also characterises fertility and meiotic chromosome behaviour in genotypes and progeny sets in heterozygous allotetraploid *Brassica* derived lines, and indicates that genotypes of the parents and H1 hybrids are affecting chromosome pairing and fertility phenotypes in the H2 population. The identification and characterisation of factors influencing stability in novel allohexaploid *Brassica* populations will assist in the development of this as a new crop species for food and agricultural benefit.

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Introduction

The genus *Brassica* is one of 51 genera in the tribe Brassiceae belonging to the crucifer family, and is the most economically important genus within this tribe (Rakow 2004). The *Brassica* oilseeds; *B. napus, B. rapa, B. juncea* and *B. carinata*, provide 12% of the world's supply of edible vegetable oil, placing them third behind palm and soybean (Zhang and Zou 2006). Vegetable *Brassica* species include *B. oleracea* and *B. rapa*, while the seeds of *B. nigra* and *B. juncea* are also used as a condiment and *B. rapa, B. juncea, B. carinata* and *B. napus* are used as oilseeds. *Brassica* species are rich in dietary fibre, vitamin C, and phytosterols, and contain beneficial anti-carcinogenic compounds. In addition, the use of *Brassica* species as renewable raw materials has attracted growing interest for the biofuel and chemical industries (Chen et al. 2011).

The *Brassica* genus is an interesting model for allopolyploid formation in agricultural crops, as six agriculturally significant species share a genomic and evolutionary relationship. The predecessors of the diploid species *B. rapa* (A genome, $2n=20$, Chinese cabbage and turnip), *B. nigra* (B genome, $2n=16$, black mustard) and *B. oleracea* (C genome, $2n=18$, cabbage, cauliflower, broccoli) are hypothesised to have given rise to the allotetraploid species *B. juncea* (A and B genome, $2n=36$, leaf mustard, Indian mustard), *B. napus* (A and C genome, $2n=36$, oilseed rape, canola) and *B. carinata* (B and C genome, $2n=34$, Ethiopian mustard) through pairwise hybridisation. This relationship is referred to as the *Brassica* U's triangle, and is an evolutionary example of allopolyploidy (Morinaga 1934; UN 1935).

Polyploidisation has played a major role in plant evolution and speciation, allowing adaptation over a wide ecological landscape and increased "hybrid vigour" relative to progenitor diploids (Ramsey and Schemske 1998; Leitch and Letch 2008; Song et al. 2012). For example, tetraploid cotton is a successful polypoid and is preferred in fibre production for its longer, stronger and finer fabric compared to its diploid relatives (Udall and Wendel 2006). Similarly, successfully developing an allohexaploid *Brassica* ($2n=$AABBCC) could benefit from the positive effects of polyploidisation over its allotetraploid relatives for agricultural benefit (Chen et al. 2011).

Previous attempts to create *Brassica* allohexaploids involved induction of somatic chromosome doubling of triploid ABC interspecific hybrids to form AABBCC allohexaploids, with *B. rapa* ($2n=$AA)$\times$*B. carinata* ($2n=$BBCC) being the most common cross combination (Iwasa 1964; Pradhan et al. 2010). The studies conducted mainly investigated the use of allohexaploid *Brassica* as a bridge to transfer useful traits, such as disease resistance, seed colour and cytoplasmic male sterility, into cultivated allotetraploids, and to resynthesize the allotetraploid species from their diploid progenitors; however, these lines were often characterised by chromosomal instability and poor seed set (Sjödin and Glimelius 1989; Arumugam et al. 1996; Meng et al. 1998; Li et al. 2004). Howard (1942) found that *Brassica* crosses from *B. rapa*$\times$*B. carinata* showed improved fertility over a few generations, whilst Iwasa (1964) found low fertility in hybrids up to the fifth generation using similar crosses. Recent studies with *Brassica* allohexaploids from different genotype combinations, including the *B. rapa* and *B. carinata* crosses, suggest increased fertility and stability may arise in subsequent generations (Tian et al. 2010; Zhou et al. 2016).

An alternate approach to create allohexaploid *Brassica* involves production of unreduced gametes, rather than somatic doubling, to increase the ploidy level (Mason et al. 2010). The allotetraploid U's Triangle species (UN 1935) can be crossed in a pairwise fashion to produce trigenomic hybrids with the unbalanced genome complements AABC, BBAC and CCAB. Hybrids were found to produce various frequencies of unreduced gametes (gametes with the somatic chromosome number; i.e. chromosome complements AABC, BBAC, and CCAB) which were hypothesised to be passed on to the next generation in a round of crossing with the third allotetraploid species to produce allohexaploid *Brassica* AABBCC. This approach was only partially successful; one near-allohexaploid hybrid was produced and characterised (Mason et al. 2012). In spite of the various attempts in creating an allohexaploid *Brassica* species, a completely stable allohexaploid *Brassica* (AABBCC genome $2n=54$) remains elusive, and fertility and meiotic stability over subsequent generations have only been examined in a few studies (e.g. Tian et al. 2010; Zhou et al. 2016).

Chromosome behaviour during meiosis must be under strict genetic regulation to facilitate correct segregation of chromosomes into daughter cells, which can be a challenge in polyploids which contain more than two pairs of chromosomes (Cifuentes et al. 2010b). A proper sorting-out mechanism for these chromosomes is, therefore, necessary to avoid illegitimate associations that would otherwise lead to aneuploidy. Information on factors influencing meiotic stability in allohexaploid *Brassica* remains sparse, and more studies regarding stability and fertility of hexaploids are necessary to help establish a stable and fertile allohexaploid species. In this study, fertility and meiotic stability were assessed in homozygous allohexaploid A2 progeny produced through the cross *B. rapa*$\times$*B. carinata*, and in heterozygous allohexaploid H2 progeny derived from different genotypes in the cross (*B. napus*$\times$*B. carinata*)$\times$*B. juncea*.

