Homologous and homoeologous recombination in *Brassica napus*

Anne Marie Chèvre[1], Frédérique Eber[1], Martine Leflon[1], Stéphane Nicolas[1], Zhiqian LIU[1], Marie-Odile Lucas[1], Olivier Coriton[1], Nicolas Pouilly[1], Jean-Claude Letanneur[1], Cyril Falentin[1], Michel Renard[1], Maria Manzanares-Dauleux[1], Hortense Brun[2], Régine Delourme[1], Eric Jenczewski[3]

[1] UMR INRA-Agrocampus Rennes, Amélioration des Plantes et Biotechnologies Végétales, BP35327, 35653 Le Rheu cedex France
[2] UMR BiO3P, BP35327, 35653 Le Rheu cedex France
[3] Station de génétique et d'Amélioration des Plantes, INRA – Institut Jean-Pierre Bourgin, Route de St Cyr,, 78026 Versailles cedex, France Email: anne-marie.chevre@rennes.inra.fr

Abstract

Broadening the genetic diversity of oilseed rape is a major issue. This can be achieved either by crossing *B. napus* ($A_nA_mC_nC_n$, 2n=38) with its progenitors, *B. rapa* (A_rA_r, 2n=20), *B. oleracea* (C_oC_o, 2n=18) or by inducing rearrangements between the constitutive A and C genomes of *B. napus*. In all cases, it is important to monitor homologous and/or homoeologous recombination. When dealing with the progenitors it is possible either to cross them directly with *B. napus* or to produce synthetic forms ($A_rA_rC_oC_o$, 2n=38). Using *B. rapa* (A_rA_r, 2n=20), we have shown that the level of homologous recombination between the A genomes is highly dependant on the genomic structure of the hybrid (diploid, digenomic triploid, tetraploid) and on the genetic background. The characterization of synthetic oilseed rape forms and their selfed progeny indicated that chromosome rearrangements occurring between the genomes depend on the genetic backgrounds and on the way the amphidiploids were produced (colchicine-doubling of F_1 AC hybrids or through unreduced gametes). The impact of such chromosome rearrangements will be compared to genome dynamics of natural *B. napus*. In fact, homoeologous recombination between A and C genomes is controlled by a major gene *PrBn* plus a network of genes. With this genetic system, translocations can be induced, preferentially between the regions of primary homeology in oilseed rape haploids. Our results constitute a general framework to apprehend the evolution and establishment of polyploid species as well as the mechanisms of genetic exchanges between related species for genetic improvement of oilseed rape.

Key words: oilseed rape, *Brassica napus, Brassica rapa, Brassica oleracea*, recombination, cytogenetic, BAC FISH, genetic mapping

Introduction

Oilseed rape (*Brassica napus*, AACC, 2n=38) is an allopolyploid species resulting from multiple hybridization events (Song et al., 1992) between *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18). This species shows a regular diploid-like meiotic behavior and a disomic inheritance that occur in spite of large genomic duplications carried by each of *B. napus* constitutive genomes. Actually it is now largely demonstrated that A and C genomes derive from a common ancestor after several rounds of polyploidization and chromosome rearrangements giving rise to large duplicated regions within and among each genome (Truco et al., 1996; Parkin et al., 2003). Additionally, alignment of genetic maps performed for each progenitor and for oilseed rape revealed that some A and C linkage groups of *B. napus* are homeologous along their full length whereas some others show only segmental homeology (Parkin et al., 2003; 2005). The genomes of the diploid species seem to be conserved in the allopolyploid species; Parkin and Lydiate (1997) observed that the majority of loci exhibited a disomic inheritance in the progeny of an hybrid between a synthesized oilseed rape and a natural one, demonstrating that *B. rapa* and *B. oleracea* chromosomes were each recombining with corresponding A-genome and C-genome homologues in *B. napus*, respectively. However, the genetic distances are twice higher in diploid *B. rapa* than on the A genome of the natural oilseed rape (Teutonico and Osborn, 1994).

This conservation of the progenitor genome structure as well as the large duplications among and between genomes allow the use of homologous and homoeologous recombinations to enlarge the genetic basis of oilseed rape. Homologous recombination can be promoted between A or C genomes either by direct crosses between the progenitors and oilseed rape or by the production of synthetic form but nothing is known on the impact of the ploidy level and of genotype structure on the rate of homologous recombination. Homoeologous recombination is involved in the first stages of oilseed rape stabilization, as it has been shown that chromosome rearrangements occur in the advanced selfing progeny of synthetic forms (Song et al., 1995; Pires et al., 2004). Homoeologous recombination can also induce translocations in natural oilseed rape varieties (Lombard and Delourme, 2001; Osborn et al., 2003; Udall et al., 2005). The role of a genetic control of homoeologous pairing between A and C genomes, due to *PrBn* for Pairing regulator in *B. napus* (Jenczewski et al., 2003) in such rearrangements is unknown.

