GCIRCB11*05

Brassica and Oilseeds Research in Norwich

Communications from the Brassica and Oilseeds Research Department
JI Centre
Colney, Norwich, NR4 7UJ, U.K.

Lipid and starch synthesis in developing oilseed rape embryos

S Rawsthorne, M J Hills, F Kang, D J Lacey, C L Morgan, D H Hobbs

Embryos of oilseed rape (*Brassica napus*) accumulate storage oils and proteins as they develop although the onset of oil synthesis is earlier. Furthermore, starch also accumulates transiently before oil accumulation is completed. These storage compounds all rely on the metabolism of sucrose, which is imported by the developing embryo, to produce the carbon skeletons from which they are made[1]. The biochemical pathways between sucrose and the synthesis of storage oils and starch are complex and involve enzymes located in different subcellular compartments[1]. Both the starch and fatty acids which are used for storage oil synthesis are produced in the plastids of the cell while the sucrose is initially broken down into a range of carbon compounds in the cytosol. Furthermore, the modification of fatty acids and assembly of the triacylglycerol (TAG: storage oil) involves enzymes present in the endoplasmic reticulum (ER).

Studies of the activities of glycolytic and other enzymes in plastids isolated from developing oilseed rape embryos suggested that a range of metabolites could be imported from the cytosol and used within the plastids for the synthesis of either starch, fatty acids or both. In order to determine whether these cytosolic metabolites could cross the plastidial membrane, isolated plastids were incubated with ^{14}C-labelled compounds and the fatty acids and/or starch were then isolated and the amount of ^{14}C that had been incorporated was determined. The most effective substrate for starch biosynthesis was glucose-6-phosphate (G6P) although other hexoses and hexose phosphates were also utilised (Fig. 1).

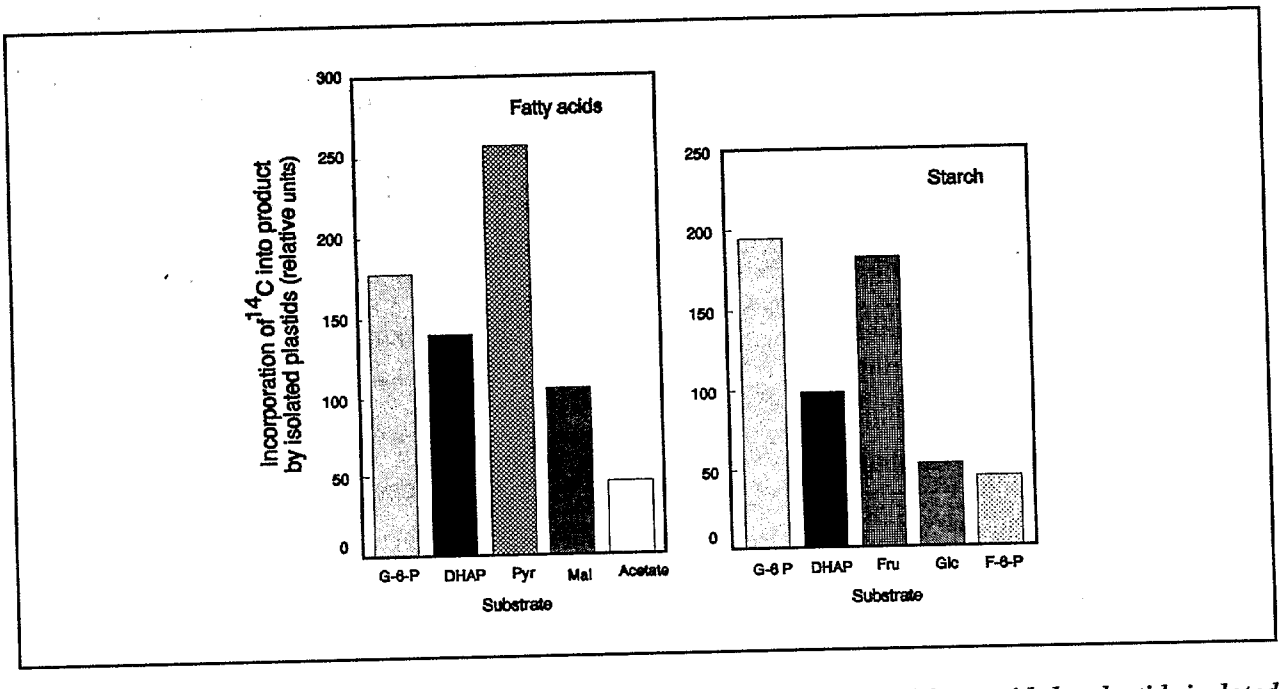

Fig. 1. Uptake and incorporation of ^{14}C-labelled metabolites into starch and fatty acids by plastids isolated from developing embryos of oilseed rape (G-6-P: glucose-6-phosphate; DHAP: triose phosphate; Fru: fructose; Glc: glucose; F-6-P: fructose-6-phosphate; Pyr: pyruvate; Mal: malate)

