

Extending the rapeseed genepool with resynthesized *Brassica napus* L. I: Genetic diversity

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Received: 23 June 2011 / Accepted: 17 October 2011 / Published online: 9 November 2011
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Abstract Resynthesized *Brassica napus* L. could be employed to increase the genetic variation in the narrow oilseed rape genepool and to establish a genetically distant genepool for hybrid breeding. One important criterion for selecting appropriate resynthesized lines is their genetic distance to adapted breeding material. In this study we estimated the genetic distances in a wide collection of 142 resynthesized lines and 57 winter and spring rapeseed cultivars from Europe, North America and Asia using RFLP marker data. The highest in-group genetic distance (0.36) was observed in 142 resynthesized lines compared to 0.21 in winter, 0.23 in summer, and 0.28 in Asian genotypes. The group of adapted breeding material clustered into three groups of winter-, spring-type, and Asian genotypes. Resynthesized lines did not form distinct subgroups in the cluster analysis, but large differences were revealed in the genetic distance of resynthesized lines to the

adapted winter oilseed rape genepool. The highest distance to winter oilseed rape was found in resynthesized lines with parental lines from the Asian genepool as *B. oleracea* convar. *botrytis* var. *albolabra* (Bail.) Sun or *B. rapa* ssp. *chinensis* (L.) Hanelt.

Keywords *Brassica napus* · Genetic distances · Genetic diversity · Resynthesized line

Introduction

The narrow genetic base of oilseed rape (*Brassica napus* L.; $2n = 38$; AACC) limits the prospects for hybrid breeding since the optimal utilization of heterosis requires complementing genepools. The present breeding material was derived from very few interspecific crosses between cabbage (*Brassica oleracea* L.; $2n = 18$; CC) and turnip (*B. rapa* L.; $2n = 20$; AA) that occurred spontaneously some centuries ago. Hybridizations between different subspecies of *B. rapa* and *B. oleracea* allow the construction of resynthesized *B. napus* with favorable alleles from the unadapted ancestral parents. Examples for this strategy of introgression of single traits are reviewed by Qiong et al. (2009). Apart from single traits, the utilization of artificially resynthesized *B. napus* could be one option for increasing genetic variation by means of the development of genetically distinct genepools for hybrid breeding. Becker et al. (1995) investigated genetic

Electronic supplementary material The online version of this article (doi:10.1007/s10722-011-9772-8) contains supplementary material, which is available to authorized users.

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distances between resynthesized lines and *B. napus* varieties with RFLP and allozyme markers and suggested that resynthesized lines might be a valuable source for broadening the genetic base of the present breeding material of *B. napus*. Seyis et al. (2003) analysed genetic distances of six families of resynthesized lines, spring oilseed and fodder rape cultivars with AFLP-marker. Udall et al. (2004) described the introgression of resynthesized *B. napus* into hybrid spring canola as a source of novel alleles for the improvement of canola spring hybrids. However, the resynthesis lines poor agronomic performance and undesired seed quality traits such as high seed erucic acid and glucosinolate content complicated the direct introduction of resynthesized lines into hybrid breeding programs. Backcrossing genetically diverse resynthesized lines into well adapted breeding material to establish a heterotic genepool for hybrid breeding is a labor intensive approach and a long-term perspective, and appropriate resynthesized lines would have to be selected carefully. One important criterion for the selection of such lines is their genetic distance to adapted breeding material. Therefore, in this study, we have evaluated the genetic distance in a wide collection of 142 resynthesized lines, which are based on nearly all cultivated types of *B. oleracea* and *B. rapa* as parents, and in 57 winter and spring rapeseed cultivars from Europe, North America and Asia using molecular markers.

Materials and methods

Plant material

A collection of 199 *B. napus* genotypes included 142 resynthesized *B. napus* lines originating from the Georg August Universität Göttingen, Freie Universität Berlin, Dansk Planteforaedling and Svalöf Weibull AB (Online Resource 1). The *B. napus* resyntheses were obtained from hybridizations of different *B. oleracea* and *B. rapa* subspecies (Table 1, Online Resource 1). The set of genotypes was completed with 32 winter- and 13 spring-type *B. napus* varieties and 12 genotypes of Asian origin (Online Resource 2, Fig. 2). The taxonomic classification was carried out according to Hanelt (2001).

