

Expanding the gene pools of *Brassica napus* and *Brassica juncea*

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ABSTRACT

There is still wide diversity to exploit within *Brassicaceae* gene pools, providing options to overcome genetic bottlenecks both in domestication of the *Brassica* crops and the evolution of allopolyploid crops. These include interspecific crossing among the allopolyploid crops, resynthesis of allopolyploids from the wider genepools of the diploid progenitor crops, and introgression from the wild relatives of the secondary and tertiary genepools. The genetic variation in both the diploid and allo-polyploid cultivated species contains extensive genetic variation for key traits of interest to agriculture. There are further opportunities to transfer this variation between species, including resynthesis of the allo-polyploids from their progenitor diploids. The wild relatives also provide wider genetic variation which is largely unexploited, but are increasingly accessible with the development of genetic engineering approaches. Molecular technology offers new approaches which have been realised in herbicide resistant varieties, and which can be more widely applied with development of markers and investigation of candidate genes.

Key words: *Brassica*, genepool, introgression, resynthesis.

INTRODUCTION

Genetic bottlenecks have occurred both in the domestication of crops from wild relatives and with the speciation of polyploid crops from domestic diploid crops. The conversion of rapeseed to canola quality, including the use of a single source of low glucosinolates worldwide, is one such bottleneck. These bottlenecks have potentially reduced the variability available within the cultivated *Brassica* species *B. napus* and *B. juncea*.

Lack of available variability within the primary gene pool of both these species has limited breeding progress for a number of key characters, including disease and insect resistance, adaptation to stress environments, quality and hybrid systems. Where this within-species variability has been insufficient to make progress, breeders have turned to secondary or tertiary gene pools for potentially useful genes.

Within the *Brassicaceae* family, there are several potential sources of new genetic variability, with different breeding strategies used to incorporate new variability. The success of each strategy is discussed and priorities for future breeding are identified.

BREEDING STRATEGIES FOR ENHANCING GENETIC VARIATION

Breeding strategies being used to enhance genetic variability in the allopolyploid species *B. napus* and *B. rapa* include interspecific transfer from other cultivated species within the triangle of U which schematically describes the relationships of diploid progenitors to the cultivated allo-polyploid species (U, 1935), resynthesis of *B. napus* and *B. juncea*, using either the cultivated forms or the wild forms of the diploid progenitor species, interspecific transfer from other cultivated *Brassicaceae* species, interspecific or intergeneric transfer from weedy *Brassicaceae* species, protoplast fusion, mutation and molecular techniques.

Genetic variation in the primary genepools of *Brassica*

There are still opportunities for germplasm enhancement in *B. napus* and *B. juncea* through access to primary genepools within species, including the landraces. Gene pool diversity in *B. napus* has been shown with SSR markers to cluster into four main groups, spring oilseed/fodder, winter oilseed, winter fodder and vegetable types (Hasan *et al.*, 2006). The

European Union working *Brassica* group aims to phenotypically characterise the morphology and phenology of a core collection of *B. napus*. Singh and Chandra (2005) have extensively screened germplasm of *B. napus* and other *Brassica* species for source of resistances to white rust, alternaria blight, downy mildew, mustard aphid, drought, temperature tolerance, salinity, frost, earliness, large seed, non-shattering, silique size and oil quality traits. As a much older crop, even wider genetic diversity may occur among *B. juncea* landraces.

Interspecific transfer from cultivated species within the triangle of U

U (1935) demonstrated that *Brassica* crop species comprise three diploid species, *B. rapa* (genome AA, $2n=20$), *B. nigra* (BB, $2n=16$) and *B. oleracea* (CC, $2n=18$), plus three amphidiploid species, *B. napus* (AACC, $2n=38$), *B. juncea* (AABB, $2n=36$) and *B. carinata* (BBCC, $2n=34$). The amphidiploid species originated through interspecific hybridisation between two of the three diploid species.

Interspecific transfer from other cultivated *Brassica* species within the triangle of U have been an effective way to introgress new variation, particularly when the two species have a common genome. Important recent successful examples have included the introgression of low glucosinolates and white rust resistance into *B. juncea* from *B. napus*. Introgression of characters between cultivated *Brassica* species can result from autosyndetic pairing (when the two species being crossed have a genome in common), allosyndetic pairing or both (when two amphidiploids are crossed). The historic difficulties in transferring and introgressing the excellent B genome blackleg resistance from *B. juncea* into *B. napus* related to the requirement for allosyndetic pairing between the B and C genomes.

Resynthesis of *B. napus* and *B. juncea*

Resynthesis offers opportunities for the introduction of new genes from the diploid progenitors, including both the cultivated forms of the wild forms of the diploid species. Direct commercial utilisation of resynthesized *B. napus* and *B. juncea* lines has been limited due to problems with fertility and low seed yield relative to existing cultivars. However, repeated selection within such lines, or crossing with other natural lines has resulted in the development of promising material. A number of *B. napus* cultivars derived using these methods have been released (Salisbury and Kadkol, 1989).

