R A B I D O

The Fifth AFRC PMB *Arabidopsis* Newsletter March 1991

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Plus – attached:

Arabidopsis Current Awareness List (Subscribers) (4pp)

Protocol Book (AFRC PMB *Arabidopsis* Grant Holders) or

Protocols:

From Sue Albini...
- Synaptonemal-complex spreads (3pp)

From Caroline Dean...
- M-9 sucrose medium (1p)

From Jola Maluszynska...
- *In situ* hybridisation (5pp)
- Flower-bud chromosome prep (1pp)

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From Jane Russell...
- Seed harvesting & storage (1p)

From Rosalind Slatter...
- Nuclei isolation (2 pp)

Rabido: the fifth AFRC PMB *Arabidopsis* Newsletter, March 1991.

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Protocol Book

Accompanying this newsletter is the long-awaited AFRC PMB *Arabidopsis* Protocol Book. This has been compiled by the ACM and Caroline Dean and is entitiled; "Arabidopsis: *The Compleat Guide*". Owing to the considerable cost of production, both in labour and materials, this is only being distributed to AFRC PMB *Arabidopsis* grant holders. Non-grant holding readers of this newsletter need not go into total despair, however, as most of the manual comprises protocols that have already been distributed with earlier issues of the newsletter. Also, many of those that have not previously been sent out are included as "attachments" with those newsletters that are going to non-grant holders.

The loose-leaf format of the manual has been designed to allow for ease of photocopying, as well as enabling the simple addition of new protocols and the updating of existing ones. For, despite its punning title, it is not (yet), "Everything you ever wanted to know about *Arabidopsis*, but were afraid to ask." It is hoped to update the manual by sending out new pages with each *Arabidopsis* Newsletter. In order to achieve this, however, we obviously need to receive material to distribute. So if you find a modification to a protocol useful, or adapt a non-*Arabidopsis* technique for use with the noble weed, or even devise a whole new procedure, we will be most grateful if you could let us know at the usual address by the usual means, i.e., *via* e-mail or on disc -- see "How to reach us." As incentive to non-grant holders, suppliers of (in the compilers' humble opinion) *useful* protocols will receive a free copy of the protocol book.

From Richard Williamson...

Oz Conference

Arabidopsis thaliana and the Molecular Basis of Plant Biology: a Robertson Symposium to be held at the Australian National University, Canberra, Australia on September 30 and October 31, 1991 in conjunction with the annual meeting of the Australian Society of Plant Physiologists (October 2 to 4). Invited speakers who will be attending: Tony Bleecker, Madison, ethylene responses; John Browse, Pullman, lipid biosynthesis; Ken Feldman, Tucson, insertional mutagenesis using T-DNA; Jerome Giraudat, Gif-sur-Yvette, abscisic acid responses; Gerd Jurgens, Munich, morphogenetic mutants; Maarten Koornneef, Wageningen, floral initiation; Richard Meagher, Athens, actin genes; Elliott Meyerowitz, Pasadena, homeotic genes in flowering; Kyotaka Okada, Okazaki, root growth control; Renate Schmidt, Norwich, flowering genes; Ronald Suzek, San Diego, photomorphogenesis. Plus contributed papers and posters. Accomodation available on campus in student rooms or more luxurious (and expensive) locations. Registration forms will be mailed in March to those who have registered an interest in attending. Further details from: R.E. Williamson, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia. Fax (06) 248 9995, phone (06) 249 5087, e-mail WILLIAMSON@RSBS.ANU.EDU.AU

Note that there is easily time to make the trip to Tucson afterwards for the Plant Molecular Biology Congress for those who really want to clock up the flying time.

From Chris Somerville...

Gene Nomenclature

The following was posted onto the *Arabidopsis* bulletin board in response to an enquiry about gene nomenclature in *Arabidopsis*:

At the 1987 *Arabidopsis* meeting the following recommendations were formulated.

