

Genetic investigation of origination and domestication in *Brassica napus* with virtually synthesized lines

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Abstract

The origination and domestication in *Brassica napus* has not been well characterized. We proposed a strategy in which virtual allopolyploid lines derived from different forms of parental species were characterized in molecular level to compare with natural *B. napus*. It was found that the C subgenome of natural rapeseed was related closely to the genome of cultivated *B. oleracea* and its related wild types, such as *B. incana*, *B. bourgeauii*, *B. montana*, *B. oleracea* ssp. *oleracea*, and *B. cretica*. Meanwhile, A subgenome of natural *B. napus* showed substantial genetic variation with the virtual lines that were derived from Chinese semi-winter *B. rapa*, indicating that it can be used effectively to broaden the germplasm of current *B. napus*.

Introduction

Oilseed rape (*Brassica napus*, genome AACC, $2n = 38$) is known to have originated through spontaneous hybridizations between *B. rapa* (AA, $2n = 20$) and *B. oleracea* (CC, $2n = 18$). However, the exact origination and domestication in *B. napus* have not been well characterized (Gómez-Campo and Prakash). Given that an allopolyploid contains the entire set of chromosomes from each parental species and shows stable disomic chromosome pairing behavior, the progenitors of an allopolyploid can be deduced by comparing the genotype of the natural allopolyploid with that of virtual allopolyploid lines synthesized from all variants of the parental species. In this study, we have proposed a strategy in which the parental lines and the natural allopolyploid are genotyped using DNA molecular markers, and the genotype of a virtual line is then derived from that of its parents, i.e. it is assumed that markers that are present in the parental lines are also present in the virtual allopolyploid. The genotypes of the virtual lines are then compared extensively with that of the natural allopolyploid. The objective of this study is to investigate origination and domestication of rapeseed by extensive comparisons of genetic structure among natural rapeseed and virtual rapeseed lines derived from different variants of the *B. oleracea* and *B. rapa*.

Materials and methods

The experiment was plotted into two sets. In the first set, thirty-nine *B. oleracea* cytodeme including twenty-five accessions from 10 wild types and 14 from seven cultivated types, four accessions of *B. rapa* and six accessions of *B. napus* were collected for the identification ancestral donors of the C subgenome of rapeseed. In the second set, fifty-six accessions of *B. rapa* including 48 semi-winter accessions, two winter accessions and six spring accessions, were employed to compare with the A subgenome of two spring and three winter oilseed rape accessions from Europe and four semi-winter accessions from China to investigate the effectiveness to broaden the germplasm of current *B. napus*. The accessions of the parental species and natural rapeseed were genotyped with the AFLP and SSR markers as described above. For each locus, a score of '1' was given to the virtual line if the AFLP or SSR marker was present in at least one of the parental lines, whereas if the marker was absent in both parents, the virtual line was assigned a score of '0'. In total, 156 virtual rapeseed lines were constructed from 39 accessions of the wild and cultivated forms of *B. oleracea* and the four accessions of *B. rapa* in the first experiment, and 168 virtual lines were constructed from 56 accessions of *B. rapa* and three accessions of *B. oleracea* in the second experiment.

Results

In the first experiment, 355 polymorphic bands from 11 AFLP primer pairs and 464 polymorphic bands from 83 SSR primer pairs were employed to analyze the genetic structure among the 49 accessions by PCA. The virtual and natural *B. napus* lines were grouped in the middle of the figure, whereas the accessions of *B. rapa* and the *B. oleracea* cytodeme derived into three groups were clustered

separately in the upper left and lower right corners, respectively. All six natural accessions from the three distinct gene pools of rapeseed (European winter, Chinese semi-winter, and European spring rapeseed) were clustered closely with the virtual lines derived from the accessions in group C-I classified by Mei et al. (2011), indicating that the types in group C-I or their progenies may be the ancestral donors of the C subgenome in rapeseed.

In the second experiment, 297 polymorphic bands from 66 SSR primer pairs were used to analyze the genetic distance among 68 accessions of *Brassica*. The greatest genetic distance was between natural oilseed rape and the virtual lines derived from semi-winter *B. rapa*, followed by those derived from winter *B. rapa*, whereas the least genetic distance was between natural oilseed rape and the virtual lines derived from spring *B. rapa*. This indicates that semi-winter *B. rapa* can be used more effectively than the other ecotypes to broaden the germplasm of current oilseed rape breeding material.

