PrBn, a major gene controlling homoeologous pairing in oilseed rape (*Brassica napus*) haploids

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ABSTRACT

In oilseed rape (*Brassica napus*) haploids (AC; n=19), the amount of chromosome pairing at Metaphase I (MI) of meiosis varies depending on the varieties the haploids originate from. In this study, we combined a segregation analysis in a population of haploids with a Maximum Likelihood approach to demonstrate that this variation is genetically based and mainly controlled by a gene with a major effect.

Key words: chromosome pairing, polyploidy, pairing control gene, exotic germplasm

INTRODUCTION

Controlling homoeologous pairing and recombination is of practical importance for plant breeding, in particular to broaden the genetic basis of cultivated species by introgressing exotic genetic variation from wild relatives. Indeed, a large number of successful alien introgressions have been achieved in wheat through homoeologous recombination (Friebe et al. 1996), notably by suppressing the control exerted by the *Ph1* locus (e.g. Riley et al. 1968; Luo et al. 1996 and ref. therein) that prevents homoeologous pairing (Riley and Chapman 1958; Moore 2002). It may be anticipated that a better understanding of the genetic systems regulating homoeologous pairing in other polyploid species could help in promoting and engineering introgressions in these species. Renard and Dosba (1980), Attia and Röbbelen (1986) observed that the amount of chromosome pairing in haploid plants (AC; n=19) originating from different oilseed rape varieties (*Brassica napus*; AACC; 2n=38) was variable. They identified high and low pairing varieties at the haploid stage. In this study, we show that a large part of this variation is controlled by a single gene.

MATERIALS AND METHODS

Plant materials: All haploids were isolated using microspore culture as described by Polsoni et al. (1988). A total of 13, 27 and 244 haploids were isolated from a spring Korean line (*Yudal*), a French dwarf winter line (*Darmor-bzh*), and F1 hybrids between *Darmor-bzh* and *Yudal*, respectively. Four series of haploids were produced during time and analyzed separately. We consider that haploids belonging to the same set had the same environment. For two sets, haploids were produced from both the parental lines and F1 hybrids and observed at the same time.

Meiotic observations: Floral buds were fixed in Carnoy's solution (ethanol-chloroform-acetic acid, 6:3:1) for 24h and stored in 50% ethanol. Observations on the Pollen Mother Cells (PMCs) were performed at the MI stage from anthers squashed and stained in a drop of 1% acetocarmine solution. On average, 20 PMCs (min=14, max=149) were examined for each haploids, regardless of their origin.

Statistical analysis: Statistical analyses were performed on the number of univalents because this variable measures the whole extent of pairing in a synthetic way (i.e. reflecting by subtraction the number of chromosomes associated both as bivalents and multivalents). Parental data were first analysed on their own, in order to determine to what extent variation in the amount of pairing among *Darmor-bzh* and *Yudal* haploids was genotypically determined. Based on this preliminary analysis, the offspring and parental data were then analyzed simultaneously, so that parental and offspring distributions could be compared. We considered a model with both a segregating diallelic major gene and a completely additive polygenic

background, that involved factors with fixed (genotype, series) and random effects (plants). Testing procedure was based on the likelihood ratio (LR) test statistic.

RESULTS

Analysis of the variation among *Darmor-bzh* and *Yudal* haploids

Pairing patterns in *Darmor-bzh* and *Yudal* haploids are clear-cut (Figure 1). On average, only 36.8% of the chromosomes paired in the *Yudal* haploids while more than 75% of the chromosomes were associated in the *Darmor-bzh* haploids. Averaged meiotic behaviors are: 4.8I + 6.7II + 0.07III + 0.11 IV for *Darmor-bzh* haploids and 12I + 3.4II + 0.07III + 0.007 IV for *Yudal* haploids.

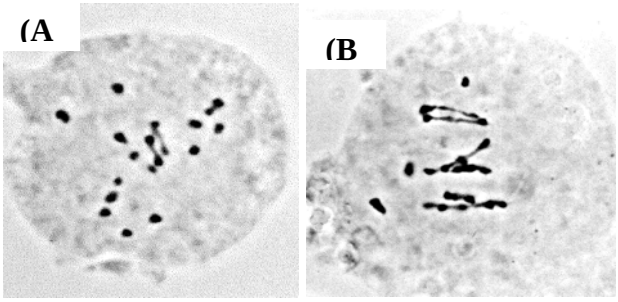

Figure 1: First metaphase of meiosis in 1% acetocarmine-stained squashes of pollen mother cells of two oilseed rape haploids produced from the parental lines: (A) low pairing in a *Yudal* haploid, with two bivalents and 15 univalents, (B) high pairing in a *Darmor-bzh* haploid, with 8 bivalents and only three univalents.