Glucose-6-phosphate is also the most effective substrate for starch synthesis by plastids isolated from developing pea embryos[2]. Triose phosphate (DHAP) also supported starch synthesis implicating metabolism via the plastidial fructose-1,6-bisphosphatase, an enzyme which is generally absent from non-photosynthetic plastids. Pyruvate was the most effective substrate for fatty acid synthesis although G6P, DHAP and malate were also utilised at a reasonable rate (Fig. 1). Acetate was used only very poorly. These experiments have shown that during the simultaneous synthesis of starch and fatty acids in rapeseed embryos, metabolites such as G6P and DHAP can be used by more than one pathway and there is therefore a partitioning of carbon within the plastid. Furthermore, the use of different carbon skeletons, which are derived from sucrose in the cytosol, for different plastidial pathways suggests that partitioning of carbon also occurs outside of the plastid. The regulation of this carbon partitioning is currently being investigated.

The first committed step in the synthesis of fatty acids is that catalysed by the enzyme acetyl-CoA carboxylase (ACCase). This enzyme had been thought to be a potential control point in storage oil synthesis in developing rapeseed embryos because its activity increased at the same time as the storage oil began to accumulate in the seeds[3]. However, using a new and more sensitive assay for ACCase we have shown that the enzyme activity increases well before storage oil begins to accumulate in developing rapeseed embryos (Fig. 2). Furthermore, its activity then remains relatively constant while the rate of oil accumulation increases (Fig. 2). The lack of correlation between these two processes calls into question the role of ACCase in regulating the synthesis of fatty acids. Nevertheless, since this enzyme lies at such an important position in the pathway we are currently using other methods to determine the extent to which it influences the rate of storage oil accumulation.

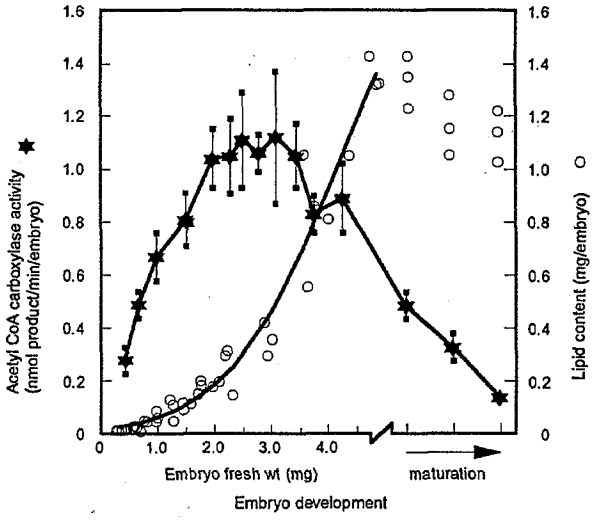

Fig. 2. Activity of acetyl-CoA carboxylase and the lipid content of developing embryos of oilseed rape.

The fatty acids synthesized by the plastid are exported as acyl-CoAs to the endomembrane system where glycerolipid synthesis occurs. Until recently it was not clear how these acyl-CoAs were transported in plants. However, we have shown that *B. napus* expresses an acyl-CoA binding protein (ACBP) gene in all tissues examined, including developing seeds. The ACBPs are thought to transport acyl-CoAs between subcellular compartments, to prevent them from binding to membranes where they act as detergents, and to protect them from hydrolysis by cytosolic thioesterases. The amino acid sequence derived from a cDNA clone encoding ACBP which was isolated from developing rapeseeds has greater than 40% identity with the ACBP from animals and yeast and contains all the amino acids thought to be essential for binding of acyl-CoA[4].

Membrane and storage lipids are synthesized from acyl-CoAs by the Kennedy Pathway[1] with the first three steps being common to both products. Diacylglycerol (DAG) lies at the branch point between membrane lipid (phospholipid) and TAG synthesis. It is not clear whether two separate pathways run in parallel for the production of these two lipid types or whether the enzymes and the DAG pool are common to both. We have characterized the membranes involved in glycerolipid synthesis using density gradient centrifugation to separate the membranes and organelles from homogenates of developing rapeseed embryos. Enzymes involved in TAG synthesis were found in two distinct membrane fractions, one having a very low density (1.045 g ml^{-1}), the other having a density more typical for ER (1.10 g ml^{-1}). Two enzymes of phospholipid synthesis, one of them an enzymic marker often used to locate the ER (choline phosphotransferase) and lysophosphatidylcholine acyltransferase, were found only in the heavier of these

membrane fractions and were almost undetectable in the light fraction. It is clear from these observations that the endomembrane system is not homogeneous with respect to its function in terms of lipid metabolism. The light fractions contain much more triacylglycerol relative to phospholipid than the ER but much less than mature oil bodies. They also do not have oleosins (structural proteins of mature oil bodies) associated with them. It therefore appears that the light membrane fraction is not derived from fragments of oil bodies produced during homogenization of the tissue. The role of the lighter membrane fraction in oil body biogenesis is not yet clear though there are several possibilities. They may be i) regions of membrane, contiguous with the endoplasmic reticulum which are specialized for the synthesis of TAG and become detached from the ER during homogenization of the cell ii) nascent oil bodies which continue to grow as TAG is synthesized to eventually become full sized oil bodies, or iii) membrane vesicles which transport TAG from the ER to the developing oil bodies whilst continuing to synthesize it. Ongoing studies should help to elucidate their origin and role.