Materials and methods

Plant material generation

Interspecific hybridisation between *B. carinata* (Indian line PGR 16789)$\times$*B. rapa* (*PAK* 85835) was performed by hand pollination in Punjab Agricultural University (PAU), Ludhiana, India. From a total of 308 buds pollinated, 42 pods and 15 putative hybrid seeds were obtained, resulting in seven viable triploid F_1 plants. Chromosome doubling to induce polyploidy was done using 0.2% colchicine in 1% DMSO on the axillary meristem at the four leaf stage, and three allohexaploid A1 generation plants obtained. The A1 lines were confirmed to have $2n=27$ chromosome complements. These allohexaploid plants were then self-pollinated to produce A2 seeds.

The *Brassica* allohexaploid (H1) population was produced by the cross (*B. napus*×*B. carinata*)×*B. juncea* at The University of Queensland, Brisbane following procedures outlined in Mason et al. (2012). The *B. juncea* parent genotypes were "JN9-04", a self-pollinated single plant selection by Janet Wroth (The University of Western Australia, Perth, Australia) from near canola quality *B. juncea* line "JN9" supplied by Wayne Burton (Department of Primary Industries, Horsham, Victoria, Australia), and inbred line "Purple Leaf Mustard" (donated by Huazhong Agricultural University, Wuhan, China). The *B. napus* parent genotypes were Australian canola lines "Boomer" (doubled-haploid (DH); Canola Breeders Western Australia), Surpass400_024DH (Canola Breeders Western Australia), "Lynx_037DH" (Canola Breeders Western Australia) and "Ag-Spectrum" (sourced from the Australian Grains Gene bank (AGG)), while the *B. carinata* genotypes were DH selections from Ethiopian lines "94024" and "1923" (sourced from the AGG).

A total of 146 H2 seeds from 12 H1 plants (described in Mason et al. 2016) were planted under field conditions at PAU India, of which 69 H2 plants germinated and were characterised. The genotype combinations in the H2 population (Table S1) are hereafter referred to as "G1", "G2", "G3" and "G4". Phenotype data for the parent genotypes were not available; however, a ten-plant average for fertility and phenotypic data was collected and available for comparison of the *B. carinata* "PC5", *B. rapa* "TL-17", *B. juncea* "RLC-1" and *B. napus* "GSC-5" genotypes as control cultivar samples. The control species were all commercial cultivar varieties.

Phenotypic characterisation

Phenotypic data for the H2 allohexaploid population (69 plants) and the A2 allohexaploid population (100 plants) were collected in the *Brassica* fields at PAU between April and May 2015. Data collected included plant height (cm), number of pods on main shoot, number of seeds on main shoot, total seed set and the seeds per 10 pods.

Pollen fertility and seed set

Ripe floral buds (near opening) were collected from *Brassica* genotypes grown at PAU, Ludhiana, India. Pollen studies were conducted with squashed anthers in 1% acetocarmine on glass microscope slides and observed under a compound light microscope. Plump, darkly stained pollen was assumed to be viable, while unstained and/or shrivelled pollen were considered as unviable. A total of 300 pollen grains were scored for each sample in both allohexaploid progenies. Fertility and phenotypic data were recorded for reference control cultivars, A2 and H2 allohexaploid progenies. Self-pollination was encouraged by enclosing racemes in bags.

Meiotic chromosome observations

Floral buds were fixed in Carnoy's II solution (ethanol:chloroform:acetic acid 6:3:1) for 72 h and stored in 70% ethanol at 4 °C. Anthers were squashed and stained in a drop of 1% acetic acid carmine solution on glass microscope slides. Sixty-nine plants from the H2 allohexaploid population and 100 plants from the A2 allohexaploid population were assessed for chromosome number. Five to twenty pollen mother cells (mode ten) were observed for each plant. Observations of the pollen mother cells (PMCs) were performed at metaphase I, and anaphase I stages and images captured using Cytovision 4.2 software Leica Biosystems. Chromosome meiotic images were observed using an Olympus BX 61 compound bright field microscope using Cytovision software. Univalent, bivalent and multivalent associations were assessed.

Data analysis

ANOVA analysis, Tukey's Honest Significant Differences test, Pearson's product moment correlations and boxplots were carried out using R version 3.2.2 (The R Foundation for Statistical Computing 2015). The (aov), (summary) R commands were run to determine Analysis of Variance (ANOVA) for the means of meiotic characteristics, chromosome numbers, fertility and plant traits per genotype and progeny sets. Tukey's Honest Significant Differences test (Tukey HSD) was used to establish significant differences between genotypes and progeny sets for each trait.

Results

Chromosome numbers and meiotic behaviour in the A2 and H2 populations

In the A2 population, the number of univalents (unpaired chromosomes) at metaphase I averaged 3 per pollen mother cell (PMC), ranging from 1 to 7 chromosomes, while the average number of bivalents (chromosome pairs) was 19 with a range of 12–27 chromosomes (Fig. 1a–c). The mean meiotic configuration was 2I + 19II with an average chromosome number estimate of 41 chromosomes (Fig. 2a). In the H2 population, the average number of univalents (unpaired chromosomes) was two per PMC with a range of 0–2, while the average number of bivalents was 24 chromosomes (Fig. 1d–f). The mean meiotic configuration was 2I + 24II with an average estimate of 49 chromosomes (Fig. 2b).

