GAMETOSOMATIC HYBRIDISATION WITHIN THE BRASSICEAE FOR THE PRODUCTION OF ALIEN ADDITION AND SUBSTITUTION LINES OF *BRASSICA NAPUS.*

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Limited genome transfer into crop *Brassicas* from distantly related *Brassiceae* might be achieved via several routes. Depending on the route taken, transfer of information from the nuclear genome may involve single genes, partial chromosomes or whole chromosomes. The transfer of single alien genes via highly targeted approaches, e.g. *Agrobacterium* (De Block *et al.*, 1989) transformation is of value in many applications, but requires that the genes to be transferred are isolated and characterised. The use of X- or γ-irradiation on diploid protoplasts to promote the transfer of partial genomes has been studied in a number of species (Gupta *et al.*, 1984; Bates *et al.*, 1987). However, while this method has proved successful in some incidences, it has a number of disadvantages. Irradiation treatments typically result in extensive random damage to the genetic material.

In the small grain polyploid cereals, extensive use has been made of nullisomic, tetrasomic and alien chromosome addition and substitution lines to promote the integration of novel genes into crops from distantly related species. These aneuploid lines have also contributed to studies of chromosome homoeology, chromosome pairing and genome analysis. The polyploid nature of cereals such as *Triticum aestivum* makes it resistant to the loss of one or a few chromosomes from homologous pairs, this facilitates the development of aneuploid lines. *Brassica napus* as an allotetraploid, should also be able to tolerate manipulation of chromosome numbers.

In conventional breeding, aneuploids are produced by artificially synthesising alloploids or backcrossing generations of interspecific hybrids. Although widely applied, this approach has a number of drawbacks. For example, long periods of backcrossing are often necessary to restore the genome of the crop parent, infertility of F_1 hybrids associated with crosses between distantly related species sometimes occurs, and pre- and post-zygotic incompatibilities that are present prevent any transfer of traits of agronomic interest.

The availability of a regenerable protoplast system in *B. napus* provides the opportunity to use somatic hybridisation techniques in more direct approaches to the production of aneuploid lines. The procedures described here are producing somatic hybrids between *B. napus* and the two distantly related species *Moricandia arvensis* and *Sinapis alba*. Gametosomatic hybridisation is an established procedure for generating triploid hybrids in *Nicotiana* (Pental *et al.*,

1988) and petunia, the procedure has not been explored in *Brassica* species. Selfing of triploid hybrids would be expected to yield a range of aneuploid lines in subsequent generations. Experiments have been undertaken to evaluate the feasibility of gametosomatic hybridisation involving *B. napus* with *M. arvensis* and *S. alba* as haploid partners. The simplest route to obtain a source of haploid protoplasts from both *M. arvensis* and *S. alba* for use in gametosomatic fusions is to isolate protoplasts from pollen mother cells at the tetrad stage. Present studies have shown that pollen tetrad protoplasts from both *M. arvensis* and *S. alba* are readily digested, resulting in the release of a high proportion of viable protoplasts which remain intact throughout the fusion procedure. The most promising fusion treatment involved the use of the chemical fusogen polyethylene glycol (PEG). Both somatic and gametic protoplasts retained their viability in the presence of PEG, although this compound has previously been reported to seriously effect microspore protoplast survival (Pental *et al.*, 1988). Electrofusion is an alternative to chemical fusion (Bates, 1985). However, this method was found to be unsuitable for fusing protoplasts of *B. napus* with those of *M. arvensis* and *S. alba*, since the high voltage required for protoplast alignment caused rapid lysis of hypocotyl-derived protoplasts of *B. napus*. Nevertherless, hypocotyl-derived protoplasts of *B. napus* are highly regenerable and therefore are ideal recipients for the transfer of alien genes via gametosomatic hybridisation. An alternative to the use of gametosomatic fusion would be to fuse haploid somatic protoplasts of the alien species with diploid somatic protoplasts of *B. napus*. Experiments are in progress to regenerate haploid plants from anther/microspore tissues of alien species as a potential source of haploid mesophyll protoplasts. A second alternative would be to fuse diploid somatic protoplasts that are at different stages of the cell cycle. This could be achieved by synchronising a high proportion of suspension cells of the alien species through treatment with metabolic inhibitors. Protoplasts derived from highly synchronous suspension cells fused with relatively asynchronous somatic protoplasts of the crop species, *B. napus*, would cause premature chromosome condensation at cell division immediately post-fusion. Thus, an increase in spontaneous chromosome elimination in these hybrids would occur, resulting in a high frequency of aneuploids of *B. napus*.

The use of aneuploid lines, especially monosomic lines, in the genetic analysis of complex traits is well established, particularly in the *Triticeae* (Snape *et al.*, 1983; Law *et al.*, 1983). These methods have greatly facilitated the study and mapping of quantitative traits and characters with low heritabilities. A pool of aneuploid lines in *B. napus* could form the basis for similar analyses of the complex genetics underlying many agronomically important traits. Such stocks could also contribute to chromosome pairing and chromosome homoeology studies. The production of alien chromosome addition and substitution lines in *B. napus* has the potential to promote the genetic analysis of important traits such as the fatty acid composition and levels of glucosinolates in extracted seed oil and meal. An understanding of the genetic control of such complex traits is invaluable in attempts to genetically improve *B. napus* via conventional breeding or biotechnology.

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