References

1 *Murphy, D J, Rawsthorne, S, Hills, M J (1993)* **Seed Science Research 3,** 79-95.
2 *Hill, L M, Smith, A M (1991)* **Planta 185,** 91-96.
3 *Turnham, E, Northcote, D H (1983)* **Biochemical Journal 212,** 223-229.
4 *Hills, MJ, Dann, R, Lydiate, D and Sharpe, A (1994)* **Plant Molecular Biology 25,** 917-920

Glucosinolates in *Brassica* and *Arabidopsis*

A Giamoustaris, I Parkin, M Trick and R F Mithen

Glucosinolates are the major secondary metabolites in *Brassica* and *Arabidopsis*. These compounds and their hydrolytic products determine the flavour of *Brassica* vegetable and salad crops, mediate pest and pathogen interactions and the feeding quality of oilseed rape meal. Recently, it has been shown that the hydrolytic product of methylsulphinylbutyl glucosinolate, which is the predominant glucosinolate in several *Arabidopsis* ecotypes, is highly effective at inhibiting carcinogens.

Studies have concentrated on elucidating the genetic basis to variation in the side chain structure of aliphatic glucosinolates. It has been shown that the diversity of aliphatic glucosinolates in *Brassica* and *Arabidopsis* is due to the interaction of one set of genes which determine side chain length and another set which modifies the side chain structure irrespective of its length. Hence a small number of genes can produce a complex glucosinolate profile. The genetic model which has been proposed is applicable to both *Brassica* and *Arabidopsis* and it is thought that the genes regulating particular parts of the biosynthetic pathway in these two taxa are likely to be homologues of each other. To support this, it has been shown that *Arabidopsis* RFLP markers mapping near to the *Gsl-elong* gene (which regulates elongation of the aliphatic side chain) also map near to the putative homologous gene in the *Brassica* genome. This suggests that this region of the genome has been conserved since the divergence of these two taxa. Current research is focused on cloning the *Gsl* genes from *Arabidopsis* in order to manipulate the glucosinolates which occur in *Brassica* crops.

A bidirectional nuclear gene promotor in *Brassica napus*

D J Murphy, C Batchelder, J Keddie, D Milioni, P Piffanelli

Although bidirectional genes are present in many prokaryotes and a few animals, few examples have been reported in the plant nuclear genome. We have recently identified a bidirectional gene in *Brassica napus* which contains two overlapping promotors driving the independent expression of two open-reading frames on opposite DNA strands.

The divergent gene was isolated from *B. napus* as a genomic DNA clone using a seed oleosin cDNA probe[1]. The clone contained 2.5kb of 5' non-coding sequence, followed by two exons encoding one of the seed oleosin protein family.

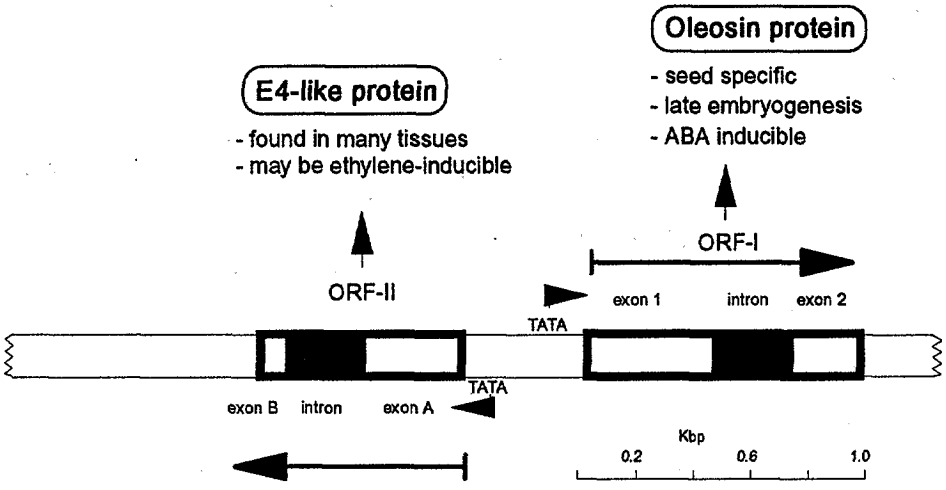

Fig. 1. The bidirectional gene found in Brassica napus and Arabidopsis thaliana.

Analysis of the complementary strand of the oleosin promotor revealed an additional open-reading frame (ORF-II) containing two exons which lie some 400b upstream of the oleosin open-reading frame (ORF-I). The ORF-II encodes a novel protein which is 76% similar to the ethylene-induced E4 gene product from tomato and 66% similar to the peptide methionine sulphoxide reductase (PMSR) of *E.coli*. A homologous bidirectional gene is also present in *Arabidopsis*.