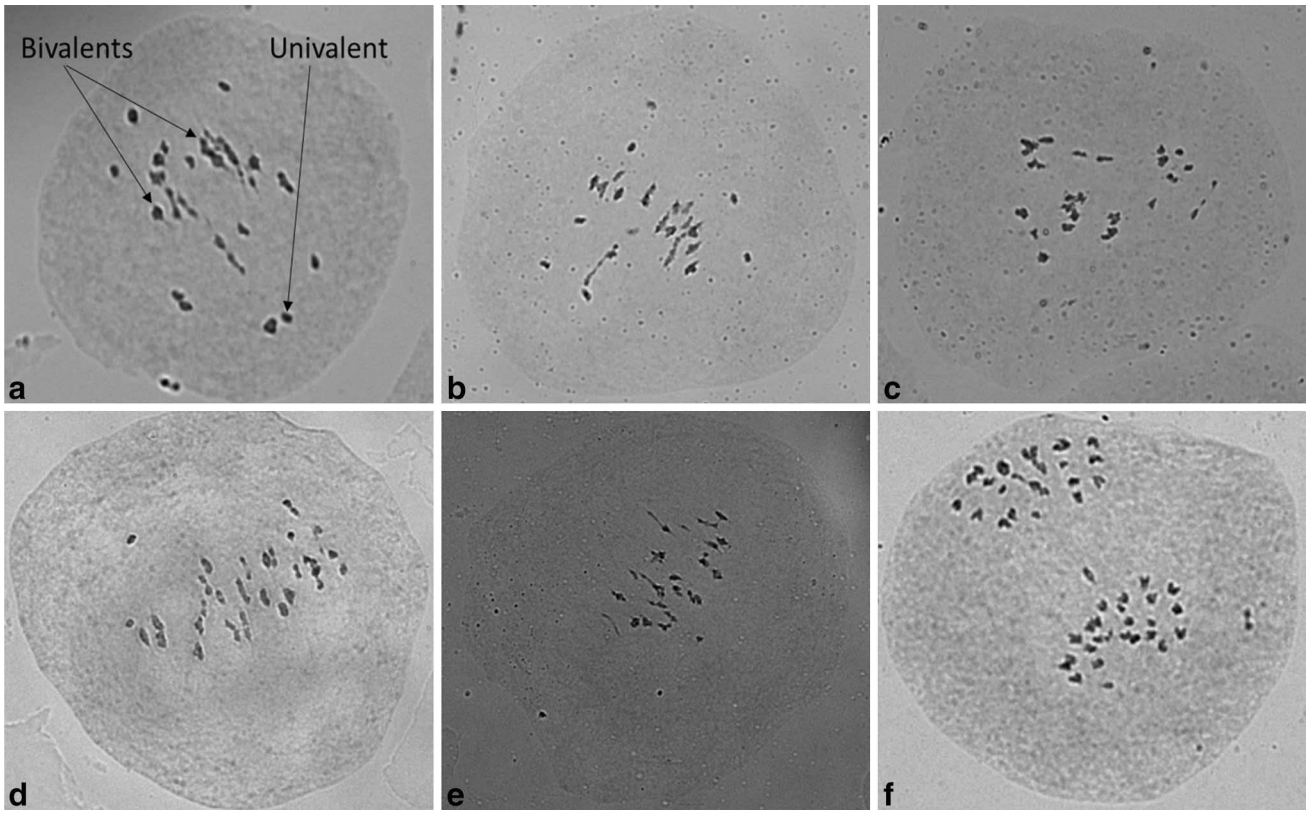

Fig. 1 Meiotic configurations in the A2 population (*B. rapa*×*B. carinata* allohexaploids), **a** 23II, 1I at metaphase-I; **b** 18II, 7I at metaphase-I; **c** 25II, 2I at metaphase I; and in the H2 population (*B. napus*×*B. carinata*×*B. juncea* allohexaploids), **d** 24II, 2I at metaphase I; **e** 25II, 1I at metaphase I, **f** 24I, 26I at anaphase 1. Magnification using ×100 objective lens

Fertility estimates and plant height in A2 and H2 population

Pollen fertility (%) in *B. carinata, B. juncea* and *B. napus* control cultivars was all >90%. Figure 3 shows infertile and fertile pollen grains in H2 population. Pollen fertility in the A2 population was lower compared to the control cultivars, averaging 13% with a range of 0–63% (Fig. 4a), while average pollen fertility in the H2 population was 64% with a range of 1–94% (Fig. 4b). Three lines in the H2 population were in the same range as the controls for pollen fertility.

The *B. carinata* and *B. rapa* control cultivars had an average of 38 and 45 pods on the main shoot, respectively, which was lower than the average of 52 pods with a range of 14–89 pods observed in the A2 population (Fig. S1A). The number of seeds on the main shoot in *B. carinata* and *B. rapa* controls was 342 and 497 seeds, respectively, which was higher than the average of 199 seeds with a range of 12–482 seeds in the A2 population. The most fertile plants exceeded the number of seeds produced by the *B. carinata* control cultivar and showed similar seed numbers to the *B. rapa* control cultivar (Fig. S1B). The number of seeds per 10 pods was 90 and 110 seeds in *B. carinata* and *B. rapa* controls, respectively, which was higher than the average of 37, with a range of 5–65 seeds, observed in the A2 population (Fig. S1C). Finally, the plant height of the *B. carinata* and *B. rapa* control was 130 and 150 cm, respectively, both control cultivars being shorter than the average of 240 cm with a range of 185–291 cm in the A2 population (Fig. S1D).

