GCIRCBUL12*12

Brassica and Oilseeds Research in Norwich

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Expression of C_3-C_4 photosynthetic characters in somatic hybrids between Moricandia arvensis and Brassica napus

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The wild crucifer *Moricandia arvensis* is a potential source of alien genes for use in the genetic improvement of related *Brassica* crop species. Of special interest is the C_3-C_4 intermediate photosynthetic/photorespiratory mechanism of *M. arvensis* which accounts for the low CO_2 compensation point of this species[1] and may result in improved water use efficiency[2]. The combination of a Kranz-like leaf anatomy and differential distribution of glycine decarboxylase in leaf cells results in efficient recapture of photorespired CO_2. Protoplast fusion enables nuclear and cytoplasmic genomes from sexually-incompatible species to be combined thereby producing hybrid cells and ultimately hybrid plants. Our aim has been to produce somatic hybrids between *B. napus* and *M. arvensis* to investigate the feasibility of transferring and exploiting these traits.

To achieve this *M. arvensis* mesophyll protoplasts were chemically fused with *B. napus* hypocotyl protoplasts. Using a slightly modified published method[3] a total of 23 plants were recovered from fusion experiments and successfully established in the glasshouse. Of these, 13 were identified as hybrids using isozyme analysis. The CO_2 compensation points, chromosome numbers, origin of organelles, fertility and phenotypic characteristics of the hybrids were investigated. *B. napus* and *M. arvensis* have CO_2 compensation points of 44 and 15 ppm, respectively. Three hybrids have compensation points of approximately 34 ppm, i.e. between those of the two parent species, and also display partial expression of the Kranz-like anatomy. One of the three plants set seed when backcrossed to the *B. napus* parent but attempts to backcross the other two have as yet been unsuccessful.

This work describes for the first time the expression of potentially useful agronomic traits in somatic hybrids between *B. napus* and *M. arvensis*. Further studies on the primary hybrids will determine the parental genome complement using genomic *in situ* hybridisation and also the localization of glycine decarboxylase in the leaves using gold labelled antibodies. Transfer of the compensation point character into a *B. napus* background is of particular significance because of its potential long term impact on plant performance in the field. Analysis of transmission and expression of this and other characters in progeny from the backcrossed hybrid is continuing.

References

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Does photosynthesis in oilseed embryos contribute to fatty acid and starch synthesis?

P J Eastmond, C L Morgan, S Rawsthorne

Fatty acid synthesis occurs in the plastids of higher plants. It requires a carbon source, energy and reducing power, in the form of ATP and reduced pyridine nucleotides (NADPH and NADH). In photosynthetic tissues ATP, NADPH and NADH, and in many cases the immediate carbon source are provided by photosynthetic electron transport and the fixation of carbon dioxide. In non-photosynthetic tissues, the plastid is entirely dependent on the cytosol for provision of these requirements for fatty acid synthesis. Developing embryos of oilseed rape are green and contain plastids which have chloroplast-like membranes[1]. It is therefore possible that photosynthesis could contribute directly to fatty acid and starch synthesis in the developing embryo. To address the extent to which this occurs, we have characterized the photosynthetic properties of the developing embryos. Whole embryos carried out photosynthesis throughout development until the start of desiccation as the embryos matured (Fig. 1). This photosynthetic capacity was positively correlated with the chlorophyll content of the embryo. As the embryo increased in size, the rate of respiration increased, but this also declined once desiccation commenced.