1. Underline or italicise genotypes.
2. The wild type genotype is capitalised (eg. MEY1)
3. Alleles are designated by a dash followed by a number (*e.g.*, mey1-3). If no allele is specified, it is assumed to be 1 (*i.e.*, mey1-1 = mey1).
4. The genotype of mutant alleles is lower case (*e.g.*, mey1).
5. All gene symbols should be three letters.
6. Genes with mutant alleles of similar phenotype can (but need not) be given the same three letter designation followed by a different number (*e.g.*,. mey1, mey2).
7. Phenotypes are designated by the gene symbol which is not underlined but has the first letter capitalised (*e.g.*,. Mey+ is wild type, Mey- is mutant). The + or - can be superscript or on the same line.
8. Uniparentally inherited mutations should be enclosed in parentheses. Things get a bit confusing here since many authors have followed an earlier suggestion that chloroplast genes follow the bacterial convention (*e.g.*, psbA).
9. There is no special designation for a dominant allele.
10. If you want a citation for the usage see: *Plant Cell* **1**, 50 (1989) or *Science* **241**, 1088 (note 10).

From Luca Comai...

Collecting *Arabidopsis* Seeds

This was also on the *Arabidopsis* bulletin board:

We have developed a simple, effective and cheap seed collection device that is made from a PET soda bottle. This

continued on page 20 (News/Trivial Pursuits)...

From Sue Albini...

Synaptonemal complex spreading: an ultrastrucural approach to chromosome analysis in *Arabidopsis thaliana.*

The months since the last report have been a phase of technical development. The search for improvements in the yield and quality of surface spread prophase I pollen mother cells of *Arabidopsis* continues. Good light micrographs of all stages of meiosis have been produced. By assessment of chromosome pairing behaviour at metaphase I of meiosis, it will be possible to detect genic mutations that cause univalence and chromosome mutations such as interchanges. A method for *in situ* hybridisation of SC complements with DNA probes is in the development stage. The materials and methods involved in the basic technique of DNA-DNA *in situ* hybidisation to plant chromosome preparations are being established. The first experimental runs on chromosomes and SCs are currently underway. The first results from these investigations will appear (hopefully) in the next report.

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From Ken Buck...

A novel approach to the isolation of origins of plant DNA replication using *Arabidopsis* as a model system.

We have now cultured transgenic plants from calli containing our hygromycin ORI vector with the TGMV replicase and replication origin in the absence of hygromycin. These have been analysed, using PCR followed by Southern blotting, for the presence of the vector. We have confirmed that the plants contain the TGMV region and the C-terminal 2/3 of the hygromycin gene. Both these regions appear to be present as single copies in the genome. Testing for the N-terminal 2/3 is in progress. The plants should soon be large enough for the next stage of the project, *i.e.*, protoplast isolation and selection on hygromycin media to isolate those cells in which intramolecular recombination has occurred. From recently published data we expect this to occur at a frequency of 1 in 10,000 cells at most.

We have completed the construction of a second ORI vector designed to regenerate a functional neomycin phosphotransferase gene by intramolecular recombination. The neomycin cassette has been cloned into the plant transformation vector, pGA482 in which the neomycin selectable marker gene has been replaced by a hygromycin phosphotransferase gene.

We learnt at the *Arabidopsis* meeting in Nottingham that some NEO genes are defective. Apparently this is not a problem when transforming tobacco, but can result in a much reduced transformation efficiency with *Arabidopsis*. We have used the diagnostic XhoII site to demonstrate that the pBin19 vector carrying our hygromycin ORI vector is defective, as is the NEO gene in our neomycin ORI vector. We are in the process of repairing the latter. However, we have need of a vector to replace pBin19 so that we can transfer our hygromycin ORI cassette to it. Offers of help in this respect would be gratefully received. The preferred vector would have a pUC19-type multiple cloning site as in pBin19 and a non-defective NEO selectable marker gene flanked by a promoter and terminater other than 35S and ocs repectively.