The *B. carinata, B. napus* and *B. juncea* control cultivars for the H2 population had an average of 38, 54 and 43 pods on the main shoot, respectively, while the H2 population had an average of 41 pods and a range of 19–62 pods. The average number of pods was within the range of the control cultivars, while ten of the plants in the population possessed a higher number of pods than the highest *B. napus* parent (Fig. S2A). The controls *B. carinata, B. napus* and *B. juncea* had 342, 702 and 475 seeds on the main shoot respectively, which were all higher than the average of 105 seeds in the H2 population (range of 10–450 seeds) (Fig. S2B). The average number of seeds per 10 pods in the respective *B. carinata, B. napus* and *B. juncea* controls was 90, 130 and 95 seeds, respectively, compared to an average of 24 seeds per 10 pods and a range of 2–73 seeds in the H2 population (Fig S2C). Finally, the average plant height

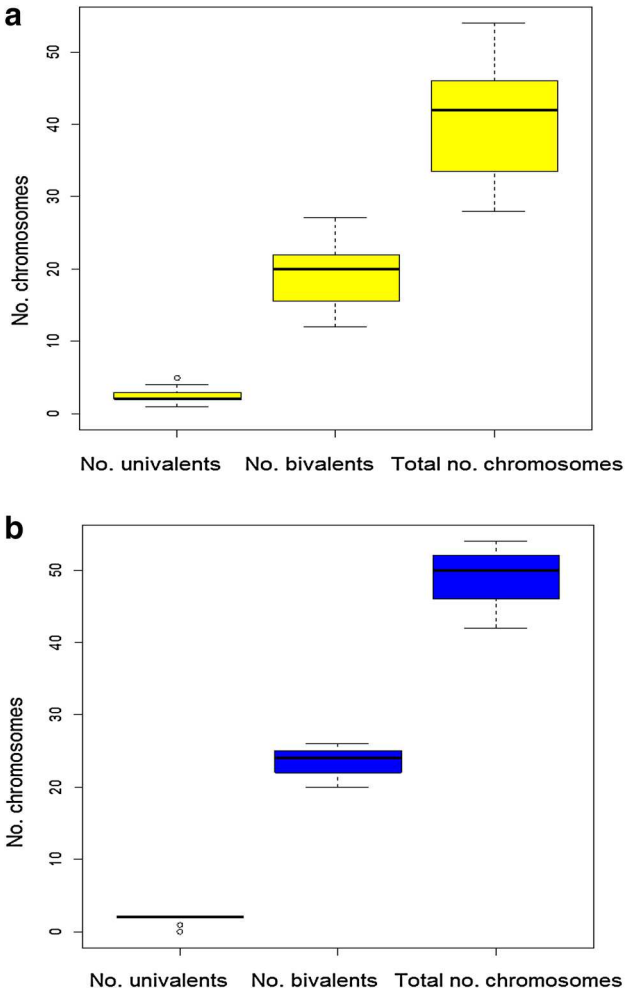

Fig. 2 Chromosome number and average meiotic behaviour in **a** an allohexaploid population (A2) derived from crosses between *B. rapa* and *B. carinata* and **b** an allohexaploid population (H2) derived from the cross (*B. napus*×*B. carinata*)×*B. juncea*

of the *B. carinata, B. napus* and *B. juncea* controls was 130, 198 and 138 cm, respectively, compared to an average height in the H2 population of 190 cm, with a range of 100–248 cm (Fig. S2D).

Correlations between chromosome numbers, meiotic behaviour and fertility traits in the A2 and H2 populations

A2 allohexaploid progeny showed a significant positive Pearson's product-moment correlation of $r=0.82$ between the number of seeds on the main shoot and seeds per 10 pods (linear regression p value <0.0001, adjusted $R^2=0.68$). No other traits were significantly correlated in this population (Fig. S3A). Correlation between various fertility traits and plant height in the H2 allohexaploid progeny showed that the number of seeds on the main shoot

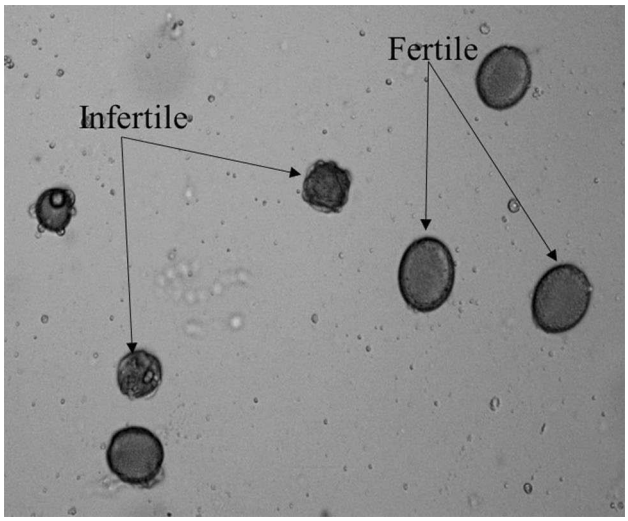

Fig. 3 Pollen fertility image showing fertile and infertile pollen grains in the H2 allohexaploid population derived from the cross (*B. napus*×*B. carinata*)×*B. juncea*

and number of seeds per 10 pods were significantly positively correlated with a Pearson's product-moment correlation of $r=0.94$ (linear regression p value <0.0001, adjusted $R^2=0.8812$). No other traits were significantly correlated (Fig. S3B).

Analysis of variance (ANOVA) for genotypes and progeny set in the H2 allohexaploid population

There were no differences observed between genotypes in the number of univalents per PMC between genotypes in the H2 population. However, significant differences were found between genotypes in the mean number of bivalents ($p=0.000017$), total number of chromosomes ($p=0.00003$) and plant height ($p=0.000448$), (one-way ANOVA; Table S2). Post-hoc analysis using Tukey's Honest Significant Differences (HSD) test found significant differences ($p<0.05$) in means of bivalents between "G1" and "G2" and between "G2" and "G3" genotypes (Fig. 5a), while significant differences ($p<0.05$) in the mean number of chromosomes were found between the "G1" and "G2" genotypes and between the "G2" and "G3" genotypes (Fig. 5b). Significant differences ($p<0.05$) in means of plant height using Tukey's HSD test were found only between the "G1" and "G2" genotypes (Fig. S4). The "G4" genotype had only a single plant and was, therefore, omitted in this analysis. Bonferroni correction for multiple testing was carried out using $\alpha=0.05$: $p<0.0062$.

