International Journal of Agricultural and Applied Sciences, June 2024, 5(1): 6-10
https://www.agetds.com/ijaas
ISSN: 2582-8053
https://doi.org/10.52804/ijaas2024.512

Research Article

OPEN ACCESS

In *Brassica rapa* meiotic recombination; chromosome axis remodeling is critical

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(Received: 19/01/2024; Revised: 17/03/2024; Accepted: 29/04/2024; Published: 20/06/2024)

ABSTRACT
The primary mechanism by which breeders generate biodiversity is meiotic recombination, which permits genetic rearrangement at every generation. It facilitates the accumulation of advantageous alleles and eliminates harmful mutations. With the development of one to very rarely more than three crossovers that are not randomly distributed, this mechanism is highly regulated. To ensure correct parental chromosomal segregation and the creation of unique allelic combinations, meiotic recombination is essential. Finding the variables affecting the rate and distribution of meiotic crossings (COs) is crucial because this process is strictly regulated, especially for plant breeding initiatives. Nevertheless, few high-resolution recombination maps exist for most crops, including the *Brassica* genus, and little is known regarding sex differences and intraspecific variation. Here, using progenies of cross populations from the crossed F_1s population, we reveal fine-scale resolution recombination landscapes for two female and one male cross in *Brassica rapa*. Parents are from very different lines, which stands for higher quality and yield.
Keywords: *Brassica rapa*, crossover, meiosis, meiotic chromosome axis remodeling, meiotic recombination

INTRODUCTION

In sexually reproducing animals, meiosis is a specialised cell division that involves separate homologous chromosomal assortment and homologous recombination (HR) to reorganise the maternal and paternal genomes. DNA double-strand breaks (DSBs) catalysed by SPO11 are the first step in the production of HR (Neale *et al*., 2005; Pan *et al*., 2011).

Brassica species might have evolved with 4 or 5 rounds of polyploidisation, so all the *Brassicas* seem to descend from a paleohexaploid ancestor. The last polyploidisation event occurred less than 10.000 years ago, by the hybridisation of three diploid ancestors of *B. rapa* (AA, 2n=20), *B. nigra* (BB, 2n=16) and *B. oleracea* (CC, 2n=18) to produce three allotetraploid species [*B. juncea* (AABB, 2n=36), *B. napus* (AACC, 2n=38) and *B. carinata* (BBCC, 2n=34)]. *B. rapa* is known as turnip rape, field mustard, or bird's rape. Cultivated forms include turnip; napa cabbage, bomdong, bok choy, rapini and rapeseed oil. This oil has become the 3rd most important source of edible vegetable oils in the world. The ancestral *B. rapa* probably originated in the Hindu Kush area of Central Asia (4000-6000 years ago). *B. rapa* has different characteristics that allow for use as a good model for the study of meiosis in *Brassicas*: its genome is relatively small about 390Mb (compared to *B. oleracea* [~630Mb]), the plants start flowering around 6-8 weeks and with TILLING mutant lines available, it has self-incompatibility for pollination and the genetic diversity within strains.

Genetic variation generated through the process of meiotic homologous recombination (HR) underpins plant breeding and efforts to deliver the rapid improvements in crops that will be required to ensure Food Security into the foreseeable future. HR is initiated by the programmed formation of DNA double-strand breaks (DSBs) by the SPO11 complex. DSBs are processed by components of the HR pathway where they are repaired as crossovers (COs), which recombine the homologous parental chromosomes, or non-crossovers (NCOs), where only short stretches of DNA are exchanged. In plants, most DSBs (90%+) are repaired as NCOs (Mercier *et al*., 2015). This limits the genetic variation that is generated in each meiotic division. Moreover, the distribution of COs, notably in cereal crops, is localized to particular chromosomal regions.

The phenomenon of chiasmata (chiasmatypie) was first hypothesized by Weissman (1885) and observed by Janssens (1909 University of Leuven, Belgium). Chiasmata co-relation with COs was proposed by Wilson and Morgan (1920). Creighton and McClintock (1931, 1935) demonstrated it using discernible chromosome features (heterochromatic knobs) in maize. Darlington (1937) corroborated Janssens discovery.