RFLP analyses

Plants for DNA extraction were cultivated in a greenhouse where a mixed leaf sample (10 g) of 10 plants per genotype was harvested. DNA was extracted in accordance with Rogers and Bendich (1988). Genomic DNA was restricted with the enzymes EcoRI and HindIII (Pharmacia Biotech) and transferred onto nylon membranes (Southern 1975). RFLP analyses were performed as described by Uzunova et al. (1995) using 71 informative DNA probes (Knaak 1996). Twenty-three probe-enzyme-combinations were selected which revealed in 199 genotypes 127 (98.5%) out of 129 RFLP-fragments as polymorphic. On average, 5.5 informative fragments were evaluated per probe-enzyme-combination.

Genetic distances among genotypes based on RFLP markers were estimated in accordance with Nei and Li (1979) using the similarity index of Dice (1945). Genotypic groups were differentiated in a cluster analysis as described by Backhaus et al. (1990; average linkage), and dendrograms were verified by cophenetic correlations as a measure of goodness of fit (Sneath and Sokal 1973). The principle coordinates analysis was performed according to Backhaus et al. (1990). All statistical analyses were performed using NTSYS-pc 2.1 software (Rohlf 2000).

Results

Genetic distances within the three groups of winter, spring-type, and Asian *B. napus* were significantly smaller than between these groups (Table 2). Mean genetic distances were 0.21 in winter and 0.23 in spring-type *B. napus*. Maximum genetic distances of 0.39 (between Accord and Mansholts) and 0.35 (between Tanto and Iris), respectively, were estimated. The highest in-group genetic variation (0.36) was observed in 142 resynthesized lines.

The principle coordinates analysis (Fig. 1) of 142 resynthesized lines and 57 *B. napus* varieties and lines from Asia and Europe revealed high genetic variation in the resynthesized lines, which were almost evenly distributed over all quadrants. Winter and spring-type *B. napus* were most easily differentiated by the second principle coordinate, which explained 6.8% of the variation. Winter *B. napus* varieties mainly clustered

Table 1 Overview of the parental Brassicaceae (taxonomic classification according to Hanelt 2001) and the extent of use as maternal or paternal genotype in 142 resynthesized lines (for details see Online Resource 1)

Taxa		Used as	
		maternal genotype	paternal genotype
<i>Brassica oleracea</i> L.	diverse	1	1
convar. <i>oleracea</i> L.	diverse	1	–
convar. <i>acephala</i> (DC.) Alef.	diverse	3	5
	var. <i>gongyloides</i> L.	3	–
	var. <i>medullosa</i> Thell.	1	1
	var. <i>sabellica</i> L.	12	5
convar. <i>fruticosa</i> (Metzg.) Alef.		–	3
convar. <i>gemmifera</i> (DC.) Gladis	var. <i>gemmifera</i> DC.	3	1
convar. <i>capitata</i> (L.) Alef.	diverse	3	–
	var. <i>sabauda</i> L.	27	–
	var. <i>capitata</i> L.	49	–
convar. <i>botrytis</i> (L.)	diverse	1	–
	var. <i>alboglabra</i> (Bail.) Sun	4	3
	var. <i>italia</i> Plenck	6	–
	var. <i>botrytis</i> L.	2	–
<i>Brassica rapa</i> L.			
ssp. <i>rapa</i> L.		5	8
ssp. <i>chinensis</i> (L.) Hanelt		4	10
ssp. <i>pekinensis</i> (Lour.) Hanelt		6	57
ssp. <i>nipposinica</i> (Bailey)		–	7
ssp. <i>trilocularis</i> (Roxb.)		1	2
ssp. <i>oleifera</i> Metzg.		4	33
<i>Brassica napus</i> L.	diverse	3	–
ssp. <i>napus</i> L.	var. <i>napus</i>	2	1
	var. <i>pabularia</i> (DC.) Rchb.	1	2
ssp. <i>napobrassica</i> (L.)		–	3

Table 2 Mean value, minimum, maximum, and standard deviation (SD) of genetic distances in and between the genetic groups of winter (W), spring-type (S), Asian (A) *Brassica napus* L. and resynthesized (R) lines

Genetic group	N ^a	Mean ^b	Minimum	Maximum	SD
W × W	496	0.213 a	0.03	0.39	0.055
S × S	78	0.231 a	0.07	0.35	0.060
A × A	66	0.279 b	0.11	0.40	0.060
R × R	10,011	0.363 c	0.02	0.65	0.075
W × S	416	0.346 d	0.17	0.50	0.054
W × A	384	0.348 d	0.22	0.49	0.054
S × A	156	0.378 e	0.24	0.50	0.054

