BRASSICA DIVERSITY AND WIDE HYBRIDIZATION

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ABSTRACT

The classification of some of the species in the tribe *Brassiceae* in different genera often does not correspond to their crossing ability. Therefore, in many instances it is possible to successfully hybridize Brassica species with those of different genera. The diversity, possible origin and geographical distribution of several Brassica species of economic importance are discussed in this section. Other topics covered include wide hybridization, both sexual and asexual, and genome structure by comparing the *Brassica* and *Arabidopsis* genomes.

DIVERSITY OF BRASSICA AND RELATED GENERA

Crop Brassicas encompass many diverse types of plants, which are grown as vegetables, fodder or sources of oils and condiments. These have been mentioned since ancient times and might have been cultivated as early as 5000 BC. The utilization of oilseed Brassicas is steadily increasing. At the present time it represents 13.2% of the world's edible oil, with a production of 210 million metric tons (Carr and McDonald 1991). Vegetable Brassicas also have great economic significance because of their wide popularity. The increased importance of these crops requires the development of basic genetic information for its application to breeding.

There is considerable confusion about nomenclature of *Brassica* species and related genera. The best approach is to classify them biologically, encompassing all the species and genera sufficiently related to the crop Brassicas as potentially capable of exchanging genes with them. The diploids species range in genomic numbers from x=7 to x=12, when including *Brassica hirta* (syn. *Synapis alba*) in this group.

The most prominent diploid species of Brassica and those of related genera, determined by genome analysis (Mizushima 1950), can be grouped as follows on the basis of chromosome numbers:

x=7	x=8	x=9	x=10	x=11	x=12
B. adpressa	*B. nigra*	*B. oleracea*	*B. campestris*	*B. amplexicaulis*	*B. hirta*
B. deflexa	*B. fruticulosa*	*B. rupestris*	*B. tournefortii*	*B. elongata*	
D. erucoides	*B. maurorum*	*B. macrocarpa*	*B. saxatilis*	*E. sativa*	
	B. spinenesis	*B. insularis*	*B. repanda*		
		B. montana	*B. barrilieri*		
		B. cretica			
		B. hilarionis			
		R. sativus			

B=Brassica, E=Eruca, D=Diplotaxis, R=Raphanus

The three *Brassica* diploid cultivated species are: 1) *B. nigra* (2n=2x=16, B genome), black mustard 2) *B. oleracea*, (2n=2x=18, C genome), cabbage, broccoli, cauliflower, Brussels sprouts, kales, kohlrabi among others, and 3) *B. rapa* (syn. *B. campestris*) (2n=2x=20, A genome) turnips, rapeseed and Oriental vegetables (Prakash and Hinata 1980). It has been early recognized that hybridization leading to amphidiploidy and aneuploidy have played important roles during the differentiation and evolution of *Brassica* species (Prakash and Hinata 1980, for review). This is well illustrated by the elegant work of U (1935) elucidating the genomic relationships among cultivated diploid and derived amphidiploid species. The three basic diploid cultivated species mentioned above, have originated the three amphidiploids, *B. carinata* (2n=4x=34, genomes BC), *B. juncea* (2n=4x=36, genomes AB) and *B. napus* (2n=4x=n=38, genomes AC). This early work was all based on cytogenetic investigation including chromosome number and pairing determination. Later, it was demonstrated that individual chromosomes could be identified by conventional cytological techniques in diploid species (Robbelen 1960) and in alien addition lines (Quiros *et al.* 1987, This *et al.* 1990 and Cheng *et al.* 1994). Furthermore, by conventional cytology it was possible to determine the physical location of some markers to their respective arms by deletion analysis (Hu and Quiros 1991). C-banding also has been successfully applied for *Brassica* chromosome identification (Olin-

Fatih and Heneen 1992) as well as *in situ* hybridization fluorescence labeling (Maluszynska and Heslop-Harrison 1993). Ohmido *et al.* (1995) propose the use of image analysis for karyotyping *Brassica* species and *B. napus* in particular (Kamisugi *et al.* 1995) as a novel tool for cytogenetic analysis.

