Allelic diversity of a novel *Brassica napus* gene pool

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ABSTRACT

The abundant genetic diversity in different *Brassica* species provides a valuable resource to expand the relatively deficient gene pool of *Brassica napus*. Recently the A^r genomic components from Chinese *B. rapa* and C^c genomic components from Ethiopian mustard *B. carinata* were introgressed into *B. napus* through interspecific hybridization, and the A^r/C^c components were enriched through two generations of molecular marker-assisted selection. In this study, the allelic diversity of 29 of these new-type *B. napus* lines, 12 from the first generation (G1) and 17 from the second generation (G2), was compared with 66 *B. napus* varieties from Australia, China and other countries based on simple sequence repeat (SSR). Hierarchical clustering and two-dimensional multidimensional scaling revealed that 23 of the new-type *B. napus* lines, including all G2 lines, formed a unique population that was distantly separated from the *B. napus* varieties. This novel gene pool had more alleles per SSR marker and significantly higher richness of private SSR alleles than the *B. napus* varieties.
Keywords: canola - interspecific introgression - allelic diversity - allelic distinctiveness

INTRODUCTION

Brassica napus L. (AACC, 2*n* = 38) is an important crop species but has a relatively narrow gene pool (Prakash and Hinata 1980; Becker *et al.* 1995). Acquiring abundant genetic variation in *B. napus* is an essential prerequisite for breeding new canola varieties adapted to changing and variable global climates. This can be achieved by expanding the gene pool of *B. napus* through the introgression of novel valuable genes from related species such as *B. rapa* (AA, 2*n* = 20) and *B. carinata* (BBCC, 2*n* = 34).

In Huazhong Agricultural University, a number of new-type *B. napus* lines were "resynthesised" from the interspecific crosses *B. napus* × (*B. carinata* × *B. rapa*) and *B. napus* × (*B. napus* × *B. rapa*). These new-type *B. napus* lines contained approximately 20-40% of the A^r and C^c genomic components and were designated as the first generation of new-type *B. napus* (G1). Recently, the A^r and C^c genomic components were further pyramided through G1 x G1 reciprocal crosses and AFLP-assisted selection and a wide variation on the A^r/C^c content, ranging from 20% to 86%, was observed in F_2 individual plants. Hundreds of lines with higher A^r/C^c content were selected and purified through single seed decent approach and microspore culture (Zou and Meng 2009). These high A^r/C^c content new-type *B. napus* lines were designated as the second generation of new-type *B. napus* (G2).

We hypothesise that this genomic introgression of A^r and C^c components will significantly broaden the genetic diversity of *B. napus* as assessed by locus-specific microsatellite markers (SSRs) and that the new-type *B. napus* lines will contain abundant private alleles (Chen et al. 2008). The potential value of this new-type *B. napus* for future genetic improvement of canola is discussed.

MATERIALS AND METHODS

The plant materials included 29 new-type *B. napus* lines including 12 of G1 lines and 17 of G2 lines; and 66 current *B. napus* varieties including 30 from China, 28 from Australia, and 8 from India, Europe and Canada.

The method for genomic DNA extraction, PCR analysis and the SSR markers used in this study are exactly the same as described previously (Chen *et al.* 2008). Allele frequency was calculated using NTSYSpc, version 2.20f (Rohlf 2006). Dissimilarity matrices were calculated as suggested by Nei and Li (Nei and Li 1979) and based on Euclidean distance. Hierarchical

cluster analysis was performed using the unweighted pair group method and arithmetic averages (UPGMA) as proposed by Sneath and Sokal (Sneath and Sokal 1973) and ordination by two-dimensional multidimensional scaling (2D-MDS) in PRIMER 6 software (Clarke and Gorley 2006). The allelic diversity was assessed for private SSR alleles in 4 populations: the current *B. napus* from Australia (AU), China (CN) and other countries including India, Europe and Canada (IEC), and the new-type *B. napus* lines (NT). The richness of private SSR alleles was defined as the average number of private alleles per accession in each population.

RESULTS

1. The G2 B. napus formed a brand new population other than current B. napus

A total of 406 polymorphic alleles were amplified using 55 SSR markers with DNA from 95 *B. napus* accessions and 5 other *Brassica* species. In order to assess the genetic distance between the 29 new-type *B. napus* lines and the 66 current *B. napus* varieties, a cluster analysis (UPGMA) was performed using dissimilarity matrices with the lowest correlation coefficient based on the proportion of shared alleles across the 406 alleles. At the genetic dissimilarity level of 0.47, 79.3% (23/29) of the new-type *B. napus* lines were clearly separated from all current *B. napus* and 5 other *Brassica* species tested in this study whereas there were 6 new-type *B. napus* lines, all of which came from G1 lines, scattered in the current *B. napus*. These results were confirmed using 2-D MDS analysis (Fig. 1). These results indicated that G2 lines tested in this study acquired abundant genetic diversity so that they formed a unique population other than the current *B. napus* collected from different countries.