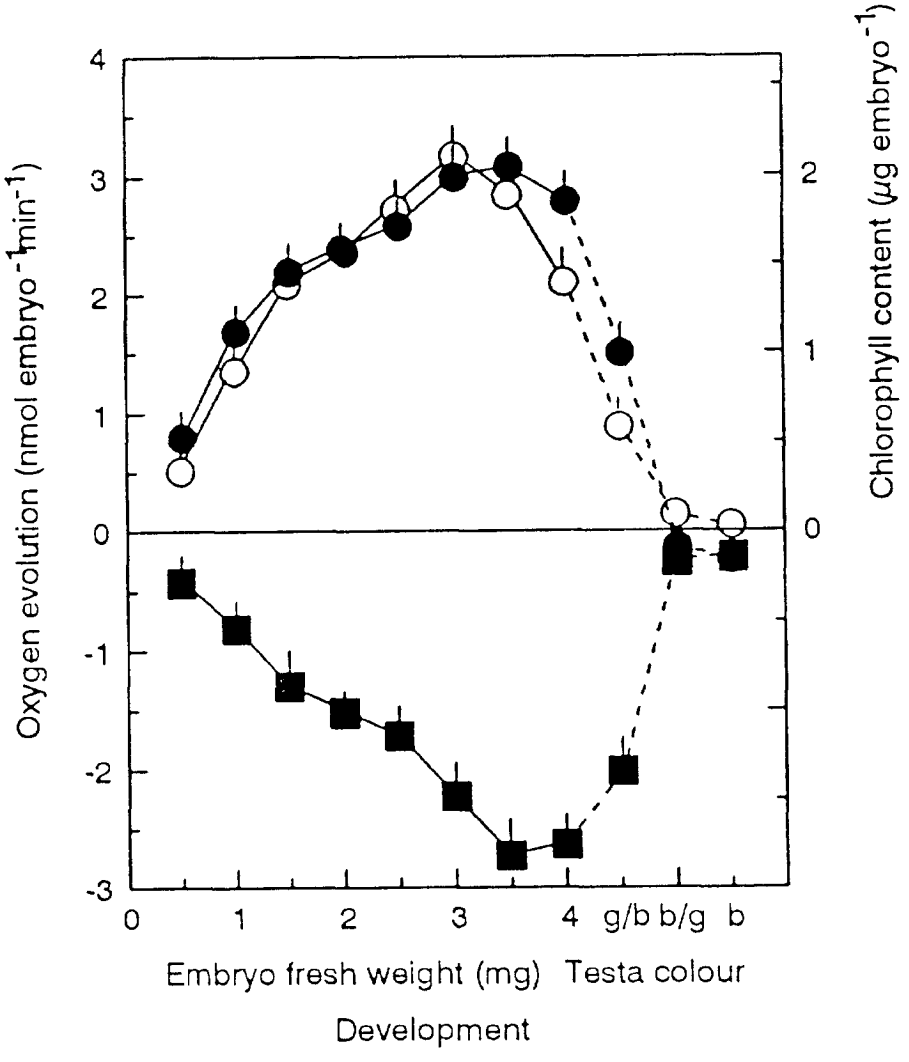

Fig. 1. Photosynthetic oxygen evolution (● nmol min^{-1}); respiratory oxygen uptake (■ nmol min^{-1}); and chlorophyll content (○ µg) per embryo during development of oilseed rape embryos. Development is expressed on a fresh weight basis up to the start of desiccation, after which testa colour is used to provide a phenotypic scale (g/b, testa green with some browning, embryo green, b/g, testa mainly brown, embryo yellowing; b, testa dark brown, embryo pale yellow.

The measurements of photosynthetic capacity described above were made at a saturating light intensity and CO_2 concentrations. In vivo, the embryo develops inside a testa which is contained within a silique. The latter is also a photosynthetic organ and will therefore intercept the incident light reducing the amount available to the developing embryos. To determine how much light is intercepted, we have measured the transmittance of photosynthetically active wavelengths of light through the silique wall during development. This remained at about 32% of incident radiation throughout development, until after chlorophyll losses in the embryo had started. At this stage, the chlorophyll content of the silique wall also declined as it became brown and dried out, and transmittance then increased. When embryos were exposed to a light level which corresponded to that inside a silique in full UK sunlight, photosynthetic O_2 evolution was much less and was essentially balanced by respiratory O_2 uptake.

To assess the contribution of light to fatty acid and starch synthesis, plastids were isolated from developing embryos. The isolated plastids were supplied with substrates in the presence or absence of light and the incorporation of these substrates into fatty acids and starch was measured (Fig. 2). Where light was provided at an intensity equal to that within the silique in vivo, the incorporation of bicarbonate was strongly dependent on light energy as this is entirely through photosynthesis. In contrast, the incorporation of a range of substrates into fatty acids and of glucose-6-phosphate into starch was limited in the light only. When ATP was added to the incubation medium in the light or the dark, rates of fatty acid and starch synthesis from these substrates were increased on average by more than four-fold.

