

ORIGINS OF Brassica napus: NEW EVIDENCE BASED ON
NUCLEAR AND CYTOPLASMIC DNAs

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The three amphidiploid Brassica species, B. juncea (nuclear genome aabb, n=18), B. napus (aacc, n=19), and B. carinata (bbcc, n=17), are believed to have originated by interspecific hybridization of the three diploid species, B. rapa (aa, n=10), B. nigra (bb, n=8), and B. oleracea (cc, n=9). This hypothesis is strongly supported by evidence from hybridization and cytogenetic studies (U 1935; Prakash and Hinata 1980; Mizushima 1980), isozyme analyses (Vaughan 1977; Coulthart and Denford 1982), and phylogenetic studies based on restriction fragment length polymorphisms (RFLPs) (Song et al. 1988).

The cytoplasmic origins of Brassica amphidiploids have been determined by restriction fragment analysis of chloroplast DNAs (cpDNAs) (Erickson et al. 1983; Palmer et al. 1983). Results from these studies indicated that B. juncea has the cytoplasm of B. rapa (A), and B. carinata has the B. nigra cytoplasm (B). However, most B. napus accessions examined had chloroplast DNA patterns that were somewhat different than those of either B. rapa (A) or B. oleracea (C) and the exact cytoplasmic origin of B. napus is still unknown.

Although evidence from previous studies supports the hypothesized origin of B. napus from 9 and 10 chromosome diploid species, it still is not known which accessions hybridized to form the amphidiploid, which accessions were the cytoplasmic donors, and whether hybridization occurred more than once resulting in different B. napus forms. To address these questions, we analyzed accessions of B. napus and of the potential diploid progenitor species for RFLPs in nuclear and cytoplasmic genomes. We were able to identify different accessions as potential progenitors of the various B. napus forms.

MATERIALS AND METHODS

Accessions from several diploid Brassica species and from the amphidiploid B. napus were analyzed for nuclear, chloroplast and mitochondrial RFLPs (Table 1). Accessions representing a range of diversity in B. oleracea and B. rapa along with one accession of each of the nine chromosome species B. montana and B. incana were included. Twenty-one accessions of B. napus were analyzed, including oilseed, rutabaga and kale types.

Total DNA was extracted from bulk samples of lyophilized leaves harvested from 24 plants of each accession. These DNAs were digested with restriction endonuclease EcoRI and Southern blots were probed with 35 anonymous nuclear DNA

clones, 5 cloned nuclear genes of known function, 12 cpDNA clones and 6 mitochondrial DNA (mtDNA) clones. The methods for detecting RFLPs are cited in Song et al. (1988). Accessions were visually compared for RFLP patterns of cpDNAs and mtDNAs to determine which accessions had common restriction fragments. Nuclear RFLPs were scored as present or absent across all accessions and phylogenetic relationships were determined by analyzing the data using the NTSYS-pc micro-computer program.

RESULTS AND DISCUSSION

The cytoplasm of accessions of the diploid Brassica species could be classified in to different types based on cpDNA and mtDNA RFLP patterns (Table 1). For the nine chromosome species, all B. oleracea accessions had the same cpDNA and mtDNA patterns (C type), B. incana had the same cpDNA pattern but a different mtDNA pattern compared to B. oleracea, and B. montana had unique cpDNA and mtDNA patterns (M type). Most B. rapa accessions had the same cpDNA and mtDNA patterns (A type), although two accessions, a wild population (UPM 4685) and 'R500', had slightly different mtDNA patterns (A1 and A2 types). Three accessions of B. rapa, two of broccoletto and one spring broccoli, had unique cpDNA and mtDNA patterns (S type).

Based on RFLP patterns of cpDNAs and mtDNAs, four different types of cytoplasm were observed among the B. napus accessions examined. Three accessions had cpDNA and mtDNA patterns found in B. rapa. Two accessions had the A type cytoplasm and 'Brutor', a French rapeseed cultivar, had S type cytoplasm. New Zealand Rawara had the C type cpDNA and mtDNA patterns found in B. oleracea. Most of the B. napus accessions, including common rapeseed cultivars, had cpDNA and mtDNA patterns which were different than any of those observed in B. rapa and B. oleracea. The cpDNA pattern was identical to that observed in B. montana; however the mtDNA pattern was not M type and appeared to be unique among all accessions examined (N type).

Results from phylogenetic analysis using nuclear RFLP data were consistent with results from the cytoplasmic DNA analysis (data not shown). The three accessions with A and S type cytoplasm clustered together as one group and the accessions with M type cpDNA and N type mtDNA formed another group. Within the M/N cytoplasm group, the rutabaga and oilseed accessions formed two separate subgroups, and the oilseed cultivars were further divided into subgroups which partially corresponded to their region of development.

The results from this study suggest that B. napus accessions were formed from at least four independent hybridization events, each with a different maternal parent. Some accessions, including the kales, probably resulted from crosses of B. oleracea with common B. rapa as the female parent. The maternal parent of 'Brutor' appears to be Spring broccoli, broccoletto or a close relative. New Zealand Rawara appears to be an unusual type that has B. oleracea as the maternal parent. However, most common rapeseed accessions appear to have arisen by crosses of B. montana or close relative as the female by B. rapa. Based on results

from this and a previous study (Song et al. 1990), B. montana or a close relative appears to be an important progenitor in the evolution of the diploid species B. rapa and B. oleracea and the amphidiploid species B. napus.

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Table 1. Accessions of Brassica species analyzed for RFLPs and their cytoplasm type.

Species	Accession name (source and collection no.) (1)	Cytoplasm (2)	
		cpDNA	mtDNA
<i>B. oleracea</i>	Kolhrabi	C	C
	Penca de povao	C	C
	Badger Inbred-16	C	C
	Portugese tree kale	C	C
	Packman	C	C
	Bomerwaldkohl	C	C
<i>B. montana</i>	wild pop. (UPM 5975)	M	M
<i>B. incana</i>	wild pop. (UPM 6560)	C	I
<i>B. rapa</i>	wild pop. (UPM 1742)	A	A
	wild pop. (UPM 4685)	A	A1
	wild pop. (UPM 1791)	A	A
	Purple Top White Globe	A	A
	BLC-198	A	A
	R500	A	A2
	Per	A	A
	Flowering pak choi	A	A
	Pak choi (WGB 005107)	A	A
	Chinese cabbage	A	A
	Narinosa	A	A
	Broccoletto (WGB 4783)	S	S
	Broccoletto (WBG 5248)	S	S
Spring broccoli	S	S	
<i>B. napus</i>	<u><i>B. robertiana</i></u> (3)	A	A
	Asparagus kale (WGB)	A	A
	Brutor	S	S
	New Zealand Rawara (WGB)	C	C
	Rutabaga (WGB 003243)	M	N
	American purple top	M	N
	Lauretina rutagaga	M	N
	Altex	M	N
	Regent	M	N
	Reston	M	N
	Major	M	N
	Hero	M	N
	Stellar	M	N
	Primor	M	N
	Westar	M	N
	Bievenue	M	N
Rubin	M	N	
Quinta	M	N	
Çreşor	M	N	

(1) UPM=Polytechnical University, Madrid; WGB=Wellsbourne Gene Bank

(2) Cytoplasm type determined by chloroplast DNA (cpDNA) and mitochondria DNA (mtDNA) RFLP patterns.

(3) Annual *B. napus* misclassified as *B. robertiana*