Whereas the oleosin (ORF-I) protein is ABA-inducible and seed specific, the ORF-II mRNA can be detected in many tissues. We are now studying the effect of ethylene on ORF-II expression. Much of the respective promotor regions of these two ORFs are contained within the open-reading frame of the complementary gene, and intriguing problems are therefore posed for the regulation of their expression, particularly if this is simultaneous.

References

1 *Keddie, J S, Hubner, G, Slocombe, S P, Jarvis, R P, Cummins, I, Edwards, E W, Shaw, C H, Murphy, D J (1992)* **Plant Molecular Biology 19,** 443-453.

The elucidation of Quantitative Trait Loci in the Brassicas

AE Arthur, MA Ford, D Keith, I Parkin, DJ Lydiate

Many traits of commercial, biological and scientific interest are quantitatively inherited, that is, they are controlled by a number of genes with small, cumulative effects rather than single, major genes. The elucidation of the quantitative trait loci (QTLs) associated with these quantitative genes in any species is dependent upon the availability of numerous genetic markers scattered throughout the genome. As in many species, the Brassicas lack the number and distribution of morphological markers to enable the mapping of QTLs or, in many cases, even genes of major effects. However, as saturated genomic maps of Restriction Fragment Length Polymorphisms (RFLPs) becoming increasingly available for the Brassicas, it is now feasible to search these genomes for QTLs associated with particular traits[1]. This will enable us to locate quantitative genes on the emerging genomic maps and analyse the contributions made by different regions of the genome to the traits in question. This will bring closer the attractive possibility of manipulating the genes associated with quantitative traits in similar ways to those now well established for genes of large effect, the major genes. However, because of the complicated genetic properties of quantitative variation, considerable effort will be required to bring the manipulation of QTLs to the level applied to the major genes.

Recombinant doubled haploid lines from two large *Brassica napus* populations derived from contrasting crosses[2] were used to investigate a range of plant characters showing quantitative variation. The lines were grown in extensively replicated field trials in two growing seasons, 1991-92 and 1993, in three contrasting environments in 1991-92 (viz. autumn planted, spring planted with and without vernalisation) and two in 1993 (autumn planted, and spring planted without vernalisation). The characters assessed included time to and node number at flowering, plant height, and traits associated with yield such as seed number, size and density in the pod, pod length and orientation. The data were subjected to statistical analyses to determine the contribution and significance of the major sources of variation associated with genotypes, environments (summarising the effects of planting times, treatments and years), the interaction between the two (the genotype x environment interaction) and residual or error variation.

The relative contributions of these sources of variation to the total phenotypic variation vary greatly amongst the characters assessed. In some cases, a significant proportion of the total variation was accounted for by the genetic component or by the environmental or the residual component. However, in other cases, the interaction between genotypes and environments was significant, indicating that, for these traits, the performance of the genotypes relative to one another depended on the environment in which they were grown. The latter traits, and those with high residual components, are particularly difficult to work with since their performances are unpredictable. Thus, characters with high genetic components were selected for further detailed studies and analysis of the QTLs as these are likely to be more reliable and easier to resolve than those with high interaction and/or residual components.

Data for these characters are being subjected to various statistical analyses to determine any association between the trait means and the RFLP markers used to construct, and therefore already located on, the genomic map. Various methods have been developed for achieving this but essentially all provide information about the extent to which the markers used in the mapping are associated with regions of the genome that explain significant variation for the trait in question. These regions are likely to contain important genes that control the expression of these traits. Despite the numerous complexities of the statistical analyses and those concerning the location of the markers on the maps, some very exciting results are emerging, offering considerable promise that the regions of the genome associated with some of these quantitative traits can be identified.

References

1 *Paterson, AH, Lander, ES, Hewitt, JD, Peterson, S, Tanksley, SD (1988)* **Nature** 335, 721-726
2 *Parkin, I, Sharpe, A G, Keith, D J, Bowman, C M, Arthur, A E, Lydiate, D J (1992)* **IPSR and JIC Annual Report,** 11-13.

A chromosome image analysis system for the identification of individual *Brassica* chromosomes.

R J Mathias, C M O'Neill, Y Kamisugi
K Fukui, K Kawakami, N Ohmido (Hokuriku National Agricultural Experiment Station, Japan)

The aim of this UK-Japan collaborative project is to adapt a computer-based **CH**romosome **I**mage **A**nalysis **S**ystem (CHIAS), originally developed by Dr Fukui to karyotype the rice genome[1], to identify the individual chromosomes of crop Brassicas and related species. The establishment of a CHIAS for the *Brassicaceae* would facilitate gene mapping, characterisation of interspecific hybrids and the introgression of novel variation, the production of aneuploid genetic stocks and genetic analysis of complex traits.

Cytogenetic techniques have made a significant contribution to the genetic analysis of the wheat, rye and barley genomes but such studies have only been practical because the *Triticeae* have relatively large chromosomes. Despite their obvious value the use of cytological and cytogenetic techniques within the *Brassicaceae* has been largely prescribed by the small size and uniform structure of the chromosomes of the Brassicas and related species. Although some chromosome addition lines have been produced by using biochemical and molecular markers to "track" individual chromosomes[2], in general cytogenetic studies in the Brassicas have been impossible.