Modern biochemical and molecular techniques are contributing substantially to our understanding of phylogenetic and evolutionary relationships of *Brassica* species and other forming part of the tribe *Brassiceae*. For example, DNA-based markers, such as RFPLs and RAPDs have served to study genomic structure, evolutionary relationships and construction of gene maps for a number of *Brassica* species (for review see Quiros *et al.* 1994). Another technique extensively used for phylogenetic inferences and cytoplasm characterization is chloroplast DNA restriction site variation. This approach has been applied by Warwick and Black (1995) to test the validity of certain sub-tribal classifications and to demonstrate that the tribe *Brassiceae* has a monophyletic origin within the Cruciferae. In addition, isozyme electrophoresis, is reported by Anderson and Warwick (1995) as a tool to determine ploidy status of several species and to study phylogenetic relationships.

GEOGRAPHICAL DISTRIBUTION ORIGIN AND DIVERSITY OF CULTIVATED BRASSICA:

The genus *Brassica* has mainly a Mediterranean distribution, but it expands to Africa and Asia. The distribution of the cultivated species is as follows:

1) *B. nigra.* This species was cultivated as a spice as early as 3000 BC. Although it may originate in Asia Minor-Iran, it is now widespread throughout Europe, Africa, Asia, India and the Far East (Hemingway 1979). Investigation of 2n=16 *Brassica* species by molecular markers have not disclosed close relationships of these to *B. nigra.* However, the closest species to *B. nigra* seems to be *Synapis arvensis*, a 2n=18 species (Truco 1993). An intriguing strain of rapeseed in China, called Xinjiang wild rape has been previously assumed to be *B. nigra.* However, Li and Guan (1995) present cytogenetic evidence that this species is not *B. nigra*, but a close relative of 2n=18 chromosomes. The same conclusion is reached by Wang (1995) using anatomical and biochemical analyses. This species fits the characteristics of *S. arvensis,* which opens the possibility that northern China is a center of origin and evolution for the B genome.

2) *B. oleracea.* Wild forms of this species are found in Northern France and England. Related species are endemic of the Mediterranean basin. A great array of different crops have been domesticated in various western European regions, such as Greece and Rome where some of these crops might have cultivated since the 1st century AD (Sauer 1993). Conversely, Chinese kale, (*B. alboglabra*) seems to have been domesticated in China. A great diversity of types exist in *B. oleracea* and its relatives, not only for plant morphology but also for karyotypic structure (Kianian and Quiros 1992b).

3) *B. rapa.* Wild forms are found in western Europe, western USSR, Afghanistan, Pakistan, Asia, Transcaucasus and Iran (McNaughton 1979). This species has been domesticated as different crops in different regions; turnips in Europe, leafy vegetables in China and Japan and oil types in India, Central Asia, and Afghanistan. Sauer (1993) report that the oilseed type sarson was cultivated in India as early as 1500 BC. Different forms of this species might have been cultivated as roots and vegetables as early as 5000 BC in China (Li 1983). Little is know about the evolution, domestication and introduction of the different *B. rapa* crop types. Hybridization studies have shown genetic differentiation between some, reflected by low fertility in F1 hybrids and maternal inheritance for some traits (McGrath and Quiros 1991, 1992). Based on erucic acid content, Wang *et al.* (1995) argues that the province of Anhui in South China is the center of origin of *B. rapa* (*B. campestris*), from where it may have spread to the rest of China, India, Middle East, Africa and Europe. Reiner *et al.* (1995) based on botanical, linguistic and historical research, discuss the probable origin in Europe of turnip and oilseed type from wild forms of this species.

The geographical distribution of the three allotetraploids derived from the cultivated diploids are:

4) *B. carinata,* is found and cultivated in North Eastern Africa and Ethiopia in particular, where it is sympatric with *B. nigra* and cultivated kales (*B. oleracea*). It is likely that hybridization leading to its origin took place in that region (Hemingway 1979).

5) *B. juncea.* Central Asia-Himalayas is a primary center of diversity for this species, with migration to China, India and the Caucasus (Hemingway 1979). The Middle East, where *B. rapa* and *B. nigra* grow wild might have been the place of origin of this species, in particular Asia Minor and southern Iran. Most likely hybridization between *B. nigra* and different forms of *B. rapa* have taken place, which is reflected in the great variability observed in *B. juncea.* Perhaps it is the oldest of the cultivated amphidiploids, since it is

mentioned since ancient times. *B. juncea* is grown for oil-seed usage in India and is gaining importance in Canada and USA as an alternative to rapeseed *B. napus*. Wang *et al.* (1995), presents an analysis of the distribution of *B. juncea* in China, and on the basis of erucic acid content, concluding that the Yunan province may be a center of origin for this species.

