Genetic Improvement of *Brassica napus* by Wide Hybridisation

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Introduction

In the past two decades, *Brassica* crop species have become one of the world-wide most important sources of vegetables and oil. This development was accomplished by substantial progress in breeding and biotechnology as well as by modernisation of cultivation practices. *B. napus* (2n=38, genome AACC), which encompasses oilseed rape, swede and some fodder crops, is a natural amphidiploid that originated from several independent spontaneous hybridisation events between the diploid species *B. rapa* (2n=10, AA) and *B. oleracea* (2n=18, CC). The limited geographic range of *B. napus* combined with intensive breeding has led to a narrow genetic basis in this species. In contrast, *B. rapa* and *B. oleracea* are both highly polymorphic, being represented by numerous important vegetable, oilseed and fodder crops with a worldwide distribution. Hence, *B. rapa* and *B. oleracea* offer a much broader genetic variability that can be exploited for *B. napus* improvement via experimental hybridisation ("resynthesis") from the original progenitors assisted by biotechnology. During the past 50 years, numerous efforts have focussed on exploring "novel" germplasm and developing basic *B. napus* breeding stocks by using genetic resources of *B. rapa* (turnip rape, turnip, Chinese cabbage, Indian sarsons) and *B. oleracea* (kales, cabbages, Brussels sprouts, kohlrabi, cauliflowers, sprouting broccoli, wild kales, etc.) for resynthesis of amphidiploids. Moreover, the genepool for genetic improvement of *B. napus* can be broadened even further by interspecific and intergeneric hybridisation with other crucifers, many of which exhibit valuable resistance and quality traits.

Development of a *B. napus* core collection

In order to optimise the exploitation of genetic resources in plant breeding the EU-funded project "*Brassica* collections for broadening agricultural use, including characterising and utilising genetic variation in *Brassica carinata* for its exploitation as an oilseed crop" (RESGEN CT99 109-112) has been initiated. RESGEN aims to conserve, document, characterise, evaluate and rationalise European collections of the four important *Brassica* crop species *B. oleracea*, *B. rapa*, *B. napus* and *B. carinata*. The overall aim of the project is to increase knowledge about the genetic resources available within these species and to improve the utilisation of their gene pools in Europe by plant breeders and growers. To achieve this, core collections are being established for each of the four species, with the intention of providing coverage of the maximum possible variation available within existing material in a representative set of well-characterised genotypes. In addition to seed regeneration, extensive characterisation of the four core sets with respect to important agronomic and phytopathological traits is being carried out, with results to be made freely available via a central database (Bras-EDB) (cf. BOUKEMA & van HINTUM 1999). With the objective of creating a *B. napus* core collection representing a broad variability of all available accessions in the Bras-EDB, 338 summer type *B. napus* accessions, including oilseed rape varieties, fodder and green manure forms as well as „exotic“ types, incl. vegetables (ssp. *pabularia*), Hakuran, Couve Nabica, were grown in the field in 2000. In the vegetation period 2000/2001 a total of 857 *B. napus* winter type accessions were sown for the same reason, including a set of genotypes displaying a biannual growth habit (vernalisation requirement) in the former spring trial. The extensive phenotypic and quality data is being used to select accessions representing the variability within the species *B. napus* and to build up a reliable core collection of 150-200 accessions including swede or rutabaga types (ssp. *rapifera*). Besides morphological and quality assessment of the material, the main task of the *B. napus* subgroup is the evaluation of the core collection regarding resistance to clubroot disease (*Plasmodiophora brassicae*) and pests, such as flea beetles (*Psylliodes chrysocephela*, *Phyllotreta* spp.), stem weevils (*Ceutorhynchus* spp.) and field slugs (*Deroceras* spp.) (LÜHS *et al*. 2001).

Resynthesised *Brassica napus* as a genetic resource

This stategy of developing synthetic *B. napus* forms has provided important basic germplasm for further improvements of seed yield, disease and pest resistance as well as relevant seed quality traits (SEYIS *et al*. 2001). This favourable route in breeding rapeseed has been further supported by the application of allozyme and molecular markers showing that synthetic *B. napus* lines are often genetically intermediate between their parental diploid species but are very divergent from natural *B. napus* forms (SONG *et al*. 1993, BECKER *et al*. 1995, VOSS *et al*. 1998).