Chiasmata are the physical manifestation of crossovers at Diakinesis and Metaphase I meiotic stages. The frequency and localization of chiasmata can be inferred by the bivalent configurations observed at the end of Metaphase I when the spindle creates the bipolar tension

needed to orientate the homologous chromosomes to opposite poles at Anaphase I. This tension united with the sister chromatid cohesion and the position of the chiasmata will produce different configurations (Sybenga, 1975). COs sites can be visualised as chiasmata at diakinesis (DK) and metaphase I (MI). Chiasmata seem to be localised preferentially in distal regions (close to telomeres) with very little in interstitial or proximal regions in *Arabidopsis*, *Brassica* and *Triticum* species. This is a serious constraint for plant breeders. In this work, we have analysed cytogenetically different aspects of *B. rapa* meiotic recombination to have a better insight into its control and how it could be manipulated.

MATERIALS AND METHODS

Cytology methods

Fluorescence in situ hybridization

With a few minor adjustments, cytological techniques were performed according to Armstrong *et al*. (2009) description. Using Texas Red and Atto488 (NT labelling kits, Jena Biosciences), 5S (pCT4.2; Campell *et al*., 1992) and 45S (pTa71; Gerlach and Bedbrook, 1979) rDNA fluorescence in situ hybridization (FISH) probes were tagged by nick translation. Five anthers per slide were used for chromosomal spreading in *B. rapa* for immunostaining; the material was disrupted with a brass rod during the first four minutes of the eight-minute digesting process in a moist chamber at 37 °C; and 1.5% lipsol was used for the spreading process.

Table 1. DNA isolation and sequencing

Material	Information
B. rapa r-o-18	Laboratory control / Fully Sequenced
B. rapa 26155	Quality oily traits
B. rapa 26156	Yield
B. rapa 26155 x 26156	F_1 hybrid combining both yield and quality

RESULTS AND DISCUSSION

Pollen mother cells from *B. rapa* r-o-18 were used to analyse the different stages of meiosis and produce a meiotic atlas.

The localization of the chiasmata was inferred from the different shapes of the bivalents at metaphase I (Sybenga, 1975). Distal chiasmata (d) were located close to the telomeres, and interstitial chiasmata (i) were located more internally along the length of the chromosome arm (two different regions were classified as i1 closer to the telomere and i2 closer to the centromere). Proximal chiasmata were located near centromeres but at *B. rapa* were never observed.

Thus, this metaphase I contains 10 bivalents, 5 rings and 5 rods with a total of 15 chiasmata. The localisation can be classified as 11 distal (d), 3 interstitial 1 (i1) and 1 interstitial 2 (i2). This higher number of distal chiasmata (crossover/recombination) is very similar to that observed in other plant species (Mercier *et al*., 2015). This distal crossover localization has serious consequences on the probability of introgression of genetic traits that might be located in interstitial and even proximal locations.

Fluorescence in situ hybridization using 45S and 5S rDNA probes was carried out to identify some chromosomes: A1 with 45S (green) and 5S (red) rDNA signals, A3 with also 45S and 5S signals but the 45S signal being smaller than in A1, A6 just with 45S rDNA signal and A10 just with 5S rDNA signal. Another bivalent with a 5S signal was observed that has not previously been identified (Waminal *et al*., 2016).

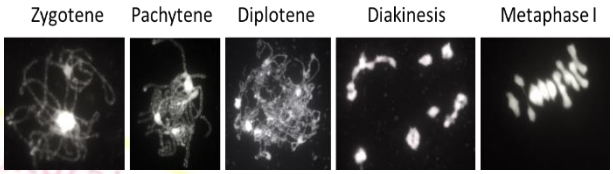

Fig 1. Different stages of meiosis at meiotic atlas

At metaphase we analysed the meiotic configurations observed, mainly ring and rod bivalents as in other species (Sybenga, 1975).

Fig 2. Meiotic configurations at the meiotic atlas

The localization of the chiasmata was inferred from the different shapes of the bivalents at metaphase I (Sybenga, 1975). Distal chiasmata (d) were located close to the telomeres, and interstitial chiasmata (i) were located more internally along the length of the chromosome arm (two different regions were classified as i1 closer to the telomere and i2 closer to the centromere). Proximal chiasmata were located near centromeres but at *B. rapa* were never observed.