T.D. Jones & K.W. Buck; Dept. of Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB.

From Neil Butt...

Cell cycle control genes in *Arabidopsis.*

Since returning from Nottingham our group has been actively pursuing several lines of research in order to identify genes involved in the mitotic cell cycle.

Screening of the Clontech genomic library in the EMBL 3 vector has resulted in the isolation of a homologue to the *S. cerevisiae* CDC34 gene. This gene encodes a highly conserved ubiquitin carrier protein that is thought to be involved in the G1 to S transition during the mitotic cycle (Nature, 1991). Although sequencing of the entire genomic clone has not been completed, a 70bp intron has been identified within the gene. Further screening of a Clontech cDNA library has generated several putative positives which are currently being analysed.

Screening with other gene homologues has suggested that the cdc13 cyclin gene from *S. pombe* is also present within the *Arabidopsis* genome. The *S. pombe* cdc13 gene (gift from P. Nurse, Oxford University) is presently being used to screen a λDASH library kindly donated by D. Coates (Leeds University).

PCR technology has also started to bear fruit with the putative cloning of the *S. pombe* cdc25 homologue. Reactions have generated bands of the expected size range which have been found to cross-hybridise with *S. pombe* cdc25. The products have been cloned and initial sequence looks promising. This product is also being used to screen both the Leeds Genomic library and the Clontech cDNA library.

Attempts are also being made to set up *Arabidopsis* cell suspension cultures within the department. The protocols for this were kindly sent to us by N. Blackhall (Nottingham University) and have presently generated callus material which will soon be used to inoculate suspension medium. This technique is being employed to produce a cDNA library of actively dividing cells to facilitate the isolation of additional cdc genes.

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From David Coates...

Molecular biology of the regulation of the plasma membrane calcium transporter in *Arabidopsis* and *Zea.*

Work on the *Arabidopsis* calcium ATPase is progressing - we are in the process of identifying and characterising clones identified using a ➸

700 bp PCR amplified fragment obtained using the nested PCR approach described at Nottingham. This same PCR product has been cloned into a plasmid vector, and is being sequenced. More news when we have a real result. All other aspects of the biochemistry are moving along satisfactorily.

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From George Coupland...

A two-component transposon tagging system in *Arabidopsis.*

The system we are using for transposon tagging was described at length in the last Newsletter. Since November, we have been consolidating the data described there. We have performed Southern blots on DNA extracted from 12 streptomycin resistant individuals which from their phenotype were assumed to have inherited germinal excisions of *Ds*. These excisions were driven by the fusion of the CaMV35S promoter to the *Ac* open reading frame. When probed with the streptomycin resistance gene all 12 did indeed carry the band expected if the *Ds* had excised. However, when the *Ds* was used as a probe only two of the lines retained the *Ds*. This suggests that *Ds* is being lost from these lines at a higher frequency than expected. We hopefully will be able to overcome this problem, by using a hygromycin resistance gene within the *Ds*. These experiments are now underway.

'This suggests that *Ds* is being lost at a higher frequency than expected'

In addition, we now have four independently transformed lines that carry the fusion of the soybean heatshock promoter to the *Ac* open reading frame. These segregate three kanamycin resistance progeny for each kanamycin sensitive, as expected for single locus insertions of the T-DNA. These are now being crossed to *Ds* carrying lines to determine the efficiency with which the HS promoter fusion drives transposition.

J. Swinburne, L. Balcells, K. Ingle, C. Recknagel, S. Scofield, J. Jones & G. Coupland.

Isolation of the flowering-time gene *fg.*

Jo has spent January in the New Zealand sun, so this report will be shorter than the last one. As described in the November Newsletter we are chromosome walking to *fg*. We now have two YAC contigs, which we think flank *fg*, and the last three months have been spent extending them. With the help of two cosmid contigs provided by Brian Hauge (Boston), we have been able to lengthen our contig containing the markers 6833 and chalcone synthase to around 400kb, with chalcone synthase approximately in the middle. A cosmid clone is located at one end of this contig, but this hybridises to no new YACs in the Grill and Somerville Columbia library and so we have made 10 copies of the Landsberg library and are now ready to screen this. At the other end we have a YAC which we are now using as a template for an inverse PCR experiment to isolate a probe from the other end of this contig. From our second contig which is approximately 160kb long and contains the RFLP marker 5962 we are now ready to go to the library with inverse PCR probe isolated from the ends of the YACs located at the termini of the contig. Although our walk has not progressed very far since the last Newsletter, we are now in a position to make several steps in the next couple of weeks.