In the present paper, we will present a synthesis of the data acquired on the main factors (1) controlling homologous recombination between A genome of *B. rapa* and of *B. napus* and (2) affecting homoeologous recombination during the first stage of synthetic stabilization as well as those modifying the pattern of homoeologous recombination within natural *B. napus*.

The results will be discussed in relation to oilseed rape genetic improvement.

Materials and Methods

Two *B. rapa* varieties were used, a doubled haploid varieties, Z1 (gently provided by AAFC Canada) and an old forage variety, Chicon among which one plant, C1.3 resistant to two *Leptosphaeria maculans* isolates, was selected. Three *B. oleracea* varieties were chosen to produce synthetic oilseed rape forms, two doubled haploid varieties RC var alboglabra, HDEM var italica and C10, a kale pure line. The two *B. napus* pure lines, Darmor a European winter type and Yudal a Korean spring type, have been used to establish a reference genetic map (Lombard and Delourme, 2001).

The schemes of crosses are presented figures 1, 2 and 3.

The haploid production was performed as described by Polsoni et al. (1988). Cytogenetic methods are described in Leflon et al. (2006a). Pathological tests at the cotyledon stage for L. *maculans* resistance as well as the isolates used were presented in Leflon et al. (2006b). The molecular methods and markers used have been presented in Lombard and Delourme (2001), Piquemal et al. (2005), Leflon et al. (2006b), Liu et al. (2006), Nicolas et al. (2006), Delourme et al. (2006).

Homologous recombination

Homologous recombination between A genomes of *B. rapa* and B. napus allows gene introgression and genetic analysis

Pathological tests for the detection of blackleg specific resistance genes allowed the selection of one resistant plant, C1.3. Using the pathological and molecular analyses of its backcross progeny, reported as 2x in figure 1, we showed that at least two specific resistance genes are located on the R7 linkage group of *B. rapa*. Pathological tests with several isolates containing different patterns of *AvrLm* genes are needed for their identification. So, Doubled Haploid lines (4x DH in figure 1) from F_1 hybrids ($A1A^DCC$) obtained by crosses between the synthetic line implying C1.3 (A1A1CC) and Darmor oilseed rape variety were produced. Several cotyledon tests per line, combined with molecular analyses of a set DH lines revealed that (1) the resistance genes correspond to *Rlm1* and *Rlm7* and (2) that the *B. rapa* genes were introduced through homologous recombination on the corresponding *B. napus* linkage group (N7) (Leflon et al., 2006b).

Homologous recombination rates between A genomes depends on the ploidy level and on the genotype.

Recombination rates along the R7 linkage group of *B. rapa* have been analyzed using 19 molecular markers in the backcross progeny of different F_1 hybrids obtained from the same parental *B. rapa* varieties but showing different genomic structure (A1A2, A1A2C and A1A2CC) (Figure 1). Firstly, we checked that homologous recombination occurs preferentially between A genomes in a digenomic triploid hybrid (Leflon et al., 2006a). Then, we demonstrated that recombination rates were 6.1 and 3.2 fold higher in digenomic triploid hybrids as compared to the diploid and tetraploid hybrids, respectively. Similar increases of recombination rates were found using *B. napus* as digenomic triploid hybrids ($A1A^DC$ or $A1A^YC$): these latter recombined 3.4 to 1.83 times more than the corresponding tetraploids ($A1A^DCC$ or $A1A^YCC$).

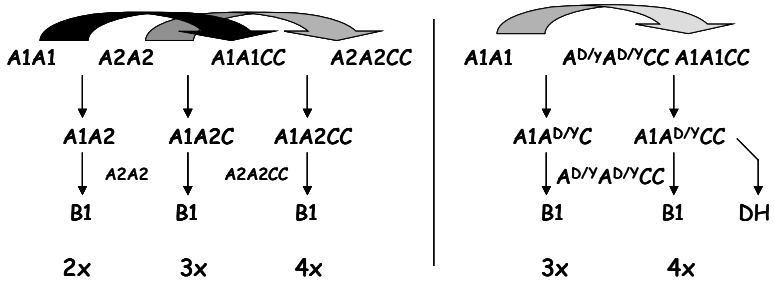

Figure 1: Production of segregating populations for the analyses of homologous recombination rates between the A genome of *B. rapa* (A1A1,C1.3 or A2A2, Z1) using synthetic oilseed rape lines (A1A1CC and A2A2CC) and between A genome of *B. rapa* and of *B. napus* (A^DA^DCC, Darmor or A^YA^YCC Yudal)

Homoeologous recombination

Homoeologous recombination during the first stages of stabilization of newly synthetic forms depends on the genotype of the parents and on the way of production of S0 initial amphidiploid forms.

We have produced F_1 hybrids representing three different combinations of B. oleracea and *B. rapa* genotypes: EMZ (HDEMxZ1), RCC or CRC (RCxC1.3 and reciprocal) and C10Z1 (C10xZ1). The analysis of their meiotic behavior at MI revealed a high level of chromosome pairing which is genotype dependent. Similarly, homoeologous recombination between A and C genomes in the S0 amphidiploid was shown to occur at a rate that depends on both the genotype and the way the amphidiploid were produced i.e. either colchicine doubling or through female unreduced gametes of the F_1 hybrid (Figure 2). The impacts of such rearrangements were confirmed by molecular analyses of the S1 and S2 progenies in which deletions of markers were detected on some specific genomic regions.