Non-uniform chromatin condensation is commonly observed as an uneven staining pattern in small plant chromosomes at prometaphase[3]. This uneven chromatin condensation pattern (CP) has been exploited as a diagnostic tool, along with chromosome length and arm ratio, to characterise the small chromosomes found in rice[1] and soybean[4]. This collaborative project has established that the same technology is effective for the karyotyping of the genomes of the diploid Brassicas of U's triangle[5]. Root tip chromosomes are prepared by an "air-dried spread" technique which results in preparations where chromosomes are spread out with minimal structural distortion. Video images from suitable spreads are aquired via a high resolution CCD TV camera from photomicrographs, or directly from the microscope, and processed by the CHIAS. Initial processing eliminates background noise from the image eg. the effects of uneven illumination and voltage fluctuations. Chromosome/background boundaries are then defined to outline the chromosomes and the chromosome images are extracted. Finally a line is drawn along the midrib of one, or both, of the chromatids of each chromosome and the CHIAS measures the staining intensity (CP) under this line. The resulting CP when averaged over several samples has proved a powerful diagnostic indicator that, when combined with other image data, is characteristic for individual *Brassica* chromosomes.

Preliminary karyotypes for *B. nigra*, *B. oleracea* and *B. rapa* have been prepared[6]. Currently the project is optimising the protocol for use with *Brassica* species and extending the method to the karyotyping of the allotetraploid *B. napus* and other Crucifer species. Clearly, this new system for rapid chromosome recognition has opened up a new dimension in the analysis of the *Brassica* genome. Work is in progress to exploit the CHIAS in karyotyping aneuploid lines of crop Brassicas produced via dihaploid x haploid and interspecific crosses.

References

1 *Fukui, K, Ijima, K (1992)* **Miscellaneous Publications of the National Institute of Agrobiological Resources (Japan) 4,** 1-25
2 *Chevre, AM, This, P, Eber, F, Deschamps, M, Renard, M, Delseny, M, Quiros, CF (1991)* **Theoretical and Applied Genetics 81,** 43-49
3 *Fukui K (1989)* **Chemical Biology 27,** 303-307
4 *Kanagisawa, T, Tano, S, Fukui, K, Harada, K (1991)* **Theoretical and Applied Genetics 81,** 606-612
5 *U, N (1935)* **Japanese Journal of Botany 7,** 389-482
6 *Fukui, K, Ohmido, N, Mathias, RJ (1993)* **Proceedings of the 57th Annual Meeting of the Botanical Society of Japan 313**

Assaying and exploiting synteny between *Arabidopsis thaliana* and *Brassica* species

A Cavell, J Oldknow, I Parkin, J Rogers, C Dean, D J Lydiate and M Trick

Genetic maps have been developed for a number of different plant species over many years. A good genetic map, with dense and even marker coverage over all linkage groups, can facilitate a wide variety of studies including the cloning and manipulation of genes which control characters of agronomic interest. In some species, notably *Arabidopsis thaliana* and rice, genetic maps are being integrated with physical maps derived from the cloning of entire genomes in overlapping YAC and cosmid libraries. Moreover, recent developments in comparative genetic mapping have revealed that basic gene order along chromosomes (synteny) is substantially conserved amongst diverse taxa, making the work on physical mapping of model genomes relevant to many crop species.

We are gauging the extent of synteny between *Arabidopsis* and *Brassica* species. By comparing homologous regions of intergenic DNA we can study mechanisms of genome evolution and exploit synteny for gene isolation. Synteny relationships can indicate whether similar mutant phenotypes in different species are analogous or caused by variations in truly homologous genes. For instance, a curly-leaved *Arabidopsis* mutant isolated through transposon insertion by Dr G. Coupland and colleagues (Molecular Genetics Department, JI Centre, Norwich) appears to be homologous to an allele producing a similar effect in a segregating *Brassica* population. Agronomic traits in a crop plant such as *Brassica napus* may not always have an obvious counterpart in *Arabidopsis* but, where an uncharacterized gene is revealed, its fine mapping and isolation will be aided by shuttling between the genetic and physical maps of the two genomes.

We are measuring synteny at different levels of resolution by genetically mapping *Arabidopsis* probes in our *B. napus* populations. The *Arabidopsis* probes are of several types, including established RFLP markers, YAC end-probes, cosmid fragments and cognate cDNA clones. In one study we have targeted a large contig, more than 2.2 Mbp long, on *Arabidopsis* chromosome 4 that was assembled by our colleagues in the Molecular Genetics Department. We have identified regions of synteny on two homoeologous linkage groups in *B. napus* (Fig. 1) which reflect its amphidiploid origins. Other regions of synteny are disclosed which support the hypothesis of an internal triplication within the unit *Brassica* genome. In other work, we are examining a region of *Arabidopsis* chromosome 5 which contains genes controlling glucosinolate biosynthesis common to both plants (see Giamoustris *et al.* below). A wider survey, using a new set of *Arabidopsis* genomic probes, will paint a broader picture of synteny on all chromosomes. However, it is already emerging that short-range synteny, such as that seen for the chromosome 4 contig, is the rule with perhaps the majority of interruptions corresponding to the breakpoints defining the internal architecture of the *Brassica* genome.