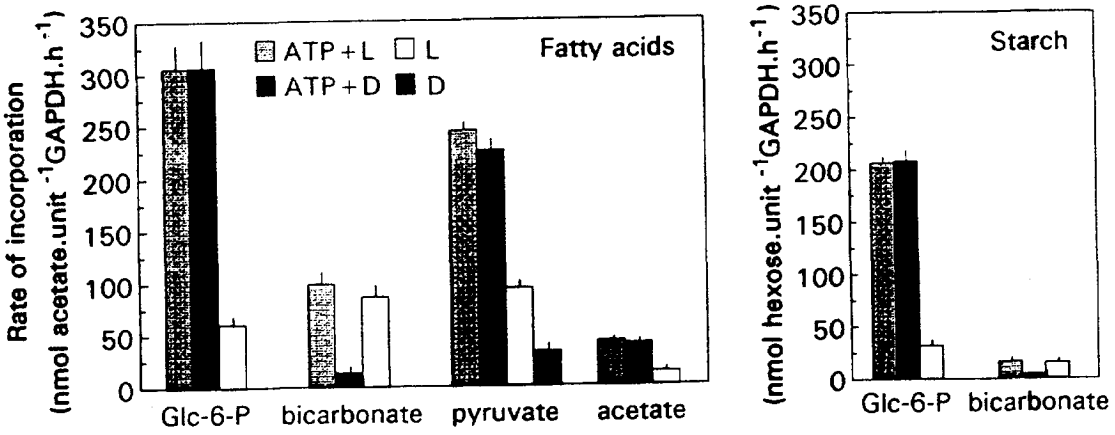

Fig. 2. Effect of light and the supply of exogenous ATP on the rates of fatty acid and starch synthesis from a range of substrates by plastids isolated from developing embryos of oilseed rape. Plastids were isolated from embryos with an average fresh weight of 2.5 mg.

Our studies clearly demonstrate that although the developing rapeseed embryo is photosynthetically competent, its capacity to utilize this in the synthesis of starch or fatty acids is limited. The fixation of CO_2 into either product is much less than for other carbon substrates and in the latter case, the incorporation of these substrates is much lower in the light alone than in the presence of ATP. We conclude that the plastids of oilseed rape embryos must depend to a very large extent on transport of carbon skeletons, ATP and reducing power from the cytosol to enable fatty acid and starch synthesis.

References

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Chromosome transmission and centromere mapping in *Brassica* allotriploids

A L Kelly, D J Lydiate

Many plant species, including several important crops, are polyploids. That is, they contain several complete copies of ancestral chromosome sets. For example, wheat is a recent allohexaploid (it contains the complete genomes from three diploid ancestors), maize is an old tetraploid (it contains modified genomes from two diploid ancestors) and oilseed rape (*Brassica napus*) is a recent allotetraploid. Polyploid species are often derived from relatively few hybridization events and this means that they normally acquire only a subset of the genetic diversity present in their diploid ancestors. Theoretically it should be possible for this diversity to be transferred into new polyploid species via crosses with their diploid ancestors. However, such crosses form uneven genomes, such as triploids and pentaploids, that typically experience problems at meiosis. Triploids and pentaploids usually form either unreduced gametes (gametes with the somatic chromosome complement) or gametes from which some or all of the odd genome has been eliminated (gametes that resemble those of the diploid parent).

B. napus (AACC; oilseed rape with 19 chromosome pairs) is an amphidiploid species that contains an A genome derived from *B. rapa* (A'A'; turnips with ten chromosome pairs) and a C genome derived from *B. oleracea* (C'C'; cabbages with nine chromosome pairs). *B. napus* can be crossed easily with *B. rapa* to form an allotriploid (AA'C; 29 chromosomes). We have analysed the progeny formed by crossing such an allotriploid with *B. napus* using both conventional cytology (in collaboration with Professor John Parker, University of Reading; to determine the number of univalent chromosomes) and marker technology (to trace the inheritance of each end of all 29 linkage groups derived from the allotriploid). The investigation established that meiosis in the allotriploid resulted in gametes with a wide range of chromosome complements. The A-genome chromosomes paired, recombined and segregated normally, to provide each gamete with an intact A genome, while the C-genome chromosomes remained unpaired. However, each C-genome chromosome had a significant (>50%) transmission frequency. Thus, the A-genome chromosomes of *B. rapa* and *B. napus* pair and recombine efficiently in the allotriploid and pollen with a wide range of C-genome chromosome complements is viable and proficient at fertilization. These results suggest that it should be relatively easy to introduce genetic variation into *B. napus* from *B. rapa* using an allotriploid bridge and conventional crosses.