Analysis in the H2 allohexaploid population (H1-006, H1-014, H1-015, H1-016, H1-020, H1-022, H1-023, H1-040, H1-044, H1-052, H1-058) found significant differences between progeny sets and the mean number

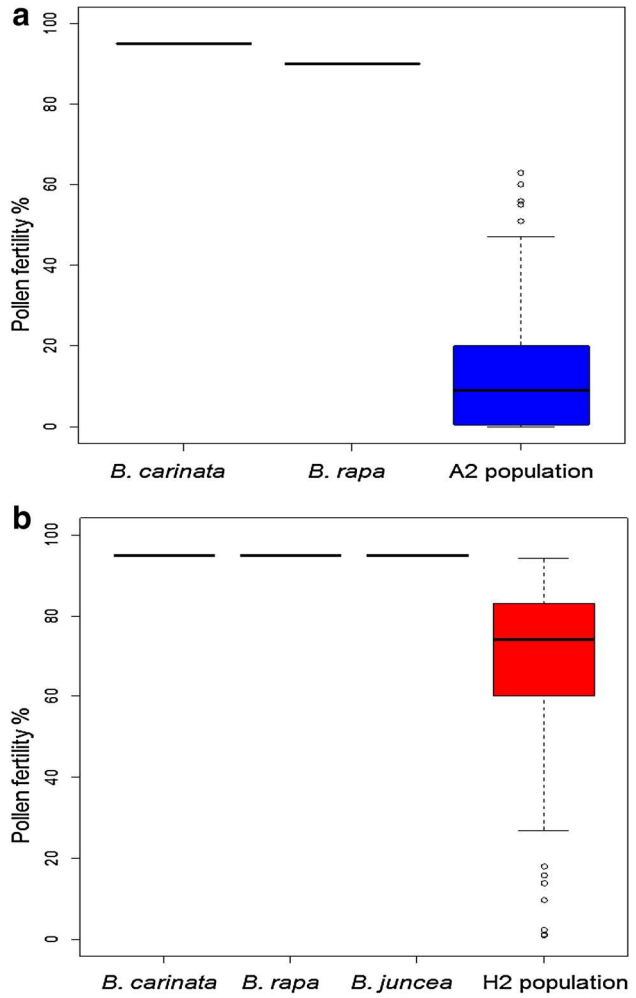

Fig. 4 Fertility traits in **a** an allohexaploid population (A2) derived from crosses between *B. rapa* and *B. carinata* and in **b** an allohexaploid population (H2) derived from different genotypes of the cross (*B. napus*×*B. carinata*)×*B. juncea*, compared against control cultivar samples *B. carinata* (PC5) and *B. rapa* (TL-17) genotypes in the A2 population and *B. carinata* (PC5), *B. juncea* (RLC-1*)* and *B. napus* (GSC-5) genotypes in the H2 population

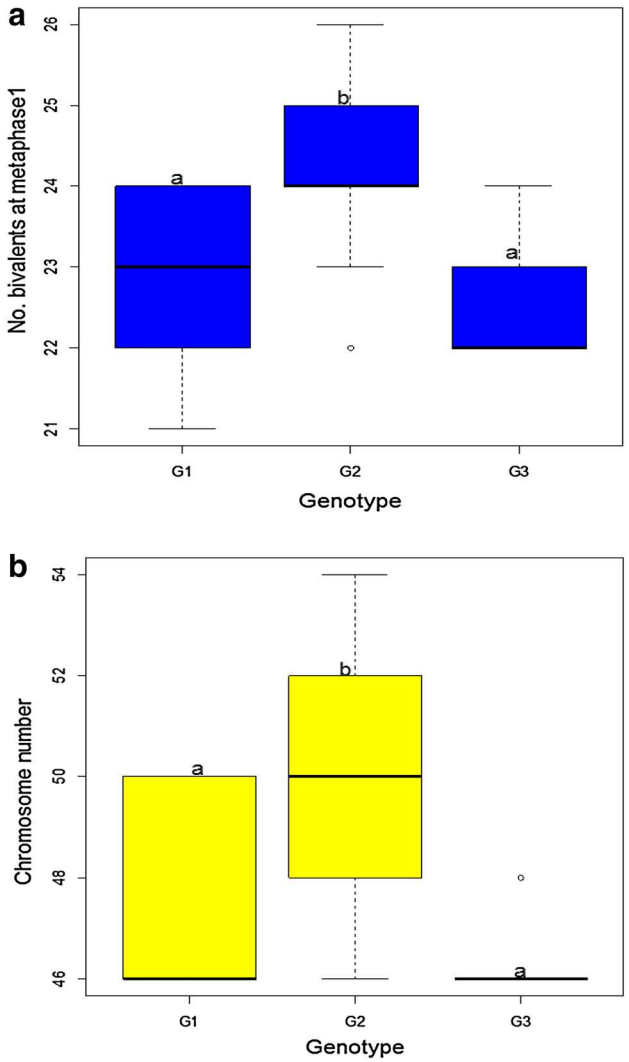

Fig. 5 The number of **a** bivalents at metaphase I of meiosis and **b** chromosome number in the H2 allohexaploid population derived from the cross (*B. napus*×*B. carinata*)×*B. juncea*, showing significant differences between genotypes ($p<0.05$, one-way ANOVA), ($p<0.05$, *a, b* Tukey's HSD)