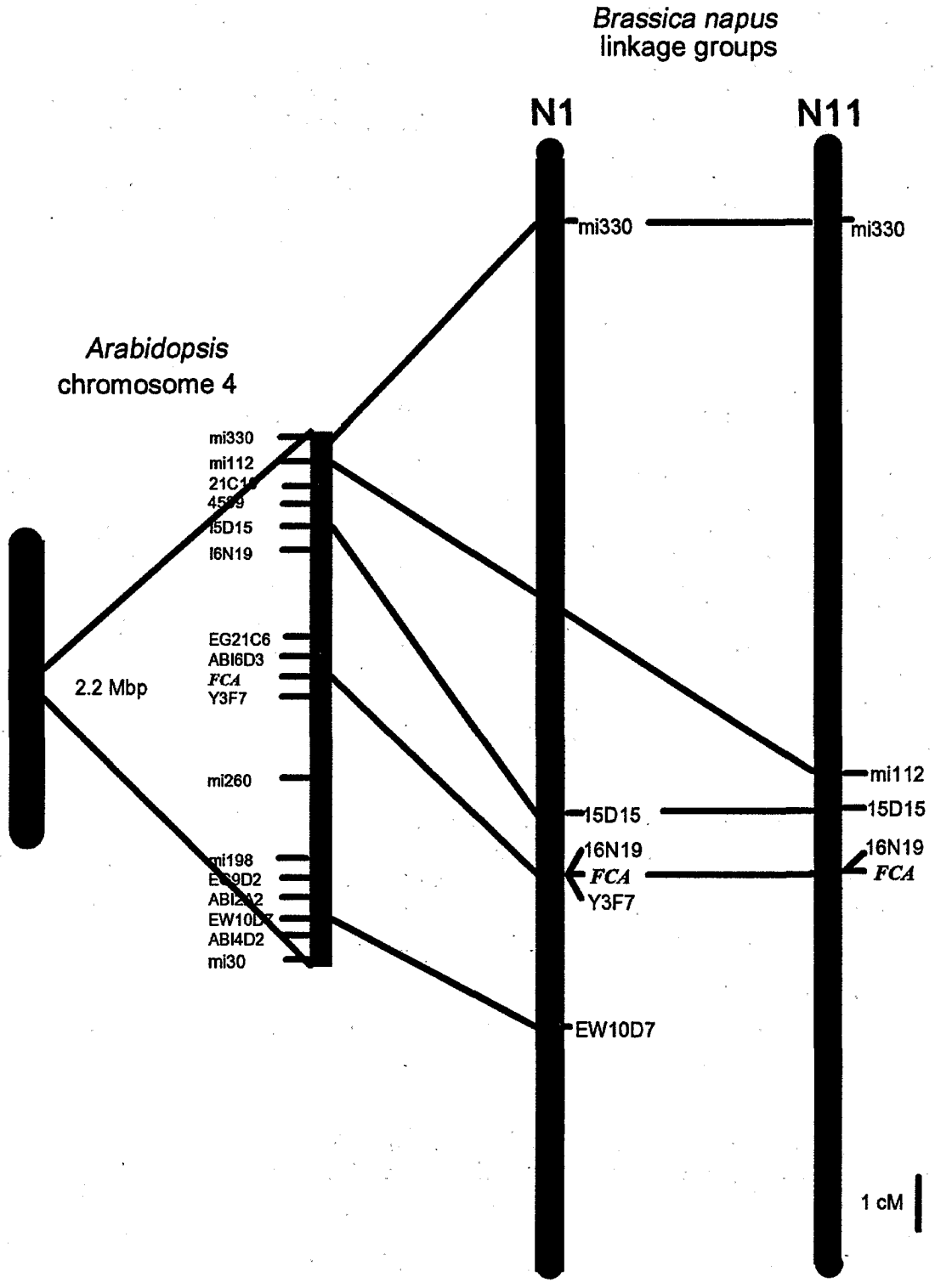

Fig. 1. Progress in aligning a large contig on *Arabidopsis* chromosome 4 with its homologues in *Brassica napus* by genetic mapping.

Transgene stability - Inheritance and expression in *Brassica napus*

N E Owen, C S Jones and P J Dale

The successful commercialization of genetically modified crops will depend on the level of transgene expression and the stability of the foreign gene in successive generations. Consequently, knowledge of transgene instability and its causes will be of considerable benefit when registering transgenic varieties. Oilseed rape (*Brassica napus*) will be one of the first crop plants to have registered transgenic varieties for widespread use and yet little is known about the stability of transgenes in this species. Transgene instability has been observed by many companies involved in the commercialization of transgenic crops[1], but little data has been published.