There was a perfect correlation between the number of C-genome chromosomes that failed to be transmitted from the allotriploid parent and the number of univalent (monosomic) chromosomes observed in the progeny of crosses with allotetraploid/amphidiploid *B. napus*. This demonstrates that each C-genome linkage group represents a distinct C-genome chromosome. It was possible to determine the transmission frequencies of individual C-genome chromosomes from the allotriploid parent through male and female gametes. These frequencies were very high through both male and female gametes, however, while there appeared to be more or less random inheritance of C-genome chromosomes through female gametes there was significant enrichment for male gametes carrying higher numbers of C-genome chromosomes (Fig. 1).

Approximately 9% of the progeny of the allotriploid X *B. napus* crosses inherited telocentric chromosomes from the allotriploid parent. These telocentric chromosomes were probably formed by the centric fission of univalent chromosomes being drawn to both poles simultaneously during the first meiotic division. The marker-analysis of these telocentric chromosomes allowed the centromeres to be positioned on four of the C-genome linkage groups (Fig. 2). Mapping centromeres is an important aspect of genome analysis because centromeres exert a strong influence over the patterns of chromosomal rearrangement that are generated and selected during evolution, and also the patterns of intergenomic (homoeologous) recombination events that can be recovered in the progeny of interspecific hybrids.

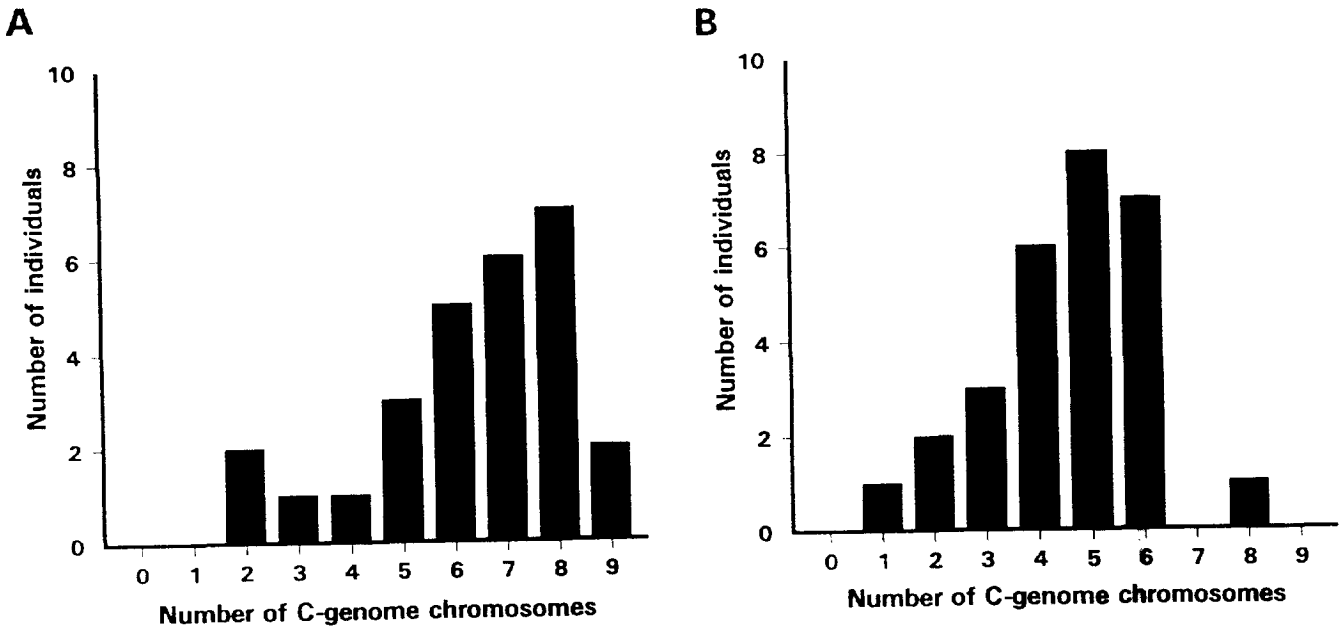


Fig. 1. Bar charts indicating the number of C-genome chromosomes transmitted by the allotriploid through male (A) and female (B) gametes.