of bivalents ($p=0.000375$), total number of chromosomes ($p=0.000678$), plant height ($p=0.000004$), pollen fertility ($p=0.00003$) and total seed set ($p=0.011$), (one-way ANOVA; Table S2). Post-hoc analysis using Tukey's HSD test also found significant differences ($p<0.05$) between mean chromosome numbers in 60% of sib lines within progeny sets in "G1" and "G2" genotypes and between 20% of sib lines within progeny sets in "G2" and "G3" genotypes (Fig. 6). Significant differences ($p<0.05$) were found in the mean number of bivalents at metaphase 1 in 30% of sib lines within progeny sets in "G1" and "G2" genotypes and between 60% of sib lines within progeny sets in "G2" and "G3" genotypes (Fig. 7). Additionally, significant differences ($p<0.05$) were found in mean pollen fertility % between 20% of sib lines within progeny sets in "G1" and "G2" genotypes, between 16% of sib lines within progeny sets in "G2" and "G3" genotypes and between 33% of sib lines within progeny sets in "G2" genotype (Fig. 8). Significant differences ($p<0.05$) were also found in total seed numbers between 3% of sib lines within progeny sets in "G1" and "G2" genotypes, between 16% of sib lines within progeny sets in "G2" and "G3" genotypes and between 13% of sib lines within progeny sets in "G2" genotype (Fig. 9). Finally, significant differences ($p<0.05$) were found in mean plant height in 40% of sib lines within progeny sets in "G1" and "G2" genotypes, between 66% of sib lines in progeny sets in "G1" and "G3" genotypes and between 50% of sib lines within progeny sets in "G2" and "G3"

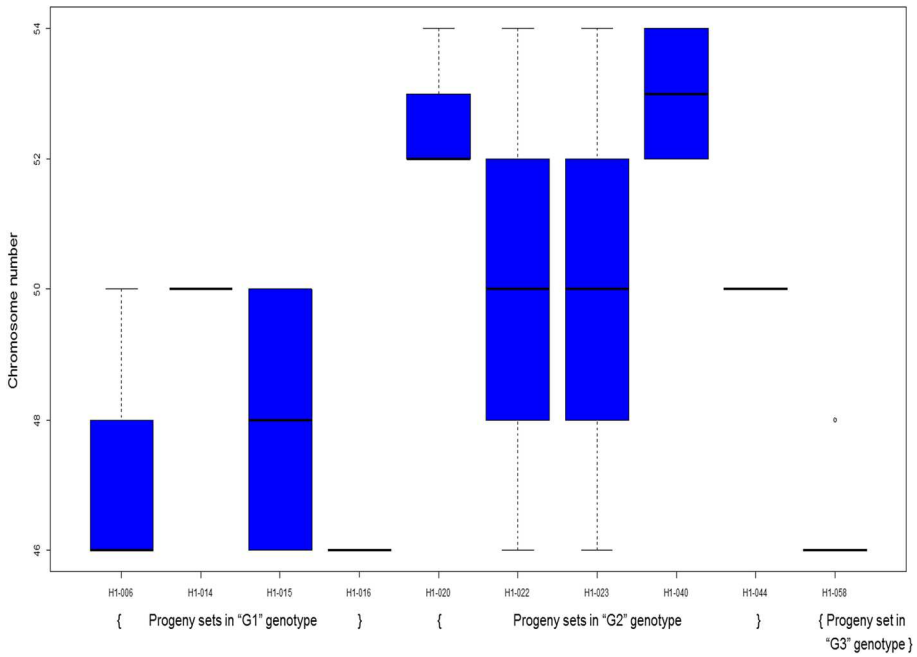

Fig. 6 Progeny sets in the H2 allohexaploid population derived from the cross (*B. napus* × *B. carinata*) × *B. juncea*, showing significant differences in chromosome number ($p < 0.05$, between progeny sets in "G1" and "G2" genotypes and between progeny sets in "G2" and "G3" genotypes, Tukey's HSD)

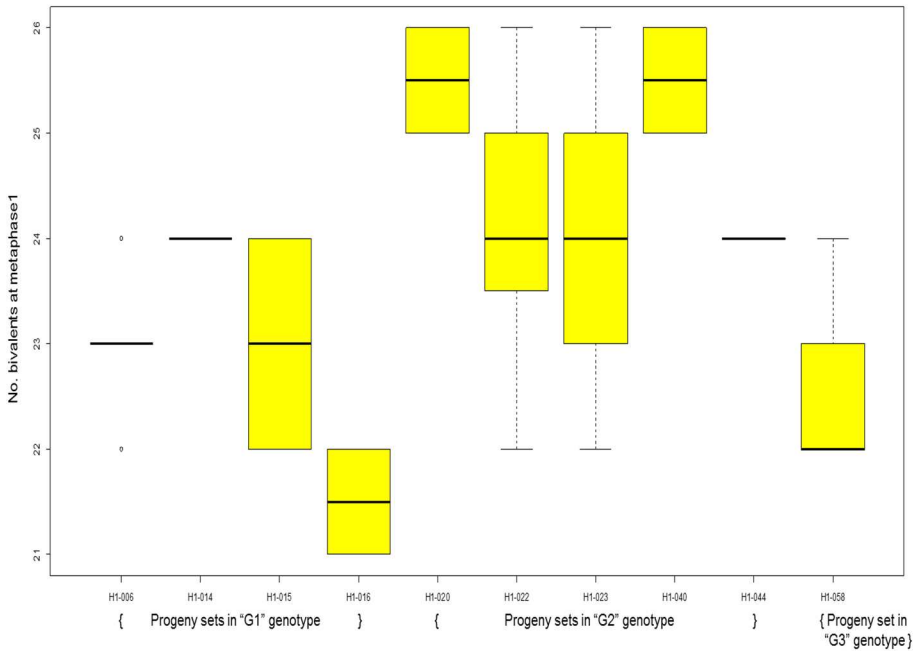

Fig. 7 Progeny sets in the H2 allohexaploid population derived from the cross (*B. napus* × *B. carinata*) × *B. juncea*, showing significant differences in number of bivalents at metaphase 1 ($p < 0.05$, between progeny sets in "G1" and "G2" genotypes and between progeny sets in "G2" and "G3" genotypes, Tukey's HSD)

genotypes (Fig. S5). Bonferroni correction for multiple testing was carried out at $\alpha = 0.05$: $p < 0.00278$.