We are currently assessing the stability of transgene expression and tissue-specificity of various promoters in successive generations of oilseed rape. Constructs containing constitutive and embryo-specific promoters, regulating common reporter genes, have been introduced into *B. napus* via *Agrobacterium*-mediated transformation (Fig. 1)[2]. Histochemical and quantitative analyses are being used to determine the stability of transgene expression in lines containing different T-DNA insertions.

Primary transformants were characterized at the biochemical level for alterations to tissue specificity or enzyme expression levels and at the molecular level to determine transgene copy number. Two lines containing constructs with embryo-specific promoters showed some alteration in tissue-specificity, with the reporter gene expressed in both flowers and leaves. PCR analysis of these lines showed the promoter to be complete. Those primary transformants containing either one or two copies of the transgene were both selfed and backcrossed. The F_1 progeny were analysed for Mendelian segregation of the transgenes and the lines classed according to the type of stability/instability observed:

I Stable
II Physical loss of transgene
III Inactivation of transgene
IV Alteration in tissue specificity
V Gross alteration in expression levels between generations

Class II and III instabilities have been observed for each promoter. In addition, primary lines containing the embryo specific promoters showed class IV instability which was stably inherited in the F_1 generation. We are currently screening the F_2 generation of the characterized lines. The significance of any instability observed during this study, and possible epigenetic effects, will be considered with reference to their potential effect on the successful commercial release of transgenic crops.

Acknowledgements
This work was funded by the Ministry of Agriculture, Fisheries and Food.

References

1 **Finnegan, J and Elroy, D** (1994) *Biotechnology 12,* 883-888
2 **Radke, S E, Turner, J C and Facciotti, D** (1992) *Plant Cell Reports 11,* 449-505.
3 **Jones, J D G, Shlumukov, L, Carland, F, English, J, Scofield, S R, Bishop, G J and Harrison, K** (1992) *Transgenic Research 1,* 285-297.
4 **Keddie, J S, Tsiantis, M, Piffanelli, P, Cella, R, Hatzopoulos P and Murphy, D J** (1994) *Plant Molecular Biology 24,* 327-340.