Discussion

Ancestors of allopolyploidy are important sources for crop improvement because they can provide beneficial alleles or genes, and widen the genetic base of *B. napus*. In this study, natural accessions of rapeseed from three divergent gene pools clustered together when compared with virtual rapeseed lines and parental species by PCA. This indicates that the amount of variation in natural rapeseed is small compared with that in the parental species, and that there is great potential to widen the genetic base of natural rapeseed by using the parental species. Our data suggest that it might be more effective to broaden the diversity of rapeseed by using wild types of *B. oleracea* in C-II and C-III, such as *B. macrocarpa*, *B. rupestris*, *B. villosa*, *B. insularis*, and *B. hilarionis* and Chinese semi-winter *B. rapa*.

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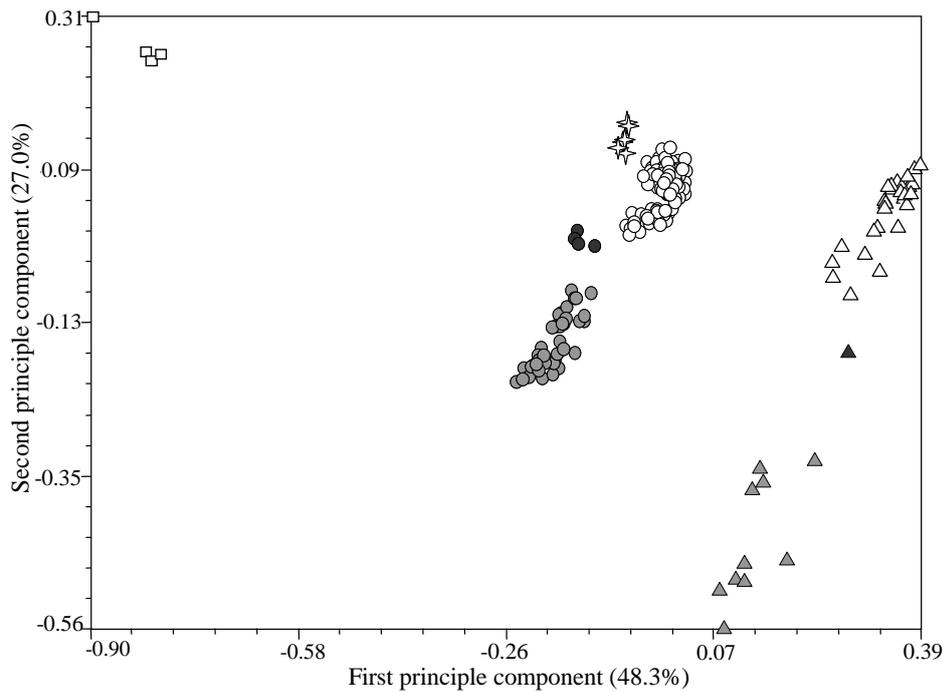


Figure 1. Associations among natural rapeseed, virtual rapeseed, and parental lines as revealed by PCA. The accessions is classified according to the results of Mei et al. (Mei et al. 2011). Open triangles, closed triangles in light grey, and closed triangles in dark grey represent the *B. oleracea* accessions from groups C-I, C-II, and C-III, respectively; open circles, closed circles in light grey, and closed circles in dark grey represent the virtual rapeseed lines derived from accessions of groups C-I, C-II, and C-III, respectively; open squares represents accessions of *B. rapa*; and open stars represent accessions of natural *B. napus*.

Table 1. Average genetic distances between natural oilseed rape and the virtual oilseed rape lines derived from different ecotypes of *B. rapa*

Natural <i>B. napus</i>	Virtual <i>B. napus</i> (Mean \pm SD)			Mean
	SW (48) ¹	S (6)	W (2)	
S (2) ¹	0.62 \pm 0.04	0.58 \pm 0.03	0.56 \pm 0.03	0.61 \pm 0.04
W (3)	0.63 \pm 0.05	0.59 \pm 0.05	0.59 \pm 0.05	0.63 \pm 0.05
SW (4)	0.70 \pm 0.06	0.68 \pm 0.06	0.63 \pm 0.05	0.69 \pm 0.06
Mean	0.66 \pm 0.06	0.62 \pm 0.07	0.60 \pm 0.05	

¹ ecotype (number of accessions). S, W and SW represent spring, winter and semi-winter type, respectively