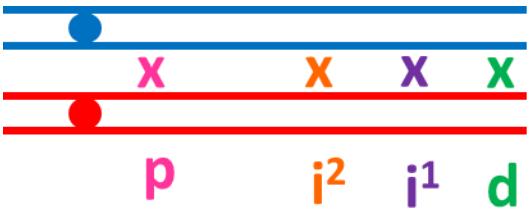

Fig 3. Chiasma frequency and localization analysis at meiotic atlas

This metaphase I cell is an example of the chiasma frequency and localization analysis done on *B. rapa*.

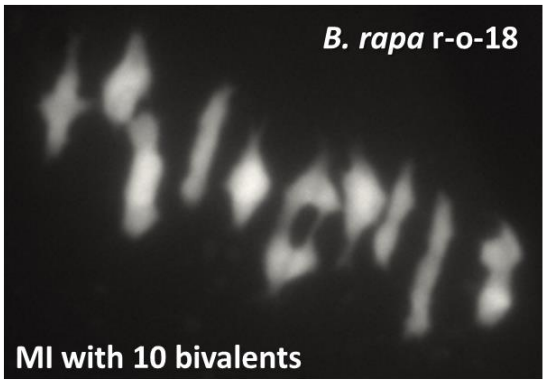

Fig 4. Chiasma frequency and localization analysis at meiotic atlas

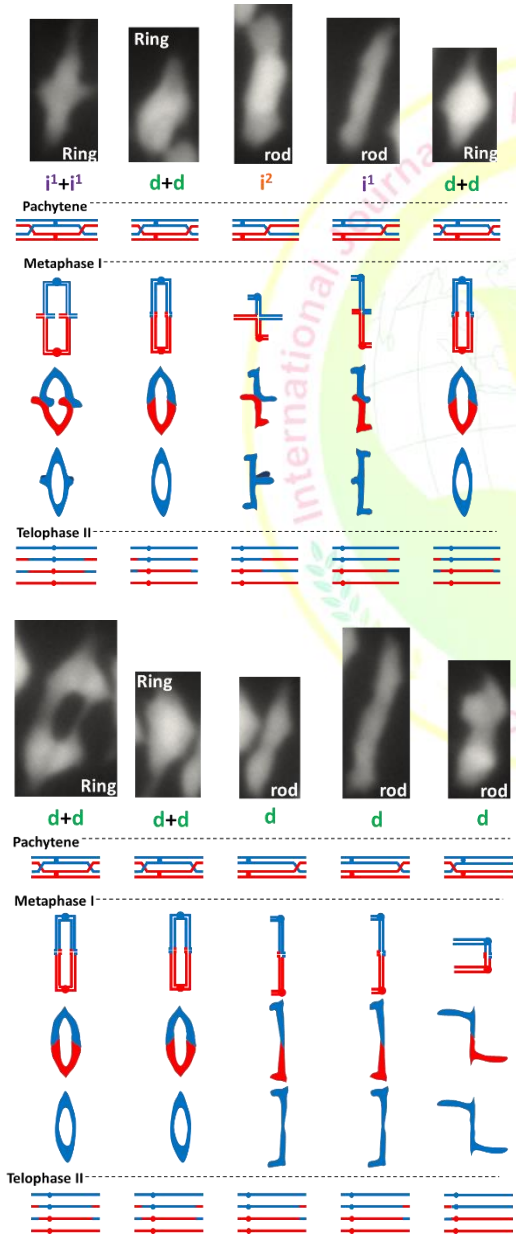

Fig 5. Chiasma frequency and localization analysis at meiotic atlas

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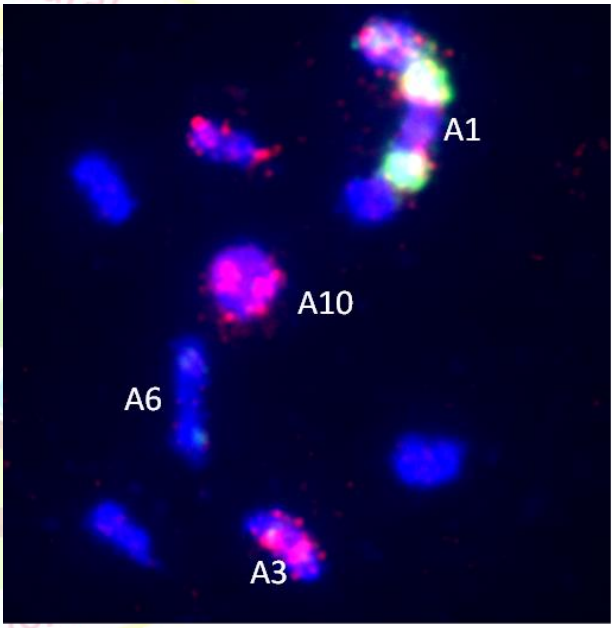
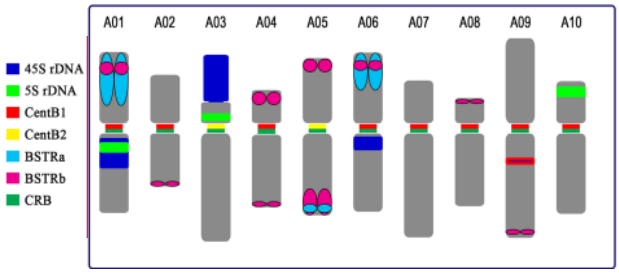


Fig 6. Fluorescence in situ hybridization using 45S and 5S rDNA probes to identify chromosomes



In *B. rapa*, recombination rates vary significantly across chromosomes; yet, Mb-scale landscapes are highly preserved across genetic backgrounds. In all *B. rapa* crosses, we found that the CO distribution was preferentially distributed towards distal regions. These findings are in line with those found in distant species like potato (Marand *et al*., 2017), tomato (Demirci *et al*., 2017; Rommel Fuentes *et al*., 2020), maize (Kianian *et al*., 2018), and barley (Dreissig *et al*., 2020), as well as those found in related species like *B. olereacea* (Pelé *et al*., 2017) and *B. napus* (Bayer *et al*., 2015; Boideau *et al*. 2022). Recombination frequency and distribution are also said to be influenced by a variety of epigenetic variables; CO incidence is associated with poor DNA methylation, low nucleosome density, and enrichment in particular histone marks. These characteristics of open chromatin were not examined in this study. Large pericentromeric regions of the chromosome, which typically exhibit a high level of DNA methylation and K3K9me2 histone marks, are home to heterochromatin and euchromatin, respectively, and are found in distal gene-rich regions (Boideau *et al*., 2022; Choulet *et al*., 2014; Li *et al*., 2019; Swagatika & Tomar, 2016). The preferred prevalence of these epigenetic