Resynthesised high-erucic acid rapeseed (RS lines)

In the course of a breeding programme we developed novel *B. napus* RS lines with very high erucic acid content (HEAR, >60% C22:1), deriving from interspecific crosses of 5 different cauliflower cultivars (BK2256, BK 2287, BK3094, BK3096 and ’Venus’) with an Indian ‘Yellow sarson’ type (*B. rapa* ssp. *trilocularis*, Y.S.), which is now used as basic material for the improvement of industrial rapeseed (cf. LÜHS and FRIEDT 1995a, 1995b). These RS lines display natural seed quality (high erucic, high glucosinolate), self fertility as well as lack of or moderate vernalisation requirement and deficiency of winter hardiness. In field trials four German cultivars (’Profitol’, ’Star’, ’Liratop’ and ’Caramba’) were used as controls and sown together with the RS lines and 37 spring oil- and fodder rapeseed cultivars. Phenotypic traits such as plant height, different leaf characters, days to flowering, flowering period, time of maturity and vegetation period were assessed along with seed yield components (e.g. number of pods/plant, number of seeds/pod, thousand-seed weight). Evaluation of the resynthesised rapeseed material was performed following the guidelines of the Bundessortenamt (Hannover, Germany), which serve for the investigation of differentiation, homogeneity and stability in rapeseed (cf. SEYIS *et al*. 1999, Fig. 1). Morphological data was used to carry out factor and cluster analysis. Scatter plots generated by principle component analysis enabled clear differentiation between the RS lines, the controls and the cultivars. For example, it is possible to distinguish between fodder rapeseed cultivars like ‘Petranova’, ‘Jumbo’ and ‘Tiger’ and the Canadian oilseed rape cultivars ‘Stellar’, ‘Oro’ and ‘Regent’ based on the evaluated characters (Fig. 1).

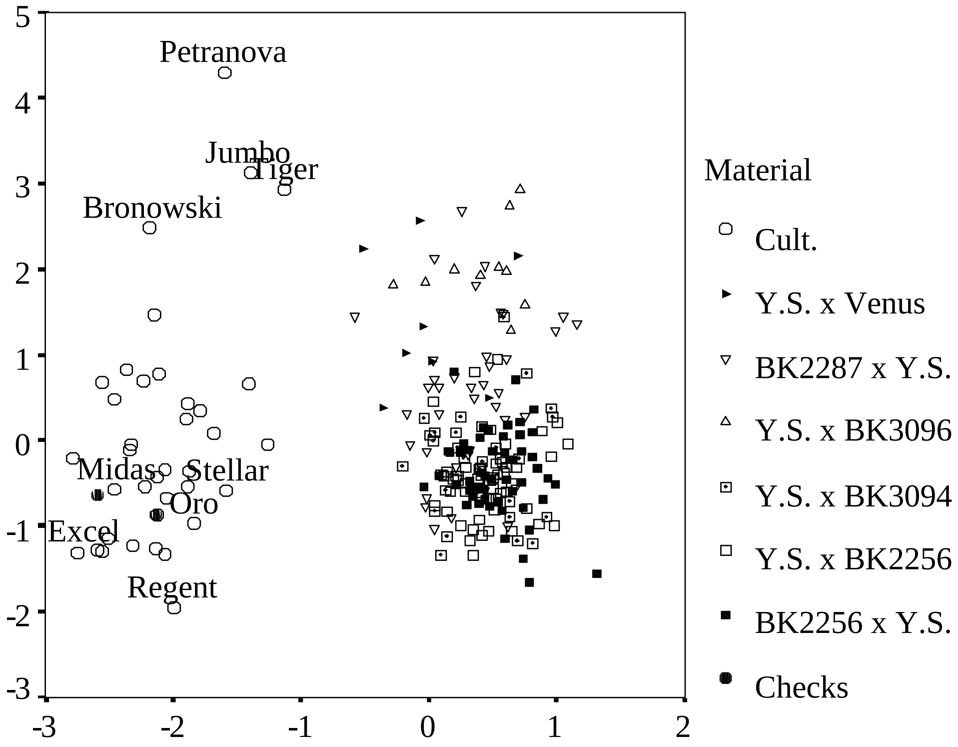

Figure 1: Scatter plot based on principle component analysis demonstrating the divergence of the novel *B. napus* material (SEYIS *et al*. 2001).