6) *B. napus*. This species seems to be of relatively recent origin, in the South West and Mediterranean regions. *B. napus* includes oil-seed types as well as swedes, thus hybridization might have taken place several times among different *B. rapa* and *B. oleracea* types or related species (Olsson 1960, Hosaka *et al.* 1990, Song and Osborn *et al.* 1992). According to Pink (1994) swede may have originated in medieval gardens where kale and turnips were grown side by side.

WIDE HYBRIDIZATION IN BRASSICA

Reports of wide hybridization attempts in *Brassica* go back to the nineteenth century, when chromosome numbers of the species in the genus were unknown (Prakash and Chopra 1991). Karpechenko (1928), popularized wide hybridization experiments in *Brassica* by creating "raphanobrassica" after crossing radish and cabbage. The successful hybridization of these two species permits to appreciate the incongruence of the taxonomic classification in the tribe *Brassiceae* with the crossing ability of species classified in separate sub-tribes and genera. This problem was re-emphasized by the work of Mizushima (1950) who produced a series of inter-generic and inter-specific hybrids for studying genomic relationships based on chromosome pairing.

The wide spread existence of species sharing same chromosome numbers in Brassica, often lead to the erroneous conclusion that these may be related and therefore could be intercrossed to produce fertile hybrids. Soon it was realized that this was not necessarily the case because many of these species were found to carry different genomes. For example, *B. nigra* is the only n=8 species that has the B genome. This lead Harberd (1972) to develop the cytodeme or ecotype concept in Brassica to encompass a group of species sharing the same chromosome number and the same genome, successfully crossing and producing fertile hybrids. For example, the *B. oleracea* cytodeme include the n=9 species *B. oleracea* and a series of wild relatives, such as *B. insularis, B. incana, B. cretica*, among others. All these species share the C genome, although karyotypic changes may be present due to reciprocal translocations (Kianian and Quiros 1992b).

Extensive activity has taken place in the area of wide hybridization in *Brassica* with the goal of transferring traits across species and genus boundaries, and the hope to synthesize new species. At this congress, we have several reports dealing with interspecific and intergeneric hybridization for various purposes. Getinet *et al.* (1995) report the hybridization of *B. carinata* and *B. juncea* and subsequent backcross to *B. carinata*. In the BC1F2 progeny, a novel glucosinolate, not present in either of the parents was detected, thus indicating that the C genome of *B. carinata* may carry glucosinolate hydroxylation genes that may express only in the presence of certain *B. juncea* genes. Bijral *et al.* (1995) successfully produced hybrids between *B. juncea* and the C3-C4 intermediate species *Moricandia arvensis* for transferring low photorespiration and disease resistance. Although these two species are classified in different sub-tribes, *Brassicinae* and *Moricandiinae*, the studies of Bijral *et al.* (1995) based on hybridization and cytogenetic studies, and those of Warwick and Black (1995), based chloroplast DNA restriction site studies, indicate that there is not enough evidence for separate sub-tribal status of these species. Similarly, Li *et al.* (1995) and Fu *et al.* (1995) report hybridization of *B. napus* and *Orychophragmus violaceus (M. sonchifolia)* for improving oil seed quality. The resulting hybrids were characterized by cytogenetic (Li *et al.*. 1995) and pollen ultrastructural (Fu *et al.* 1995) studies. Kumar (1995), reports hybridization between *B. tournefortii* and *Raphanus caudatus*. Although the resulting hybrid was sterile, amphiploidy produced fertile hybrids which could be crossed to *B. napus* and back-crossed to *B. tournefortii*, which resulted in male-sterility and cross compatibility with other species.