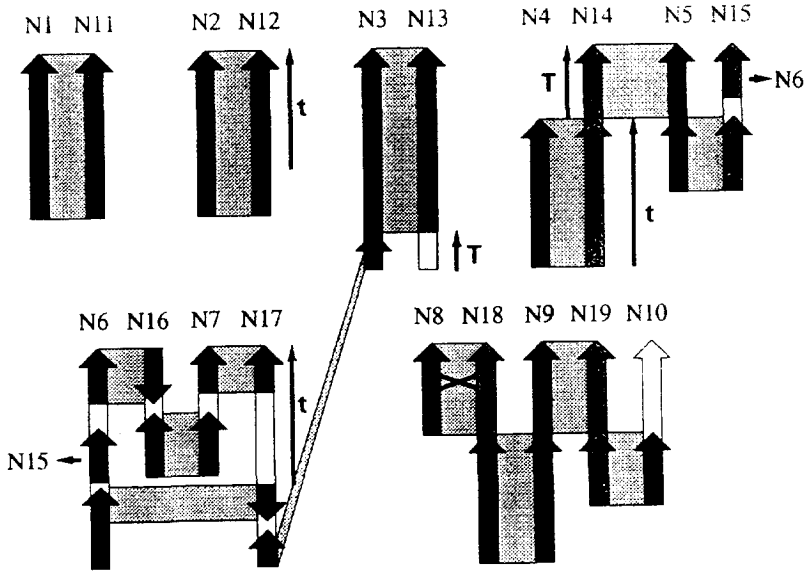


Fig. 2. Schematic diagram of the *B. napus* genome showing the homoeologous relationships (grey regions) of the A-genome chromosomes (N1-N10 in purple) and the C-genome chromosomes (N11-N19 in green) and indicating the chromosomal segments present in five telocentric chromosomes (the thin arrows marked T and t). Note that the centromeres of N13 and N14 are positioned at the points of discontinuity in collinearity between chromosomes of the A and C genomes.

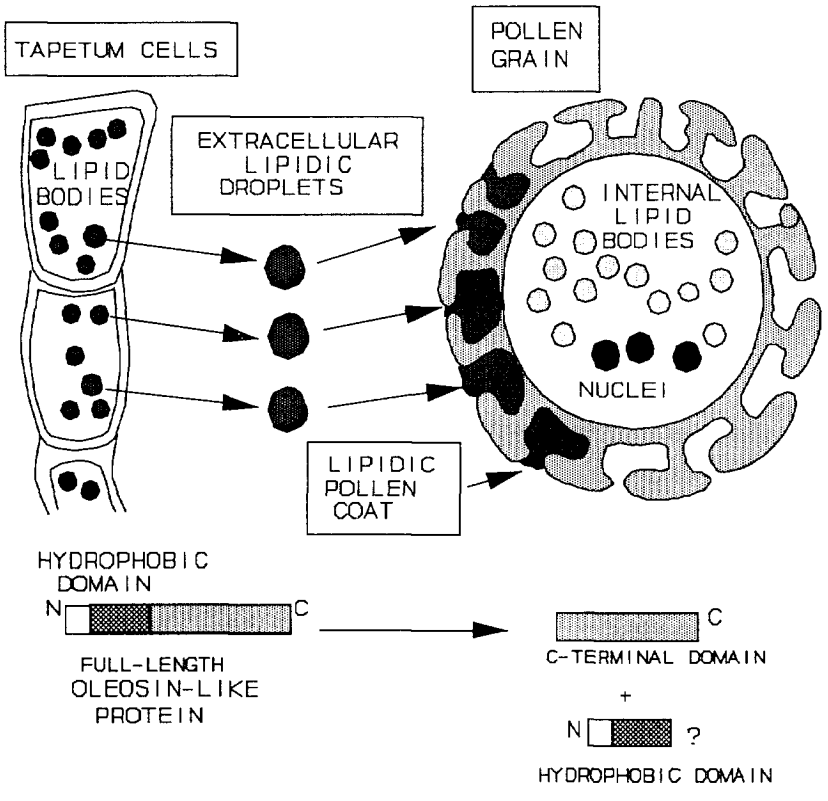
A new class of extracellular proteins found in pollen grains