Resynthesised rapeseed with improved seed quality

With regard to oilseed rape, current double-low (canola) breeding material seems to be closely related and intensive quality breeding has also contributed to narrow the genetic base of this crop species. On the other hand, the availability of effective hybridisation control systems has enabled the development of hybrid cultivars and has led to a demand for maximum diversity among breeding material. Due to its inferior agronomic performance and seed quality the establishment of a new gene pool based on artificial *B. napus* is limited and has to be considered under more long-term perspectives. One strategy to exploit novel *B. napus* in rapeseed improvement with minimum losses neither of seed quality nor genetic divergence will be new resynthesis experiments using zero-erucic *B. oleracea* forms, which we have identified as a novel source of a gene conferring low erucic acid content to *Brassica* seed oils (LÜHS *et al*. 2000). For this purpose individual zero-erucic *B. oleracea* plants belonging to the three accessions ‘Kashirka 202’, ‘Ladozhskaya’ and ‘Eisenkopf’ were crossed with different *B. rapa* quality types, viz. cv. ‘Asko’ (0, spring fodder rape), an apetalous *B. rapa* line and cv. ‘Reward’ (both 00, yellow-seeded spring type), and two 00-winter type lines (‘Q3F’ and ‘SWSP’). The efficiency of interspecific crosses was aided by embryo rescue as described earlier (LÜHS and FRIEDT 1995b). Cuttings from these hybrids were treated with colchicine in order to obtain amphidiploid *B. napus* plants. In a preliminary experiment we developed 16 amphihaploid hybrids from reciprocal crosses of *B. rapa* ‘Asko’ x zero-erucic acid *B. oleracea* forms. Fatty acid analysis of seeds from the first individual hybrid (‘Kashirka 202’ x ‘Asko’) revealed a zero-erucic acid phenotype as expected (data not shown). The results of the *B. napus* resynthesis experiments using *B. rapa* forms other than ‘Asko’ as female parent are summarised in Table 1. The highest number of amphihaploid individuals *in vitro* were obtained in those cases where the apetalous line was used as *B. rapa* parent (Tab. 1).

Table 1. Number of amphihaploid *B. rapa* x *B. oleracea* hybrids *in vitro*

Cross combination	Number of amphihaploids
‘Apetalous turnip rape‘ x ‘Kashirka 202‘	188
‘Reward‘ x ‘Kashirka 202‘	11
‘Apetalous turnip rape‘ x ‘Ladozhskaya‘	222
‘Reward‘ x ‘Ladozhskaya‘	21
‘SWSP‘ x ‘Ladozhskaya‘	10
‘Q3F‘ x ‘Ladozhskaya‘	16

Development and molecular cytogenetics of interspecific and intergeneric hybrids

Within the crucifer family, sexual hybridisation assisted by both embryo rescue and protoplast fusion have become promising routes for the introgression of desirable traits (e.g., resistance to drought, shattering, pests or diseases, and male sterility) from wide relatives into domesticated Brassicas, such as *B. napus* or *B. juncea* (cf. Fig. 2). Somatic hybrids have been developed from species belonging to the same genus, i.e. *Brassica*, as well as from members of different genera or even tribes (SIKDAR *et al*. 1990, FAHLESON *et al*. 1994, RAWSTHORNE *et al*. 1998, SKARZHINSKAYA *et al*. 1998, SCHRÖDER-PONTOPPIDAN *et al*. 1999).

To introduce beet cyst nematode (*Heterodera schachtii*) resistance into *B. napus*, intergeneric crosses were made between spring oilseed rape and nematode-resistant oil radish (*Raphanus sativus*) genotypes, using embryo rescue to overcome incompatibility barriers. During a backcrossing programme, highly resistant progeny with a minimal *Raphanus* genome component were identified by genomic *in situ* hybridisation (GISH; see SNOWDON et al. 1997). In contrast to earlier generations a sufficient number of plants were fertile, very similar to oilseed rape due to the reduced number of added *Raphanus* chromosomes, and still resistant against nematodes. Highly resistant BC3 and BC4 plants were identified with a monosomic *R. sativus* addition chromosome. These plants, which are highly fertile and show a very good rapeseed-like morphology, have now been selfed in order to generate disomic additions (VOSS *et al*. 2000).The amphidiploid species *B. napus* originated from interspecific crosses between *B. rapa* (2n=10, AA) und *B. oleracea* (2n=18, CC). By fluorescent *in situ* hybridisation (FISH) with 5S (green) und 45S (red) labelled rDNA and subsequent DAPI staining (blue) it is possible to distinguish the chromosomes of the A and C genomes in *B. napus* because of their different chromatin condensation patterns. Moreover, the rDNA hybridisation patterns allow identification of the putative homologues from the respective diploid genomes in *B. napus*. This confirms the assumption that the A and C genomes in *B. napus* are largely intact, whereby chromosome pairing and recombination can occur freely when *B. napus* is crossed with *B. rapa* or *B. oleracea* accessions exhibiting traits of agronomic interest (SNOWDON *et al*. 2001).

