Extraction of the diploid A genome and of C chromosomes from the allopolyploid *Brassica napus* (AACC, 2n=38)

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Introduction

There are two strategies available to understand structural and/or functional modifications which took place during the stabilization of polyploid species. The first involves production of synthetic forms in order to mimic the events that occurred during genome stabilization. The second involves the extraction of the polyploid's diploid component to allow the comparison with the present natural diploid species. We used two methods to extract the diploid AA genome from oilseed rape (*Brassica napus*, AACC, 2n=38) which is a natural hybrid between *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18).

Scheme of production

Firstly, AAC F1 interspecific hybrids (produced by crosses between *B. napus* and *B. rapa*) were backcrossed three times to *B. napus*. AAC plants were selected at each generation but the resulting AAC hybrids were male sterile and it was thus impossible to eliminate the C chromosomes by selfing (Fig.1.①). Secondly, the initial AAC F1 hybrids were crossed to *B. rapa* and AA plants were selected in the progeny. Additionally, *m*onosomic addition lines (2n=21) were selected (Fig. 1.②).

A genome extraction

The F1 and the two successive AAC hybrids carried theoretically 50%, 56.1% and 67.1% of A genome from *B.napus*, respectively. They allowed the production of diploid AA plants with 12.2% and 34.2% of A genome of *B.napus*, respectively. They had a regular meiotic behaviour but a morphology close to the one of *B.rapa*.

Monosomic addition lines

At each selection cycle of crosses to *B.rapa*, we selected monosomic addition lines carrying either C1, C2, C3, C5, C6, C7, C8 or C9 chromosome using flow cytometry and cytogenetic observations combined with two molecular markers per C chromosome of *B.napus*. One additional plant (2n=22) with the two additional chromosomes, C4 and C9, was selected to complete the representation of the C genome of *B.napus*. Each line had a regular meiotic behaviour. These lines are powerful tools (Fig 2) (1) to identify the impact of each C chromosome on the phenotype, and (2) to assign unambiguously BAC clones and monomorphic markers corresponding to candidate genes to a specific C chromosome.

Discussion

We can hypothesize that the first method of A genome extraction failed because of the differential evolution of A genome in a polyploid background. The second method is slower but the comparison of the AA plants from each cycle of selection will allow the analysis of the A genome by comparison to the one of *B.rapa* and *B.napus*. We will carry on the selection as well as the production of the complete set of monosomic addition lines. From those later, it will be possible to identify the structure of C chromosomes of *B.napus* and their impact on morphology.

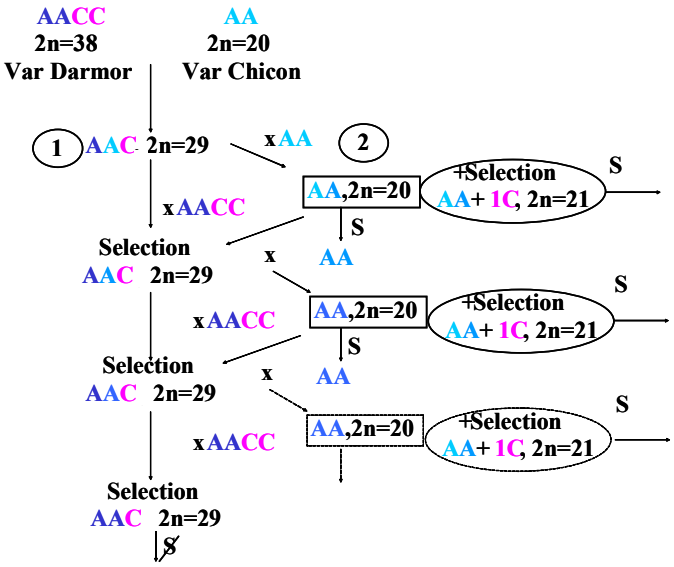

Figure 1: Production of the material either by backcrosses (1) or through alternative crosses with *B.rapa* and *B.napus* (2)

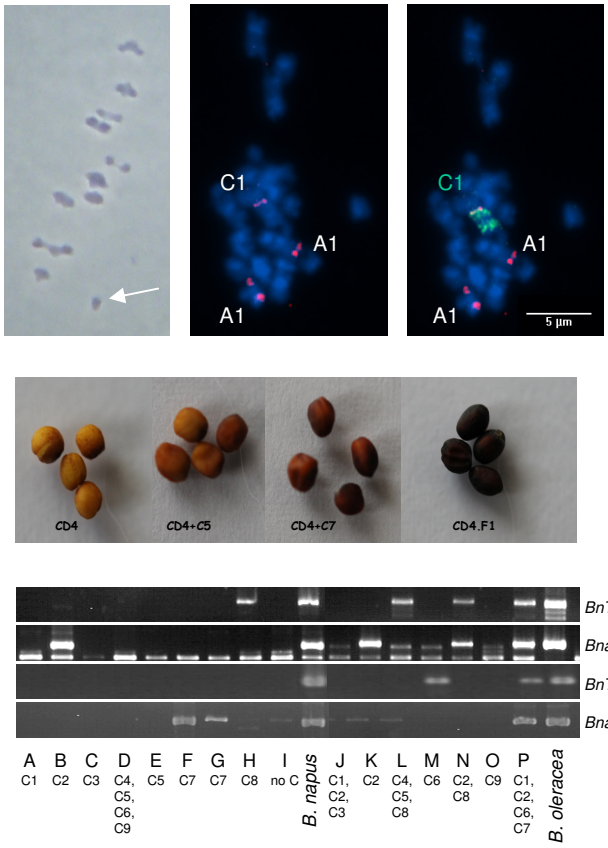

Figure 2: Monosomic addition lines: Metaphase I in meiosis, BAC FISH with a BAC KBrB055A02 (red) specific of A1/C1 chromosome and a BAC BoB014O06 (green) specific of C genome, seed morphology and location of specific genes involved in seed quality.