Although sexual hybridization permits a wide range of crosses, somatic hybridization has proved to be a useful tool not only to combine nuclear genomes but also to engineer novel cytoplasmic recombinants and cybrids with great impact in cytoplasmic male sterility research (Pelletier 1986). Coinciding with the work of Bijral *et al.* (1995), Murata *et al.* (1995) report somatic hybridization of *B. napus* and *M. arvensis* resulting in fertile hybrid plants. Somatic hybridization in combination with other techniques, such as irradiation, permit the partial transfer of genetic information from one species to another. Forsberg and Grimelius (1995) report the production of asymmetric hybrids between *B. napus* and *Arabidopsis thaliana*, species classified in different tribes. The task was accomplished by irradiating *A. thaliana* protoplasts with X-rays or UV light, or pre-treatment of these protoplasts with a restriction enzyme. Similarly, O'Neill and

Mathias (1995) generated putative asymmetric hybrids between these two species by fusing UV irradiated protoplasts of *B. napus* with iodoaetamide inactivated *A. thaliana* protoplasts.

Once that products from wide crosses are obtained, it is necessary to confirm them as true hybrids. In some cases this is feasible to do by morphological inspection, when there is expression of both parental traits. However, chromosome counts and biochemical and DNA-based markers are more reliable for this determination. As example of the application of biochemical techniques, Barcikowska *et al.* (1995) reports that glucosinolate profiles by HPLC analysis myrosinase isozymes are useful markers for hybrid verification.

GENOME STRUCTURE AND EVOLUTION IN BRASSICA

Research from our and other laboratories have demonstrated that the *Brassica* genomes are highly duplicated (Kianian and Quiros 1992a, Song *et al.* 1988, Hoeneke and Chyi 1991). It has been estimated that 50% of RFLP loci are duplicated in the diploid species. Duplicated loci observed in the cultivated species is also observed in the n=7 wild species *Diplotaxis erucoides* and *Hirschfeldia incana (B. adpressa)*, indicating that these duplications have occurred very early in the phylogeny of the *Brassica* species (Quiros *et al.*. 1988). These findings supports the hypothesis that *Brassica* diploids are actually paleopolyploids derived from ancestral genomes of fewer chromosomes. We have found that duplicated chromosomal segments are highly rearranged and dispersed in the genomes, which argues against simple polysomy, or reiteration of whole chromosomes in the *Brassica* genomes. Thus the genomes of *Brassica* cannot simply be described by formulae including a few founder chromosomes being reiterated twice or trice in the same genomes as described by Robbelen (1960), based on pachytene chromosome analysis. Quiros (1995) presents a hypothetical model postulating the events leading to the synthesis and origin of the Brassica genomes, based on hybridization and polyploidization.

Arabidopsis as a model for a simpler genome: *A. thaliana* is a related crucifer to *Brassica* species with a simple genome of only 145 Mbp (Arumuganathan and Earle, 1991), n=5 chromosomes, and approximately 12- 17% duplicated loci (McGrath *et al.*. 1993, Kowalski *et al.* 1994). Kowalski *et al.* (1994) proposed that *A. thaliana* is also a paleopolyploid, based on the existence of duplicated loci. However, its low level of duplication relative to the *Brassica* species (50% of the loci), indicates that most likely loci duplication may be due to aneuploidy and chromosomal structural changes or to other processes, rather than whole genomic duplication. Kowalski *et al.* (1994) found extensive rearrangements between RFLP maps of *A. thaliana* and *B. oleracea*, however, islands of conserved gene organization were identified. Sadowski and Quiros (1995) exploit the genetic map of *A. thaliana* (Hauge *et al.* 1993) to probe the *Brassica* genomes with an *A. thaliana* gene complex carrying five genes within a 20 Kb span (Gaubier *et al.* 1993). This complex comprises a well characterized Em-like protein coding gene and other four flanking genes, three of which were identified by sequence homology to GeneBank accessions. Genetic mapping demonstrates that the Em-gene complex array from *A. thaliana* is conserved on chromosomes C1 of *B. oleracea*, A4 of *B. campestris* and B5 of *B. nigra*.

Physical mapping studies (preliminary results) by pulsed field gel electrophoresis (PFGE) indicates that a region covering the five gene complex in *Brassica* ranges in size from >50 kb to <350 kb. Moreover, further studies on *B. oleracea* let the identification duplicated fragments of the conserved complexes on chromosomes: C1 (tandem duplication), C6, and Cx. The duplicated versions of the complex seem to have undergone structural changes such as reordering and deletion of some of the members in the complex.

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