characteristics is consistent with the U-shaped CO distribution that we observed in *B. oleracea*. We found that the CO number varied significantly among the cross combinations. The relationship between parent relatedness and CO number is not supported by our data. Indeed, a substrate with high homology may aid recombinational repair, therefore we would anticipate a greater number of COs when the two parents exhibit higher relatedness levels. This is not a typical pattern, though. It has been consistently shown that variations in CO rate exist depending on a plant's genetic background. Among our cross combinations, we did not find any significant differences in the Mb-scale CO landscapes, despite variations in CO quantities. We have demonstrated significant CO variation based on the direction and combination of the cross, which is extremely important for breeders.

CONCLUSIONS

In sexually reproducing animals, meiosis is a specialised cell division that involves separate homologous chromosomal assortment and homologous recombination (HR) to reorganise the maternal and paternal genomes. Since CO numbers are limited and skewed towards chromosome ends in many crop species, restricting recombination and access to naturally occurring genetic variety and producing linkage drag, modifying CO frequency and distribution is of particular importance for plant breeding. Since many crop species have low CO levels that are skewed towards chromosome ends, which limits recombination and access to naturally occurring genetic variety and creates linkage drag, changing CO frequency and distribution is of particular relevance for plant breeding.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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Citation: Sana-e-Mustafa 2024. In *Brassica rapa* meiotic recombination; chromosome axis remodeling is critical. *International Journal of Agricultural and Applied Sciences*, 5(1): 6-10. https://doi.org/10.52804/ijaas2024.512