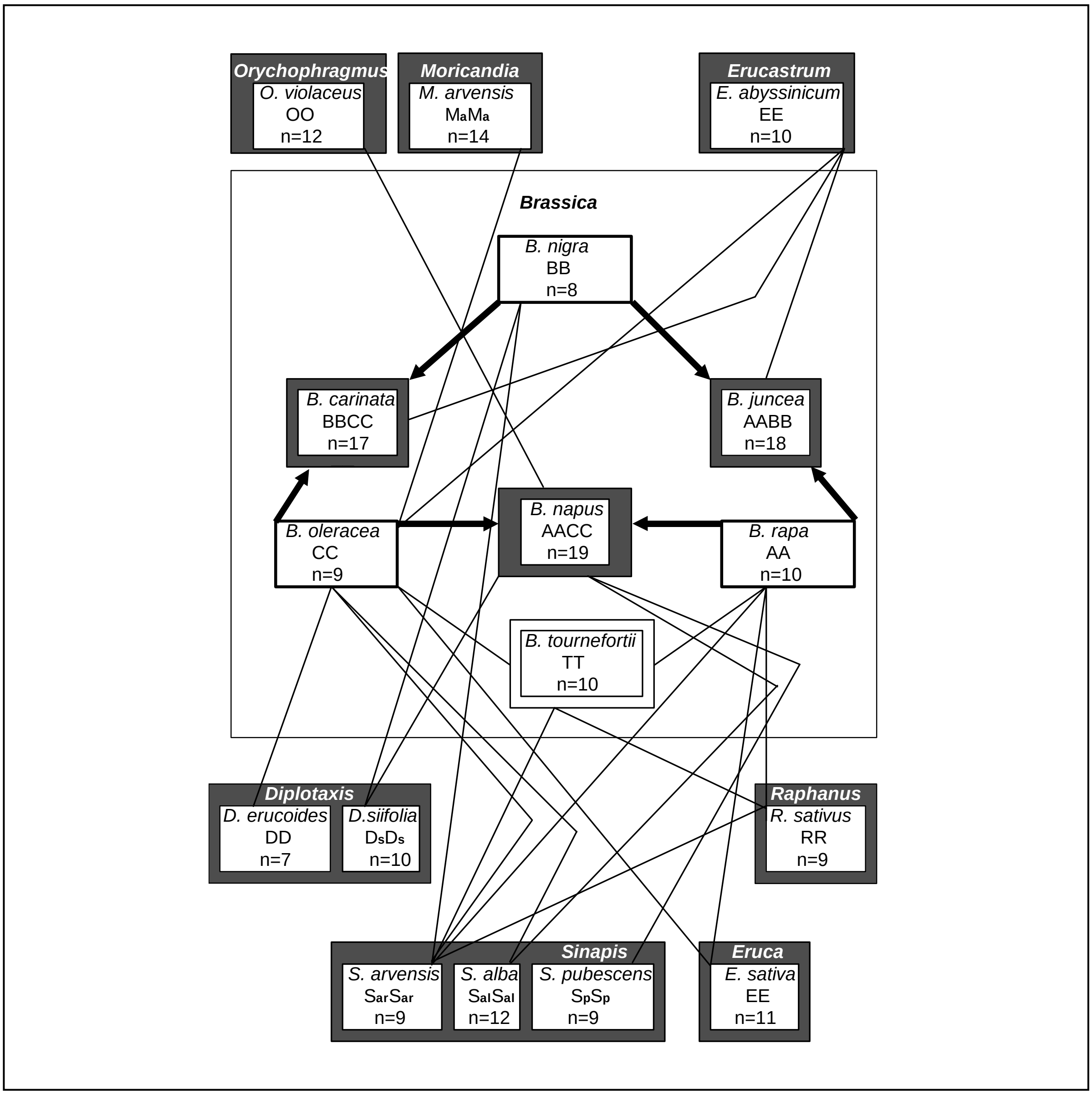

Figure 2: Genome relationships of *Brassica* species and allied genera, showing the potential for sexual hybridisation to transfer traits of interest (cf. DOWNEY & RÖBBELEN 1989, modified).

Conclusion and Prospects

Within the crucifer family, sexual hybridisation assisted by embryo rescue and protoplast fusion is now a promising tool for the introgression of desirable traits from wide relatives into domesticated *Brassica* species, such as oilseed rape. Novel genetic variation for male sterility, drought, shattering, pest and disease resistance has been frequently identified in related species and genera, and somatic hybrids provide an opportunity to exploit this expanded gene pool for improvements of *Brassica* crops in the future.

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