Our analysis of DNA isolated from recombinants containing cross-overs (see last Newsletter) on both sides of *fg* is continuing and will be reported in more detail next time.

J. Putterill, F. Robson, K. Ingle & G. Coupland; JI Centre for Plant Science Research, Cambridge Laboratory.

From Simon Covey...

CaMV infection of *Arabidopsis.*

In the last report, we expressed some excitement about the possibility that we might be able to inoculate large numbers of *Arabidopsis* seeds with CaMV simply by incubating them with virus in the presence of 2,4-D. Our more recent experiments suggest that this optimism was a little premature and we are now left with a phenomenon for which there is no obvious explanation. The initial observations were that seed recovered from CaMV-infected *Arabidopsis*, when germinated in the presence of 2,4-D, produced cotyledons which rapidly bleached and lead to plant senescence.

The effect could also be induced by incubating CaMV virions with seed from healthy plants and germinating them in the presence of 2,4-D. Controls without 2,4-D grew normally after treatment with virus. Moreover, the effect was induced by virions of a severe CaMV strain (Cabb B-JI), but not by a mild strain (Bari 1). Indications that the effect was not due to viral replication came from investigations of viral replication intermediates in the bleached seedlings. In CaMV-infected plants (including *Arabidopsis*), characteristic DNA products of reverse transcription accumulate. However, we found no such products in the bleached *Arabidopsis* plants although the possibility remained that a very small number of infected cells, that we were unable to detect, caused the effect. Confirmation that viral replication seemed not to be causative came from UV inactivation experiments. CaMV virions were titrated against UV irradiation and tested for infectivity in turnip plants. Treated virions, which did not result in infection of turnip, still produced the bleaching effect in *Arabidopsis*. However, we cannot yet exclude the possibility that *Arabidopsis* is more sensitive to infection than turnip. One conclusion from this is that the coat protein of CaMV strain Cabb B-JI (but not that of strain Bari 1) is toxic to *Arabidopsis* only in the presence of 2,4-D -- very curious! We have some ideas and will report on them and our searches for interesting *Arabidopsis* mutants in the next newsletter.

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From Andy Cuming...

Ammonium toxicity in *Arabidopsis*.

At the Nottingham meeting we reported our success in amplifying, by redundant PCR, glutamate dehydrogenase from genomic DNA of a variety of fungal and plant species, including *Arabidopsis*. Sequence analysis of the PCR products obtained confirmed their homology with other known GDH sequences. Since the meeting, we have been using our PCR fragments as hybridisation probes in order to isolate full length GDH sequences. Our screening of Christine Raines's *Arabidopsis* cDNA library is currently in progress and proceeding well. In our initial hybridisation we identified a small number of hybridising plaques whose purification has now reached the tertiary plating stage. We hope to report the successful identification of an *Arabidopsis* GDH clone in the next newsletter. We are also collecting *Arabidopsis* root tissue in order to construct a root-specific cDNA library. This is taking some time as although this author admits to having a degree in Botany, his gardening skills have never reached a particularly high pitch!

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From Caroline Dean...

(While Caroline soaks up the Australian sun, the lab soldiers on... -- ACM.)

Transposon tagging.