Significant differences existed between the two series of haploids produced from *Darmor-bzh*, which differed on average by the association of two chromosomes as a bivalent. By contrast, no differences were observed between the two series of haploids produced from *Yudal*. We estimated that up to 93% of the observed variability for the number of univalents could be attributed to differences between the parental genotypes. This result clearly indicates that a large part of the variation for the amount of chromosome pairing in oilseed rape di-haploids is genetically based.

Analysis of the whole data set, including the segregating population of haploids

The distribution of the mean number of univalents in the offspring data set was obviously bimodal and showed clear evidence for a mixture of two distinct distributions (Figure 2).

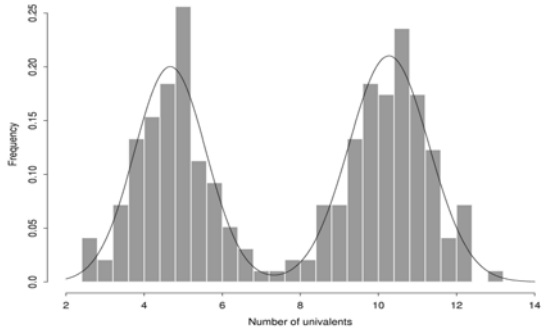

Figure 2: Comparison of the observed (histogram) and estimated (solid curve) frequency distributions of the mean number of univalents among the offspring haploids.

We first verified that the high pairing haploids in the offspring data set were as frequent as the low pairing ones. Accordingly, the distribution of the mean number of univalents in the offspring data set was consistent with the segregation of a major gene with two alleles. However LR test showed that (*i*) the mean number of univalents was significantly lower in the offspring haploids that display a low pairing behavior than in the parental *Yudal* haploids and (*ii*) the variance for the number of univalents was significantly higher among the offspring haploids that display a high pairing behavior than among the haploids produced from the *Darmor-bzh* parental line. In addition, a few haploids in the offspring data set exhibited an intermediate pairing behavior. The amount of pairing in two of these intermediate haploids was measured twice and values were similar in both measurements (data not shown).

All these results suggest that the amount of pairing at MI in haploids of oilseed rape is controlled by a major gene, named *PrBn* for *Pairing Regulator in Brassica napus*, segregating in a background of polygenic variation. We estimated that 86% of the observed variability for the number of univalents in the offspring data set was due to the segregation of *PrBn*.

DISCUSSION

Several authors have observed a variation for the extent of pairing among oilseed rape haploids (Renard and Dosba 1980; Attia and Röbellen 1986). In this study, we demonstrated that this variation is genetically based and mainly controlled by the major gene *PrBn* (***P**airing **r**egulator in **B**rassica **n**apus*; Jenczewski et al. 2003). However, the distribution of the number of univalents in the offspring data set was not consistent with the mixture of the two parental distributions, as would be expected if only *PrBn* was involved in the regulation of pairing. This pattern may have resulted from the segregation of additional weaker genes with non-additive effects that are confounded with the major gene activity or the range of chromosome pairing affinities.

Our results suggest that control of chromosome pairing in oilseed rape haploids is roughly similar to that in wheat in that major genes are involved in both cases. However, differences must be pointed out. Polymorphism observed among oilseed rape haploids is natural whereas there is hardly no natural polymorphism for *Ph1*. Secondly, *Ph1* prevents homoeologous pairing both at the haploid and diploid stage . By contrast, all *Brassica napus* accessions, regardless of the frequency of chromosome pairing in their poly-haploid forms, display regular bivalent associations and disomic inheritance. This indicates that, if the presence of *Ph1* is essential for chromosome stability and fertility in wheat, *PrBn* is not required. Alternatively, *PrBn* could contribute to the regularity of chromosome pairing in all diploid forms of *B. napus*, but the allele present in genotypes with a high pairing behavior at the haploid stage could be ineffective at the hemizygous stage, or at least less efficient than in the diploid state. This last hypothesis is tentative and clearly deserves further examination.

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