D J Murphy and J H E Ross

There are two major types of pollen produced by different plant species, i.e. entomophilous or insect-borne and anemophilous or wind-borne pollen. Entomophilous pollen is characterized by a sticky, mainly lipidic, layer which fills in the interstices of the sculpted pollen wall, or exine, and is responsible for its adhesion to insect and other vectors. This lipidic pollen coat, or tryphine, is also responsible for pollen adhesion to the stigmatic surface in many species, such as the *Brassicaceae*. Changes in the organisation of the tryphine following pollen adhesion to the stigmatic surface probably facilitate the rehydration response undergone by compatible pollen grains prior to their germination.

Our studies have shown that the pollen coat in the *Brassicaceae* has a relatively simple protein composition with one class of three major polypeptides of molecular weight 30kDa-40kDa and another class of many small polypeptides of molecular weight 2kDa-10kDa. The major 30kDa-40kDa pollen coat proteins are encoded by a complex family of genes which share significant homology with those encoding the oleosin proteins that are found uniquely in seed tissues. These proteins are therefore termed oleosin-like proteins (OLPs). Northern blotting and *in-situ* hybridisation has shown that the expression of these genes is confined mainly to the anther, and in particular to a single-cell layer - the tapetum - which surrounds the loculus and is involved in nourishing the developing microspores which give rise to mature pollen grains.

Fig. 1. Putative post-translational processing of anther-specific oleosin-like proteins (OLPs) during the transfer of tapetal lipid droplets to form the extracellular pollen coat.



Like the seed-specific oleosins, the anther-specific OLPs are characterized by a central, highly conserved 60-70 residue hydrophobic domain (Fig. 1). In the case of seed oleosins, this domain is postulated to be involved in oil-body stabilization, particularly during desiccation. Comparison of the amino acid sequences, predicted from DNA clones, of the hydrophobic domains of 27 different anther OLPs and seed oleosins indicates that, although the two classes of proteins are related, they diverged from each other at an early stage of their evolution. In addition, the anther OLPs contain very divergent polar N-terminal and C-terminal domains compared to the seed oleosins. The OLP C-terminal domains are characterized by a predominance of amino acids usually found in structural polypeptides, such as combinations of glycine and lysine, or proline and alanine, and short, regular, repeating motifs of such residues. Direct amino acid sequence analysis of polypeptides purified from pollen coat fractions of both *Brassica* and *Arabidopsis* has shown that there is an apparent post-translational cleavage of the OLPs at the junction of the hydrophobic domain and the C-terminal polar domain. Each of the three pollen coat polypeptide sequences determined to date (37kDa, 35kDa and 5kDa-15kDa) corresponds to the C-terminal domain of an OLP gene product.

As shown in Fig. 1, we postulate that oleosin-like proteins are synthesised in the tapetal cell layer, then transferred to the pollen coat at the time of tapetal breakdown. There is no ultrastructural evidence for the existence of oil bodies in the pollen coat analogous to the structures found surrounded by oleosin proteins in mature seeds, so the function of the extracellular OLPs in pollen may be entirely different from that of seed oleosins. Possibilities include a role in facilitating the passage of water through the lipidic pollen coat to allow the rehydration of the pollen grain which is a requirement for germination. The hydrophobic domain of the OLPs may also act as a carrier to transfer the extracellular lipid bodies and proteins from their site of synthesis in the tapetum to their site of accumulation on the coat of the maturing pollen grain. Once on the pollen coat, the full-length OLP may be cleaved to give rise to a mature protein corresponding to the C-terminal domain of the primary translation product. Experiments are now underway to compare the roles of the seed-specific oleosins and the anther-specific oleosin-like proteins.