Emily Lawson

In the last three months I have continued to investigate the difference in excision frequencies observed between the wild-type *Ac* element, and the *Nae*I deleted derivative. Our extensive scoring data on streptomycin indicate that the *Nae*I deleted *Ac* has a significantly higher level of excision than the wild-type element. It had been suggested that one reason for this may be a higher level of multiple insertions at one locus in the transformants carrying the *Nae*I deleted *Ac*. In order to investigate this, I have now completed an exhaustive study of the structure of all of the T-DNA insertions of transformants carrying the *Nae*I deleted element. Out of the 12 transformants investigated, 9 had single insertions at each locus. The majority had only one insertion locus, although one transformant had 3 simple, single insertions. One transformant has a tandem repeat, one an inverted repeat, and a third has no right border sequences.

In addition, I now have binary vectors where I have cloned both wild-type and *Nae*I deleted *Ac* elements into a 35S GUS fusion. These will be ready for transformation into plants imminently. Because the streptomycin assay only works in the cotyledons, we have previously been limited to looking at excision only at this early stage of plant development. The GUS fusion should yield some interesting information on the pattern of excision over the whole plant, and tell us whether the timing of excision of the two element types is very different.

> 'Our data indicate that the *NaeI* deleted *Ac* has a significantly higher level of excision than the wild-type element.'

Clare Lister

As part of the transposon-tagging project, I am mapping the positions of a number of T-DNA insertions and of *Ac* transposed from these insertions. This is being carried out to determine if *Ac* behaves in *Arabidopsis* in a manner similar to the way it does in maize, i.e., by transposing preferentially to closely-linked sites. This has important implications for the strategy of transposon-tagging.

Using the technique of inverse PCR (IPCR), I have isolated genomic DNA flanking T-DNA insertions from six lines containing either a wild-type or a modified, *Ac* element. These will be sequenced and have been hybridised to Columbia (Col) and Landsberg *erecta* (Ler) YAC libraries.

The amplified sequences will also be used to identify RFLPs between Ler and Col. They are the parents of both the F4 segregating families (from Elliot Meyerowitz) and the recombinant inbred lines, being generated here.

Once RFLPs have been identified, the positions of the insertions can be mapped relative to the known RFLP markers. The sequences flanking the transposed *Acs* will be isolated and mapped in a similar manner.

The *Arabidopsis* genome project.

Gerda Cnops & Renate Schmidt

Using the 33 RFLP markers located on the top halves of chromosomes 4 and 5, we have identified 85 corresponding YAC clones. All of the clones were sized on CHEF gels and verified by Southern blot analysis. For five RFLP markers we could not identify corresponding YAC clones in the Columbia library (Erwin Grill), but we successfully isolated one or more YAC clones from the Landsberg library (ABI). For a couple of RFLP markers we have so far only found one YAC clone. For such cases, we intend to use a third YAC library - also of the Columbia ecotype. This was kindly given to us by Eric Ward (Ciba-Geigy).

Thus far, our results are very encouraging. Analysing the YAC data we were able to identify two contact points for the two non-integrated RFLP maps - one on chromosome 4 and one on chromosome 5. Furthermore, we could identify a contiguous region of 400 - 500kb which spans 4 RFLP markers (210, 455, 326, 580) on chromosome 4 - a region of 6 cM! Right now, we are in the process of extending this contig by walking. We estimate that we have mapped YACs for at least 30% of the top halves of chromosomes 4 and 5.

The mechanism of vernalization.

John Chandler

I am continuing to screen an EMS-mutagenised population of the fca locus (this confers a late flowering phenotype responsive to vernalization) in order to isolate individuals that are no longer respond to vernalization. These mutants, if not themselves late flowering, have mutations in the perception and transduction of the cold signal. The same EMS-mutagenised fca population is also being screened for suppressor mutants of fca, which, without vernalization, flower at the same time as wild type *Landsberg erecta*.

