Brassica and Oilseeds Research in Norwich

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Genetics of glucosinolates in *Brassica* and *Arabidopsis*

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Glucosinolates are thioglycosides found in the Brassicaceae. The molecule consists of a glycone moiety and a variable aglycone side chain which is derived from an amino acid. Following tissue disruption, they are hydrolysed to an array of products many of which have important biological properties. The most prominent of these products under standard conditions are the isothiocyanates ('mustard oils'). Research at the JIC focuses on the genetic regulation of the biosynthesis of glucosinolates with aliphatic side chains derived from methionine. These are the major class of glucosinolates found in *Brassica* crops and in the leaves of *Arabidopsis thaliana*. Genes which regulate the biosynthesis of these compounds have been positioned on RFLP maps of the *Brassica* and *Arabidopsis* genome. Current research is attempting to isolate two of these genes from *Arabidopsis* by map based cloning. One of these regulates the initial entry of methionine into glucosinolate biosynthesis and the length of the aliphatic side chain, while the other regulates subsequent modifications to the aliphatic side chain.

Glucosinolates are important in both oilseed and horticultural *Brassica* crops. In oilseed rape, the level of glucosinolates in the seeds determines the value of the meal left after oil extraction. In addition, glucosinolates in the leaves and roots mediate pest and pathogen interactions. Oilseed rape lines with modified leaf glucosinolates which produce a different array of volatile compounds compared to standard oilseed rape cultivars have been developed. The effect of these modifications on specialist and generalist herbivores, and their parasites, is currently being studied.

Certain isothiocyanates have been shown to have pronounced anticancer activity in mammalian systems by the induction of phase 2 detoxification enzymes such as glutathione transferase. The most potent of these is 4-methylsulphinylbutyl isothiocyanates (sulphoraphane) derived from the glucosinolate glucoraphanin. In a collaborative programme with IFR, Norwich and industry, broccoli breeding lines are being developed which have enhanced levels of this particular glucosinolate by the introgression of novel alleles from wild *Brassica* species.

Transgene Insertion and Expression in Horticultural *Brassicas*

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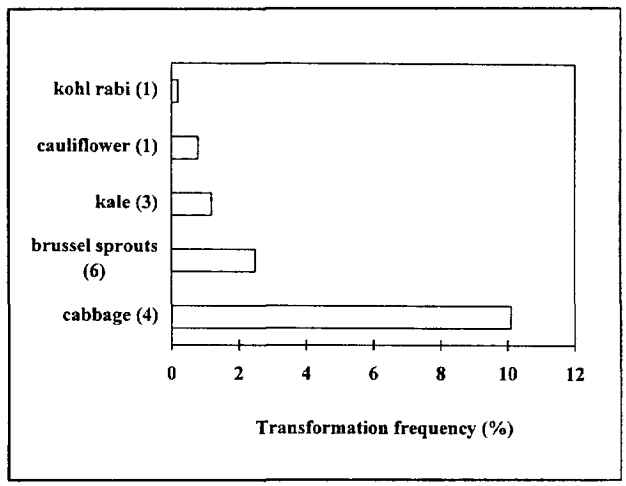
The *Brassica genus* includes several economically important vegetable and oilseed species. Of these, *Brassica oleracea* is one of the largest groups of vegetables with a worldwide distribution. The aim of our research is to develop efficient transformation in horticultural brassica species including *B. oleracea* and to study the genetics, stability and inheritance of transgenes in these crops.