The wild forms of the respective diploid parents *B. oleracea*, *B. rapa* and *B. nigra* offer considerable potential as an underexploited source of genetic variability to be utilised in resynthesis programs. Mattson (1988) resynthesized *B. juncea* using a range of *B. rapa* subspecies. Kahlon *et al.* (1999) screened both the *B. rapa* and *B. oleracea* landraces for tolerance of manganese toxicity, selecting superior accessions with which to plan resynthesis of *B. napus*.

Wild *Brassicaceae* species

Many potentially valuable traits have been identified in wild *Brassicaceae* species. Examples include disease resistance (*Alternaria* blight, blackleg, white rust, clubroot), insect resistance (flea beetles), stress tolerance (drought, cold), different quality types and C3-C4 intermediate photosynthesis (Salisbury and Kadkol, 1989; Prakash and Bhat, 2007). However, a number of barriers have been identified to their successful introgression into *B. napus* or *B. juncea*. For example, Singh *et al.* (2007) evaluated 100 interspecific or intergeneric crosses combinations between cultivated *Brassica* species and wild *Brassicaceae* and reported significant pre-fertilization barriers in 73 crosses and post-fertilisation barriers in the other 27 crosses.

Where crosses have been successful (or embryo rescue has been used), some degree of homoeologous pairing between the chromosomes of the crop *Brassica* species and the wild *Brassicaceae* species has often been reported. However, sterility in the F_1 and subsequent generations has regularly limited successful gene transfer. Despite the large number of successful crosses reported between cultivated *Brassica* and *Brassicaceae* species, and the occurrence of some homoeologous pairing, there is very little published information on the successful introgression of useful nuclear genes from wild species to cultivated species (Salisbury and Kadkol, 1989). Based on the lack of successful introgression of wild genes into the cultivated genomes, Prakash and Bhat (2007) have suggested that the use of

molecular biology to identify, isolate and pyramid these genes in cultivated species should be pursued.

In contrast to the lack of successful nuclear gene transfer, the cytoplasm of weedy *Brassicaceae* species has been successfully transferred into *B. juncea* as a component of CMS systems (e.g. Banga *et al.*, 2003).

Protoplast Fusion

Protoplast fusion can overcome sexual barriers and has allowed somatic intergenomic hybrids to be created where sexual hybrids have not been reported. Many intertribal somatic hybrids have been created between wild species and cultivated *Brassica* species, with a view to incorporating useful wild genes into the cultivated species (Prakash and Bhat, 2007). In many instances, the desirable trait is expressed in the somatic hybrids, however, introgression remains a problem due to lack of sufficient intergenomic chromosome homoeology and also the high degree of sterility (Prakash and Bhat, 2007).

The most productive utilization of protoplast fusion has been in the development alloplasmics combining cytoplasm of wild species and the crop nuclei for expressing male sterility (Prakash and Bhat, 2007).

Mutation

Mutation has contributed some important traits to the allopolyploid *Brassica* gene pool, knocking out unwanted gene function. Examples include several modified fatty acid compositions.

Molecular Techniques

Genetic engineering provides an ability to overcome natural species barriers and allow effective gene transfer where not previously possible (Salisbury, 2002). Provided this technology gains widespread acceptance, it offers *B. napus* and *B. juncea* breeders in Australia access to an incredible range of new sources of variability, either through incorporation of new genes or the switching off of unwanted existing genes. The impact of the Roundup Ready and Seedlink hybrid traits in Canada clearly demonstrates the potential of this technology. In combination with GM technology, other molecular tools, including marker assisted selection and genomics provide more effective and efficient means of accessing germplasm from secondary and tertiary gene pools that have proved difficult or impossible to access. Further, affinity of *Brassica* with *Arabidopsis thaliana* (where the genome has been fully sequenced), offers wide opportunities to search for candidate genes for priority traits.

ROLE OF ATFCC

The Australian Temperate Field Crops Collection (ATFCC) maintains a core collection of the primary gene pool of each *Brassica* species. Geographically diverse collections of *B. napus* and *B. juncea* have been evaluated for molecular diversity using AFLPs to select a genetically diverse core for maintenance and further studies (Redden, *Pers. Comm.* 2007).

In addition, the ATFCC is developing a collection of the wild forms of the diploid progenitor species and the wild *Brassicaceae* species. This germplasm is available for utilisation by breeders. An increased focus on secondary and tertiary gene pools will result from new molecular technologies that break down traditional species barriers.

THE FUTURE

Redden *et al.* (2005) outlined a strategy not only for investigating landrace diversity in these gene pools through molecular characterisation and evaluation of core collections for agronomic and oil quality traits, but also for investigating diversity for key crop traits in the parental diploid species and their wild relatives. Exploitation of wild relatives through molecular techniques offers opportunities for novel allelic variation not only in major gene traits, but also quantitative traits via introgression of QTLs into the genetic background of elite cultivars as in recent genetic enhancement of wheat.

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