Discussion

The purpose of this study was to analyse fertility and meiotic stability in novel *Brassica* allohexaploids and to determine which factors were influencing these traits. Pollen fertility, chromosome behaviour and phenotypic traits were characterised in a homozygous allohexaploid A2 population and in a heterozygous H2 population, and the traits compared to control species cultivars. The control cultivars used in this study were commercial cultivars and different from the parent genotypes used to generate the A2 and H2 populations; controls were used to make inferences. Both the A2 and H2 populations had lower average pollen viability and seed set than the control plants. This may be attributed to the fact that the hexaploid populations are still segregating for fertility and

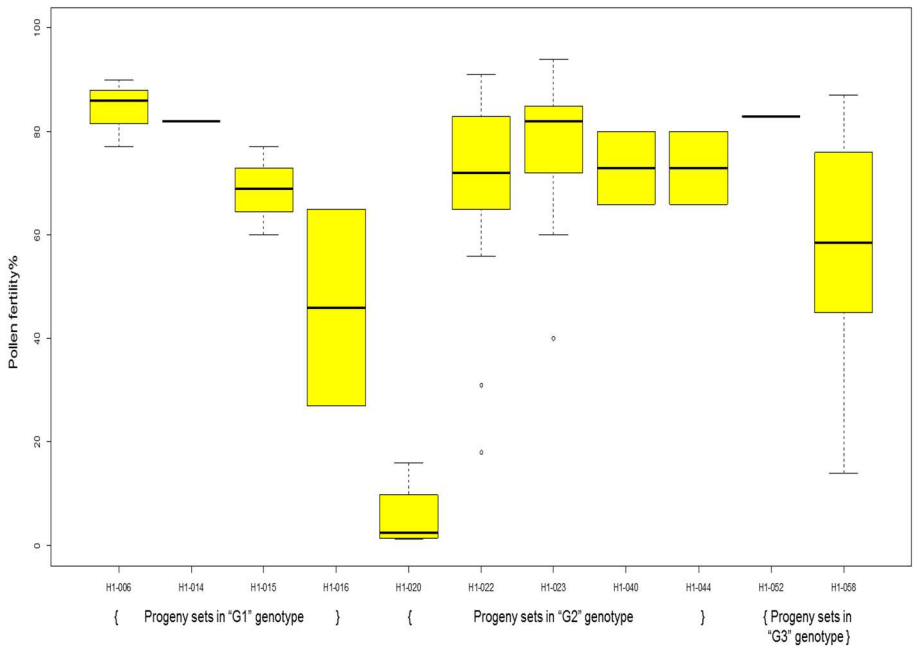

Fig. 8 Progeny sets in the H2 allohexaploid population derived from the cross (*B. napus* × *B. carinata*) × *B. juncea*, showing significant differences in pollen fertility %, ($p < 0.05$, between progeny sets in "G1" and "G2", between progeny sets in "G2" and "G3" genotypes and between progeny sets in "G2" genotype, Tukey's HSD)

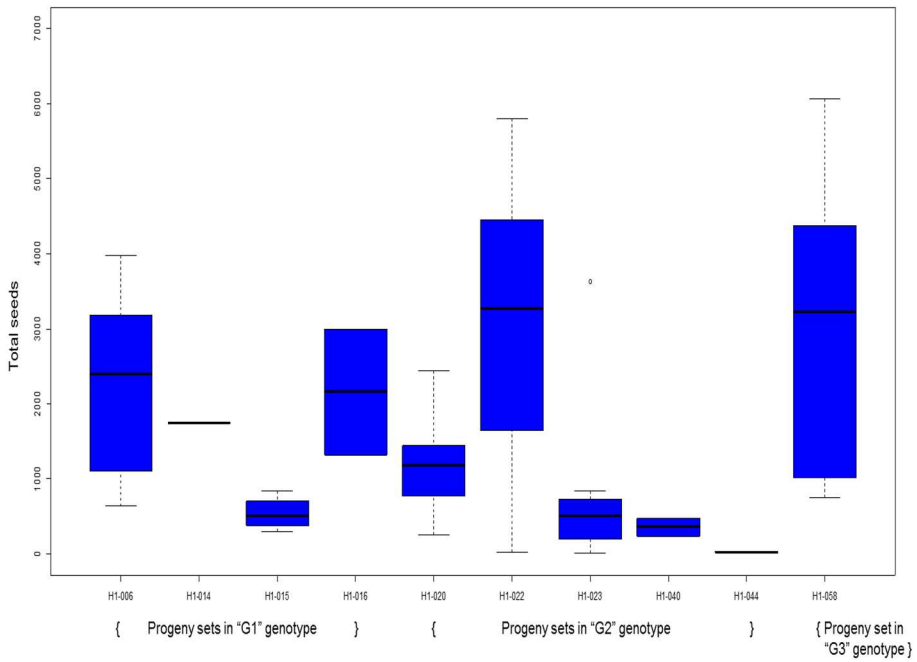

Fig. 9 Progeny sets in the H2 allohexaploid population derived from the cross (*B. napus* × *B. carinata*) × *B. juncea*, showing significant differences in total seed set ($p < 0.05$, between progeny sets in "G1" and "G2" genotypes, between progeny sets in "G2" and "G3" genotypes and between progeny sets in "G2" genotype, Tukey's HSD)

meiotic stability. However, some individuals in these populations were within or in close range to the control samples for some traits. The number of seeds on the main shoot in the H2 was much lower than the control cultivar samples, which may possibly be attributed to the more "wild-type" branching habits of the *B. carinata* and *B. juncea* genotypes used to generate the H2 population. Average plant height in both the A2 and H2 populations was higher than in the respective controls samples, suggestive of heterosis for growth traits.