^a Number of paired comparisons^b Mean values marked with the same letter are not significantly different (*t* test, *P* = 0.001)

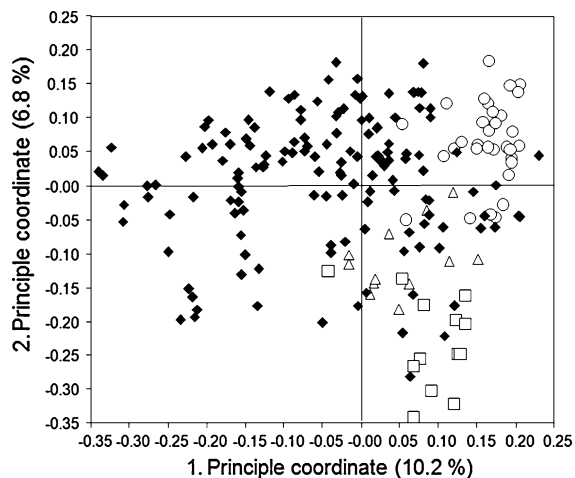


Fig. 1 Principle coordinate analysis of 142 resynthesized lines and 57 *Brassica napus* L. genotypes of European and Asian origin. Symbols are (circle) for winter, (square) for spring-type, (triangle) for Asian *Brassica napus* and (filled diamond) for resynthesized lines

in quadrant II whereas spring-type and Asian genotypes were located in quadrant III.

In the principle coordinates analysis (Fig. 1), no distinct groups could be identified within the 142 resynthesized lines. To examine the relationship between resynthesized lines of different origin in greater detail, a cluster analysis was performed; the results are presented in a dendrogram (Fig. 2).

Winter (W), spring-type (S) and Asian (A) lines were identified as groups in the dendrogram. Resynthesized lines 22, 88, and 99 with 0+ seed quality are grouped with winter lines. Resynthesized lines in group (I) and (II), all being developed at the FU Berlin, are grouped close to the winter lines group. Genotypes derived from the same interspecific cross are closely clustered as lines 36 and 125 (*B. oleracea* convar. *capitata* var. *saubada* L. × *B. rapa* ssp. *oleifera* (DC.) Metzg. *annua*) or lines 34 and 123 (*B. rapa* ssp. *rapa* L. × *B. oleracea* convar. *acephala* var. *sabellica* L.). Semi-resynthesized lines (£; 126, 127, and 128) with 50% genome of *Brassica napus* ssp. *napus* or *B. napus* ssp. *napus* var. *pabularia* (DC.) Rchb. (leaf rape, Siberian kale) were located in group I, II and VI. Resynthesized lines in group IIIb, as well as lines 31 with 110 and 12 with 63 (†) have the same *B. oleracea* form as parent. Spring-type resynthesized lines 122 and 129 (©) were identified as outcrossings with breeding material.

Brassica napocampestris types (‡), originating from crossings between *B. napus* and *B. rapa*, were genetically distant to winter oilseed rape, but they did not form one cluster. Resynthesized lines 90, 91, and 92 were derived from crosses between *B. rapa* ssp. *oleifera* (turnip rape) and *B. napus* ssp. *napobrassica* L. (Swedish turnip), whereas lines 5, 11, and 64 were derived from crosses between *B. napus* var. *pabularia* (leaf rape, Siberian kale) and *B. rapa* ssp. *pekinensis* (Lour.) Hanelt (Chinese cabbage).

The biggest genetic distances to all compared genotypes were determined for resynthesized lines in groups VII and VIII. In the latter, a cluster was formed of resynthesized lines with the Asian *B. oleracea* convar. *botrytis* var. *albolabra* (Bail.) Sun as parental line (¥). Resynthesized lines with *B. oleracea* convar. *fruticosa* (Metzg.) Alef. and Chinese cabbage (*B. rapa* ssp. *pekinensis*) as parental lines (∞) were also integrated into this group. In group VII, crossings between Yellow Sarson (*B. rapa* ssp. *trilocularis* (Roxb.) Hanelt) and cauliflower (*B. oleracea* convar. *botrytis* var. *botrytis* L.) revealed high mean genetic distances (120 and 121, ©).