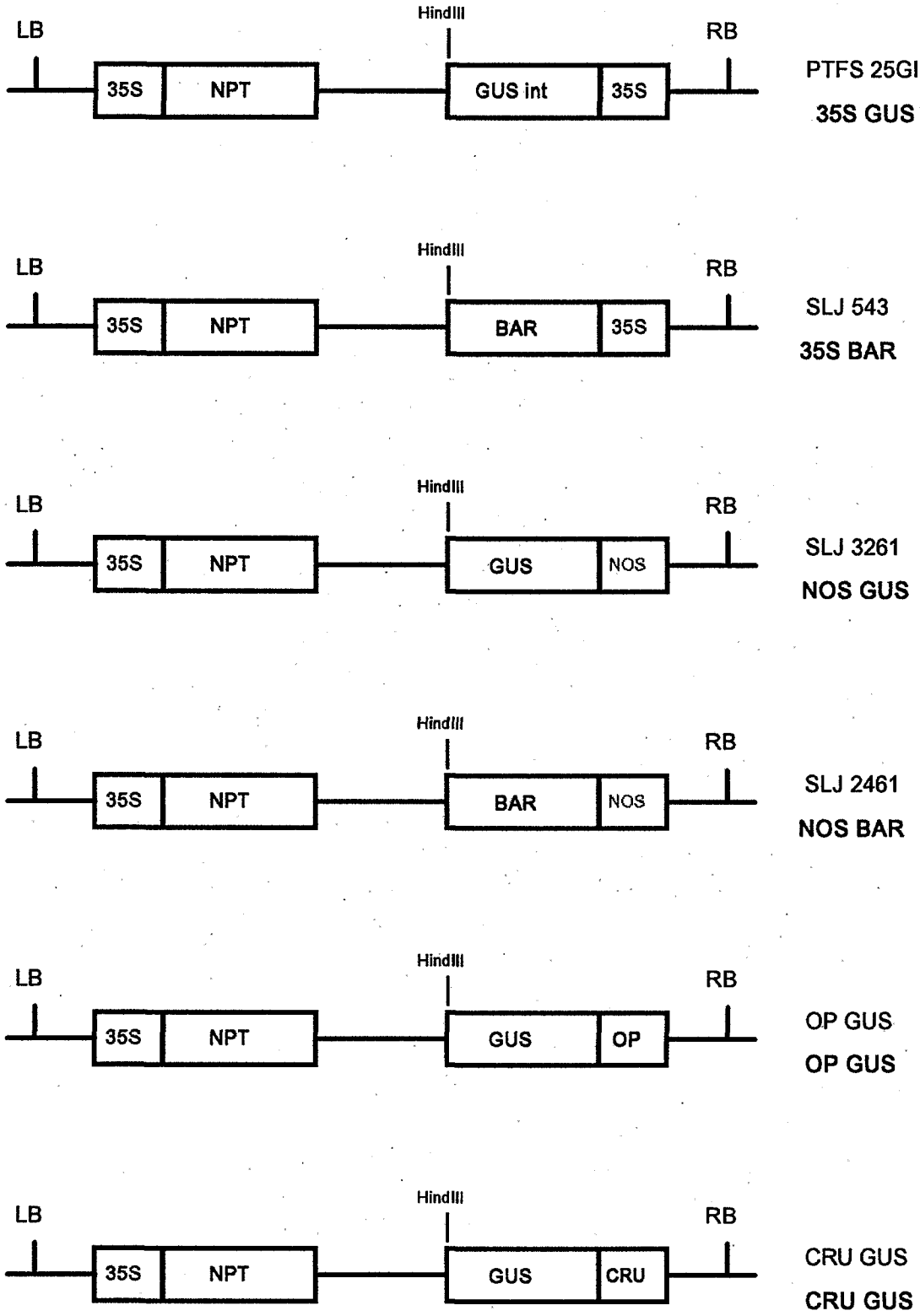

Fig 1. Constructs, based on pSLJ binary plasmids[3], introduced into *B. napus*. The constitutive promoter 35S, regulating the *gus* reporter gene and the *bar* gene (conferring resistance to the herbicide Basta); the nopaline synthase promoter (Nos) regulating the *gus* reporter gene and the *bar* gene; the embryo specific promoter cruciferin (Cru) from *B. napus* regulating the *gus* reporter gene and the embryo specific promoter oleosin (Op) from *B. napus* driving the *gus* reporter gene[4].

Transformation of the C_3-C_4 intermediate species *Moricandia arvensis* to a C_3-like phenotype

L Rylott, T Franza, S Turner, C Morgan and S Rawsthorne

Our previous studies of the C_3-C_4 intermediate species *Moricandia arvensis* have shown that the low rate of apparent photorespiration in this plant is due to two main features. The first is the development of the bundle-sheath cells (which surround the vascular tissue). In these cells there are numerous mitochondria at the cell faces adjacent to the vascular bundle centipedal faces and in close association with the chloroplasts. The second is a difference in the photorespiratory metabolism of *M. arvensis* compared to that of related C_3 species. This difference is due to the lack of one of the subunits (the P protein) of glycine decarboxylase (GDC), the enzyme which releases CO_2 during photorespiration, from the mesophyll cells[1]. We had proposed that the lack of GDC from the mesophyll and the confinement of photorespiratory CO_2 release to the bundle-sheath leads to an enhanced recapture of this CO_2 within the leaf. This in turn leads to a lower CO_2 compensation point (Γ) than in a C_3 plant[2].

In order to test our hypothesis we transformed *M. arvensis* with a gene construct (CaMV35S promoter driving a cDNA encoding the P protein of *Pisum sativum*[3]) that we believed would restore a functional GDC to the mesophyll cells. The biochemical, anatomical and physiological consequences of this transformation have been examined. Two groups of transformed plants were produced. One group showed a profound change to a C_3-like phenotype with respect to the presence of P protein in the mesophyll cell mitochondria, the increase in Γ, and surprisingly in the anatomy of the bundle sheath cells (Table 1) In particular, the organelles within the bundle-sheath cells were no longer clustered at the centripedal face and were distributed around the periphery as in a C_3 plant. In the other group of transformants the P protein was not detected in the mesophyll mitochondria and the plants otherwise resembled untransformed *M. arvensis*. The reasons for lack of detectable P protein in this latter group remains to be determined. However, these findings suggest that the restoration of GDC activity to the mesophyll cells does prevent the enhanced recapture of photorespired CO_2 in the bundle sheath cells and so gives a C_3-like Γ. More interestingly they also suggest that the development of the bundle-sheath cell is influenced by the changes to the metabolism in the leaf. We are now starting to investigate this intriguing link between the two principal characters of C_3-C_4 plants.

Genotype	P protein in mesophyll mitochondria	Gamma (μl l^{-1})	Mitochondrial attibutes in bundle-sheath cells	
			Profile area (μm^2 $cell^{-1}$)	Number of profiles ($cell^{-1}$)
M. arvensis	No	15.5	nd	30.0
MAT 5	No	10.5	9.3	24.3
MAT 6	No	19.3	6.3	25.0
MAT 3	Yes	41.1	1.4	5.4
MAT 7	Yes	44.1	2.1	8.4
MAT 8	Yes	44.5	1.7	10.5
M. moricandioides	Yes	50.5	nd	nd

Table 1. Attributes of untransformed *M. arvensis* (C_3-C_4 intermediate), *M. moricandioides* (C_3) and independent transformed lines of *M. arvensis* (MAT genotypes). The presence of the P protein was determined using immunogold labelling. Measurements of CO_2 compensation point were made on attached leaves at a saturating light intensity. The standard errors of the Γ measurments were less than % of the mean.

References.

1. *Morgan, CL, Turner, SR, Rawsthorne S (1993)* **Planta 190,** 468-473
2. *Rawsthorne, S, Hylton, CM, Smith, AM, Woolhouse, HW (1988)* **Planta 173,** 298-308
3. *Turner, S, Ireland, RJ, Rawsthorne, S (1992)* **Journal of Biological Chemistry 267,** 5355-5360