In this study, the H2 population displayed a wide segregation range for fertility traits and an average meiotic configuration of 49 chromosomes. The H2 population was generated from heterozygous "F_1" parents with alleles from each of *B. juncea, B. napus* and *B. carinata* (approximately $A^jA^nB^j$ $B^cC^{n/c}C^{n/c}$; Mason et al. 2012). Thus, we would expect variation for meiotic stability and fertility as a result of allelic segregation, because the initial hybrid was heterozygous. It is also known that natural *B. napus* must have some genetic factor/s preventing (most) non-homologous

chromosome pairing that must be absent in most genotypes of *B. rapa* and *B. oleracea,* as all synthetic *B. napus* identified to date is unstable (Song et al. 1995; Gaeta et al. 2007; Szadkowski et al. 2010). The same locus could act in allohexaploid *Brassica* that have *B. napus* parents to keep the A and C genomes from pairing.

Some variation was observed in the A2 population, which is predicted to be due to non-homologous chromosome pairing and segregation during meiosis in the homozygous A1 plants, as normal homologous pairing would not lead to allelic segregation in homozygotes. Variation observed between individuals in the H2 population could be due to either allelic segregation as a result of heterozygosity in the H1 parents, or also be due to non-homologous chromosome rearrangements. Low fertility and low average chromosome numbers in the A2 population could be due to chromosome loss via laggards or non-homologous chromosome recombination events. In some previous studies, a widespread preferential loss of C- genome chromosomes over A- genome chromosomes in allohexaploid lines was found (Ge et al. 2009; Zhou et al. 2016), while Mason et al. (2014a, b) found two lines had an excess of C genome chromosomes and loss of A genome chromosomes, suggesting that the "preferential loss" of C genome chromosomes, as stated above, could be a selection pressure effect, where loss of the C genome is better tolerated than loss of the A genome in maintaining plant viability and fertility. Evidence shows that close homoeology between the A and C genomes promotes frequent homoeologous exchanges during meiosis, which are likely to lead to instability (Nicolas et al. 2007, 2012). Gaeta and Pires (2010) propose that homoeologous interactions in alloployploids, such as resynthesized *B. napus*, can not only generate novel gene combinations and phenotypes, but also destabilize the karyotype leading to aberrant meiotic behaviour, reduced fertility and aneuploidy.

Brassica researchers have in the past created allohexaploids mainly to transfer useful traits from one species to another. An allohexaploid *Brassica* from a *B. carinata* × *B. rapa* cross with high fertility over a few generations was produced by Howard (1942), however, a later study with similar crosses found lower fertility in hybrids up to the fifth generation (Iwasa 1964). In more recent studies, Tian et al. (2010) produced similar *B. rapa* × *B. carinata* allohexaploids demonstrating an increased fertility and percentages of offspring with $2n = 54$ chromosome complements up to the fourth generation using different genotype combinations, while Zhou et al. (2016) synthesised *Brassica* allohexaploids from different crosses, and after several generations found high fertility and stable breeding behaviour in allohexaploids from *B. rapa* × *B. carinata* and *B. juncea* × *B. oleracea*, and lower fertility in allohexaploids from newly combined diploid genomes. Improved fertility in the A2 population may be achieved by using different parental crosses from different genetic backgrounds as a means of improving *B. rapa* × *B. carinata* variation for meiotic stability alleles (Tian et al. 2010).

Statistical analysis showed significant differences in cytological traits, such as chromosome number and bivalent formation, between sib lines within progeny sets as well as between genotypes in the H2 population. This implies that parent chromosome and allele complement, as well as starting parent genotype, is affecting fertility and meiotic stability in the H2 population. Although it was not possible to distinguish which particular parental-genotype combinations contributed to these differences in this study, previous work suggests *B. napus* is likely to harbour allelic variation for meiosis traits. Cifuentes et al. (2010a) found that two different meiotic phenotypes in *B. napus* for the *PrBn* allele came from different parent *B. oleracea* genotypes. In related studies, Sheidai et al. (2006) found cytogenetic variability in canola (*B. napus*) cultivars to be genotype specific. Differences in meiotic behaviour were found between three AABC (*B. juncea* × *B. napus*) genotypes (Mason et al. 2010), while variation between CCAB (*B. napus* × *B. carinata*) hybrid genotypes was found for homoelogous and homologous recombination frequencies and A-B, B-C and A-C pairing (Mason et al. 2011).

To date, limited data exists on meiotic behaviour in *Brassica* allohexaploids. Further cytogenetic analysis is required in subsequent populations of allohexaploids to identify particular genotypes exhibiting stable meiotic characteristics. Coupling this meiotic phenotyping with genotyping analysis in future may allow identification of underlying genetic mechanisms involved in meiotic stability, and bring researchers closer to making a stable allohexaploid *Brassica* for agricultural benefit.

Author contribution statement MM and MG conducted the meiotic and phenotypic assessments, MM analysed the data with input from AM and wrote the manuscript. MM, AM, SB, MG and JB revised the manuscript. AM, CA, SB and JB developed basic genetic resources and provided input into experimental design and AM, CA, JB and SB supervised MM and MG.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the study complies with the current laws of the country (India) in which they were performed.

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