Discussion

Earlier evaluations of the genetic diversity and distance of resynthesized lines and adapted *Brassica napus* varieties integrated only few resynthesized lines (Diers and Osborn 1994; Song and Osborn 1992; Seyis et al. 2003). Becker et al. (1995) observed a mean genetic distance of 0.35 for resynthesized lines in an evaluation of 17 resynthesized lines and 24 rapeseed cultivars, which was confirmed in this study with a mean genetic distance of 0.36 estimated in 142 resynthesized lines (Table 2).

The group of resynthesized *B. napus* was not clustered into distinct subgroups. All genotypes showed a high mean genetic distance with the exception of some lines which originated from the same interspecific cross. A clear differentiation corresponding to their *oleracea* or *rapa* parental types was not obvious. Becker et al. (1995) and Voss et al. (1998) described groups of spring and winter resynthesized lines. In this study spring-type genotypes were identified in the groups V to VIII (Fig. 2) with mean genetic distances of 0.39, which were distinct to winter-type resynthesized lines. These lines often have

their origin in crosses with Asian *B. rapa* (ssp. *pekinensis*, ssp. *chinensis* (L.) Hanelt), or annual forms of *B. rapa* (ssp. *oleifera*, ssp. *trilocularis*) and *B. oleracea* convar. *botrytis* (var. *botrytis*, var. *italia* Plenck, var. *alboblabra*).

The group of adapted breeding lines clustered into three groups of winter- and spring type *B. napus* and genotypes of Asian origin as described earlier by Becker et al. (1995), Knaak (1996), Lombard et al. (2000), and Qian et al. (2006). The cluster of Asian genotypes has its genetic origin in population varieties from Europe and England (Becker et al. 1999) which were crossed with oilseed and vegetable forms of *B. rapa* from China. In principle coordinates analysis, the varieties ‘Italy’ and ‘Xiangyou11’ were positioned between winter and spring-type *B. napus* (Figs. 1, 2). For ‘Italy’, the name refers to the European origin and ‘Xiangyou11’ is the only Chinese variety with 00-quality originating from European spring-type varieties. Qian et al. (2006) discussed the introgression of Chinese semi-winter lines as hybrid parent for the European winter oilseed rape genepool and estimated a genetic distance based on AFLP-marker data of 0.36 within 9 Chinese semi-winter varieties and of 0.39 to European winter oilseed rape varieties, compared to 0.28 and 0.35, respectively, in the present study.

The distance between *B. napus* resynthesized lines and varieties could be explained by diploid parental lines, different to those of the resynthesized lines investigated here, or, with a restructuring of the merged amphidiploid genome within the first generations of the resynthesized lines (Song et al. 1988). Szadkowski et al. (2010) observed in the very first meiosis of a resynthesized *B. napus* many meiotic-driven genetic changes influenced by the cytoplasm background inherited from the progenitors.

The genetic distances between spring- and winter-type and Asian phenological groups are promising as a basis for inter-pool hybrids between these genepools. Butruille et al. (1999) tested winter oilseed rape as a hybrid parent for spring-types and found these, except for a delay in maturity, a valuable complementary genepool in the breeding of spring-type hybrids. Qian et al. (2009) discussed Chinese semi-winter types as hybrid parents for winter oilseed rape and reported high heterosis for seed yield. Nevertheless, the poor frost tolerance and differences in vernalisation requirement and time of flowering complicate the use of spring-type and Asian *B. napus* as a corresponding

genepool for winter oilseed rape hybrid breeding. Resynthesized *B. napus* is winterhardy and genetically distant to the European winter oilseed rape.

Becker and Engqvist (1995) suggested the use of resynthesized rapeseed to develop a complementary genepool for hybrid breeding of European winter oilseed rape. To introduce canola quality, crosses with adapted material and subsequent backcrosses to resynthesized material are required, followed by recurrent selection for agronomic performance. The present study has revealed large differences in the genetic distance of resynthesized lines to the winter oilseed rape genepool. To analyse the relationship between genetic distance and yield heterosis and to measure the amount of heterosis, 44 of the 142 resynthesized lines were crossed with two adapted cultivars and tested for field performance. The result of these studies will be presented in a separate publication.

Acknowledgments The authors thank Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung (GFP) and the German Federal Ministry of Food, Agriculture and Consumer Protection for financial support and Sonja Yaman for the excellent technical assistance.

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