To achieve the full potential of genetic modification, genotype independent regeneration and transformation systems are required. An efficient shoot regeneration procedure has been developed for hypocotyl and cotyledonary explants of six vegetable types of *B. oleracea* for which more than 80 % of explants regenerated one or more shoots. Selected lines were used to develop a transformation system. Cotyledon explants from sterile seedlings were inoculated with *Agrobacterium tumefaciens* containing an nptII selectable marker gene (which confers resistance to amino glycoside antibiotics such as kanamycin) and the ß-gucuronidase (*uidA*) reporter gene. Following cocultivation with *Agrobacterium*, explants were transferred to kanamycin containing medium to select for transformed shoots. Fertile transgenic lines (T° generation) were successfully produced from at least one genotype of five vegetable types of *B. oleracea*. Shoots expressing the *uidA* marker gene have now also been produced from two genotypes of broccoli. Transgenic lines were analysed for *uidA* expression using histochemical and quantitative assays. Histochemical staining revealed intense blue coloration in individual transgenic plants. PCR analysis confirmed the presence of the *nptII* and *uidA* genes. Transformation frequencies of up to 10 % were obtained where transformation frequency is expressed as the percentage of original explants regenerating one or more transformed plants. Figure 1 shows the range of transformation frequencies obtained for each vegetable type. More than 150 independent transformed plants have been produced and this material is currently being analysed for transgene copy number and stable inheritance in the T1 generation.



A Description of Genome Synteny between *Arabidopsis thaliana* and *Brassica napus*

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The *Brassica genus*, which includes the diploid species *B. rapa*, *B. nigra* and *B. oleracea* that form importrant oilseed, vegetable and fodder crops, is closely related to *Arabidopsis thaliana* which has become adopted as a model for both physical genome analysis and the study of plant growth and development. Although this phylogenetic affinity is reflected by 87-% conservation of homologous gene coding sequences, their genome organisations are markedly different. *Arabidopsis* has a compact genome of 145 Mbp, with little gene duplication or repetitive DNA, making it well-suited to gene isolation with map-based or chromosome walking techniques. In contrast, the diploid *Brassica* species have genome sizes around 650 Mbp with complex, internally duplicated structures. Furthermore, some important *Brassica* crop species are actually amphidiploids formed by evolutionarily recent hybridisations. For instance, *B. napus* (oilseed rape) comprises the : *B. rapa* (A, n = 10) and *B. oleracea* (C, n =9) genomes.

Some recent studies in the Graminae and Solanaceae have shown that collinearity of marker order, or synteny, can exist over large genetic intervals even between quite diverged species. Where synteny can be established with a physically mapped and sequenced model organism, gene identification in crops species may be greatly facilitated. We have

undertaken systematic comparative genetic mapping between *B. napus* and *Arabidopsis* allowing defined segments of the *Arabidopsis* genome to be simultaneously compared with the A and C genomes of *B. napus* and *vice versa*. This has enabled us to deduce a likely evolutionary history of the Brassicaceae and to assess the feasibility of shuttling between *Arabidopsis* and *B. napus* maps for gene isolation.

Arabidopsis probes comprising ESTs, *PstI* genomic clones, cosmids and YAC endprobes were selected which mapped to three regions of the *Arabidopsis* genome : the top of chromosome 3 (approximately 30 cM) ; two thirds of the long arm of chromosome 4 (10 Mbp or approximately 30cM) and the top of chromosome 5 (approximately 60 cM). These were hybridised to the *B. napus* mapping population so identifying regions of the genome homologous to the targeted *Arabidopsis* segments. *B. napus PstI* genomic probes which detected RFLP loci in those regions were then genetically mapped in *Arabidopsis* or directly hybridised to the YAC contig from *A. thaliana* chromosome 4.

Each segment of the *Arabidopsis* genome tested was found to be represented by three distinct, syntenic regions in each of the two constituent diploid genomes of *B. napus*, making six copies in all. For example, a region on the long arm of chromosome 4 has homologues on the *B. napus* linkage groups N1 and N11, N3 and N17 and N8 and N18, each pair being full or partial homologues from the A and C genomes. Within each of these regions, the marker order was either perfectly conserved or interrupted by simple rearrangements. Our results thus indicate substantial conservation of gene collinearity at the macro level, suggesting that physically mapped clones and DNA sequence data from *Arabidopsis* will of direct used in the identification and isolation of genes in *Brassica* crops. This study has also highlighted the pattern of intra-genomic duplication in *Brassica*. It has yielded strong evidence that the extant *Brassica* diploids descend from a hexaploid common ancestor whose genome complexity was similar to that of present day *Arabidopsis*.