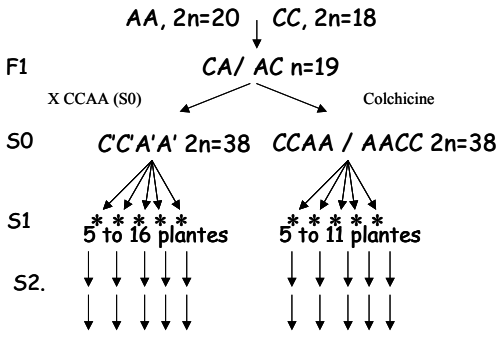

Figure 2: Production of synthetic lines from two *B. rapa* varieties, Z1 and C1.3 and three *B. oleracea* varieties, RC, HDEM and C10 either through colchicine doubling or from female unreduced gametes.

Homoeologous recombination still occurs in natural oilseed rape

We first obtained evidence that homoeologous chromosome pairing (at metaphase I) in B. napus haploid is genetically controlled by examining the segregation pattern for pairing behaviour in a population of haploids produced from F_1 hybrids between two natural B. napus lines with high- and low-pairing behaviours at the haploid stage (at metaphase I). We observed that the parental metaphase I pairing patterns were inherited in a Mendelian fashion, supporting the presence of a major gene that determines the homoeologous chromosomal pairing in haploids (Jenczewski et al., 2003). This major genetic determinant, named PrBn for Paring Regulator in B. napus, was recently located on linkage group N19 and shown to display incomplete penetrance (Liu et al., 2006). Three minor QTL having slight additive effect and epistatic interactions were detected, demonstrating that the hereditary components of homoeologous chromosome pairing are polygenic rather than monogenic.

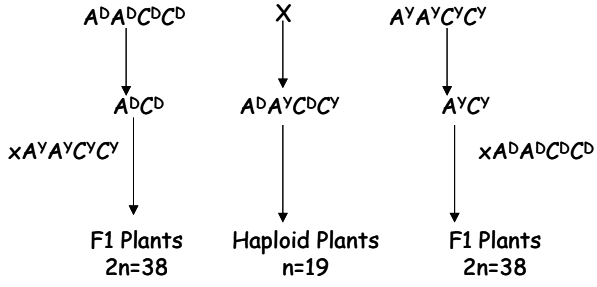

Figure 3: Production of progenies from two oilseed rape varieties, Darmor $A^DA^DC^DC^D$ and Yudal $A^YA^YC^YC^Y$, used either to analyse the genetic determinism through haploid plants produced from $A^DA^YC^DC^Y$ hybrids or to assess the effect of the genetic control on homoeologous pairing from F1 plants obtained from haploid plants showing a high (haploid of Darmor, A^DC^D) or low (haploid of Yudal, A^YC^Y) level of chromosome pairing.

We then showed that this genetic system is able to promote homoeologous recombination in natural oilseed rape varieties. Molecular analyses were performed with co-dominant markers evenly distributed over the oilseed rape genome on 2n=38 F_1 plants produced from haploids showing either a high (Darmor, A^DC^D) or a low (Yudal, A^YC^Y) level of chromosome pairing at MI (Figure 3). Losses (non-transmissions) of molecular markers were detected in these F_1 plants and provided evidence that at least 50% of these rearrangements were due to homoeologous recombination generating Homoeologous Non Reciprocal Translocations (HNRT) (Nicolas et al., 2006). The averaged proportion of marker loss ranged from 1 to 5% depending on the parental haploid plants, Yudal or Darmor, respectively. A higher proportion of HNRT per plant (3 vs 1) was detected in the progeny of high pairing haploids (from Darmor) than in the progeny of low pairing haploids (Yudal). In addition, the frequency of rearrangements depended on the genomic regions, at least in the progeny of Darmor haploids.

Discussion

We showed that it is possible to transfer genes of interest from the A genome of *B. rapa* into the A genome of oilseed rape through homologous recombination. However, using the same genotypes for the A genome, the rate of homologous recombination is dependent on the genomic structure of the F_1 hybrids. Using triploid hybrids, it becomes possible to manage introgression restricted to the region of interest. The genetic control of chromosome pairing carried by the oilseed rape, which is under complex genetic determinism, seems to affect homologous recombination in digenomic triploid hybrids and we demonstrated that it regulates homoeologous recombination between A and C genomes in haploids of oilseed rape, which occurs spontaneously in this species. The HNRTs generated by homoeologous recombination during the first stages of synthetic stabilization and in natural oilseed rape varieties induce deletion of genomic regions and duplication of the corresponding homoeologous regions. Such mechanisms broaden the genetic diversity that is available for oilseed rape improvement.

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