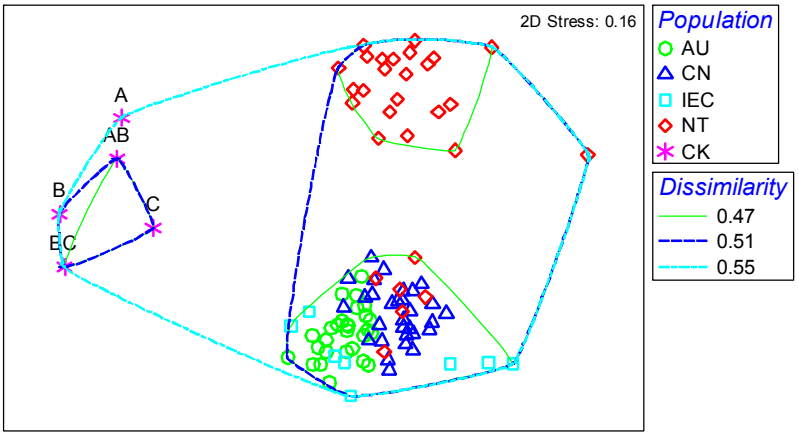

Fig. 1. 2-D MDS analysis based on 406 alleles identified in 95 *B. napus* accessions and 1 accession each from 5 other *Brassica* species (CK, *) revealed that 23 of the new-type *B. napus* (NT, ◇) formed a unique population other than the current *B. napus* from Australia (AU, ○), China (CN, △) and other countries including India, Europe and Canada (IEC, □). A, *B. rapa*; B, *B. nigra*; C, *B. oleracea*; AB, *B. juncea*; BC, *B. carinata.*

2. The new-type B. napus population has higher allelic abundance and richer private alleles

A total of 330 alleles were detected among 95 *B. napus* accessions, of which 211 were found in the AU population, 234 in the CN population, 195 in the IEC population, and 277 in the NT population. The average number of alleles per marker were in the following order: NT (5.13 ± 2.40) > CN (4.33 ± 1.86) > AU (3.91 ± 1.74) > IEC (3.61 ± 1.81). This indicated that the allelic abundance of the new-type *B. napus* was significantly higher than all current *B. napus* populations tested in this study.

The number of private alleles observed in each population differed very significantly from what was expected, with 0.36 alleles per accession in AU, 0.43 in CN, 0.75 in IEC and 1.93 in NT (Table 1). The new-type *B. napus* population had a greater richness of private alleles than any current *B. napus* population. The occurrence frequency of private alleles against the number of SSR alleles detected in each population showed the similar result (Table 1): one private allele

was found in every 5 NT alleles whereas on average one private allele could only be detected in every 20 alleles or more in the 3 current *B. napus* population. This highly significant difference on the richness of private SSR alleles suggested that the new-type *B. napus* population was distinctive from the current *B. napus* populations due to the alien allele introgression from *B. rapa* and *B. carinata*.

Table 1. Summary of the private alleles (PA) detected in 4 different *B. napus* populations and their richness according to the number of accessions and the number of alleles in populations, respectively.

Population	no. accessions	no. alleles	no. PA	no. PA/accession	no. PA/Allele
AU	28	211	10	0.36	0.05
CN	30	234	13	0.43	0.06
IEC	8	195	6	0.75	0.03
NT	29	277	56	1.93	0.2
Chi-square test [a]				51.18***	52.03***

[a] Contingency tests were carried out for the number of observed private alleles in populations, relative to expected number which was calculated from the ratio of the number of accessions and alleles, respectively, in each population to the total number, multiplied by the total number of private alleles). A significant Chi-square test indicates significant departure of observed from expected with degrees of freedom equal to 3 (*** = P<0.001).

Of the 330 alleles detected among 95 *B. napus* accessions, however, 143 alleles were present across all the 4 populations, indicating that 43.33% of the alleles were common alleles. Together with 42 NT alleles shared with 2 of the 3 current *B. napus* populations and 36 NT alleles shared with 1 of the 3 current *B. napus* populations, there were 66.97% (221 of 330) of the *B. napus* alleles were shared between NT and the current *B. napus* populations, indicating that the new-type population NT was still within *B. napus* species.

DISCUSSION

This study demonstrated that the *B. napus* gene pool was greatly expanded by the introgression of the genomic components from *B. rapa* and *B. carinata* via interspecific hybridization and marker assisted pyramiding. After pyramiding of the exotic genomic components in the original new-type *B. napus* (G1 lines), the new generation of new-type *B. napus* lines (G2 lines) obtained higher content of A^r/C^c derived from *B. rapa* and/or *B. carinata*. In comparison with the current *B. napus* populations, there were more number of alleles per SSR marker and significantly higher richness of private SSR alleles in the new-type *B. napus* population. As a result, all the G2 lines formed a unique population which was prominently separated from all current *B. napus* collected from Australia, China and other countries including India, Europe and Canada. Therefore, the new-type B. napus, particularly the G2 lines, could be regarded as a new-type unique *B. napus* gene pool in terms of the abundance of allelic diversity and the degree of allelic distinctiveness. This new-type broader *B. napus* gene pool could provide novel genes potentially important to *B. napus* sustainable development to meet the changing climate and extreme environments.

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