In an attempt to find a function for the fca gene product, a range of double mutants is being constructed. These comprise fca with various starch, lipid and hormone mutants, in an effort to perhaps find a block in a particular pathway with which fca might be involved. In particular, the vernalization requirement and response of double mutants of fca with ga1, gai, aba, and abi1, 2 and 3 will be tested. This is to investigate the effect of the second mutation on the phenotype of fca, and the possible relationship between the fca gene product and gibberellic acid or abscisic acid physiology during vernalization.

Lore Westphal and Rob Ewing

We are mapping the fca locus (late flowering mutation, chromosome 4) using Meyerowitz's RFLP markers. Fca in Landsberg was crossed to Columbia and Niederzenz. Late flowering F2 individuals were then selfed. DNA from most (approximately 200) of the resulting F3 families has been prepared (approximately 200). We are scoring these F3 families for recombination between the fca locus and 5 RFLP markers in the vicinity.

(Lore has just started as an EC BRIDGE-funded post-doc. She was previously at UC Davis working with Richard Michelmore on RFLP mapping in lettuce – ACM.)

Genetic and physiological analysis of vernalisation requirement in the winter annual Stockholm.

Jonathan Clarke

Current work concentrates on the mapping of the loci *Fri* and *kry*. Having obtained complex segregations in the F2s, 50 randomly selected F3 families have been progeny tested. Unfortunately, we have been unable to determine the genotypes of these F3s from the segregation data obtained; with the distributions clearly skewed by the interactions of segregating modifiers. It still remains to be seen if we can use these lines to map these loci against RFLP markers and so we are also generating introgressed (near isogenic) lines.

The Quantitative Trait Loci Analysis (QTL) of Stockholm's late flowering character is about to start; we now have F2 seed from the Stockholm/Landsberg *erecta* cross and we are in the process of growing up the F2 plants for our F3 families. In the interim, polymorphism data on the parents is being amassed using the Meyerowitz RFLP markers. We now have our controlled environment rooms up and running and anticipate using a 12h (short day) and a 12h + 4h (day extension) cabinet combined with our temperamental vernalization chamber as our environmental variables.

A new string to our bow is a recently initiated collaboration with Dr. Jeremy Harbinson at ATO/Agrotechnologie, Wageningen. We hope to continue and extend our initial investigations into the changes in photosystem II efficiency in some of the late flowering lines.

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From John Doonan...

Identification and analysis of genes regulating the cell division cycle in plants.

Screening of Clontech *Arabidopsis*. λgt11 cDNA continues, apparently without the problem of Protein A clones (cf Nottingham meeting). Sequencing is commencing.

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From Gary Foster...

Regulation of *Ds* transposition in higher plants and evaluation of rapid techniques for the cloning of flanking DNA.

The isolation of a plant gene of interest for which the product is unknown, but a mutant phenotype has been observed, is classically carried out either by chromosome walking or transposon tagging. An improvement on both these approaches might be achieved by cloning a YAC vector into a modified *Ac* maize transposon element, thus allowing tagging and subsequent gene rescue without the need for library screening. In subsequent generations, the preference for *Ac* to transpose to linked sites could be utilised to capture YAC clones to a specific region of the chromosome, and thereby provide target DNA for a chromosome walk. A modified YAC4 vector has been cloned into the EcoRI site of Bin19 and then introduced into tobacco and *Arabidopsis*. The His stuffer of the YAC has been replaced with a NotI site so that high molecular DNA from transformants can be partially digested and then recircularised into large circles. On cutting with NotI, a functional YAC will be created from those circles that contain the integrated YAC sequences. We are currently demonstrating the rescue of these molecules from plant DNA by transformation in yeast. Cloning of the modified YAC into the transposable element is now being carried out so as to remove the origin of replication of pBR322, which the YAC vectors are based around, as this has been found to be unstable when included in some binary vectors.

Dr. Gary Foster, Dr. Rod Scott, Dr. John Draper, Mr. Mike Roberts, & Mr. Rob Blundell; Botany Department, University of Leicester, LEI 7RH .
Tel: 0533-523393; FAX: 0533-471001

From Ian Furner...

Towards a molecular genetics of apical development in *Arabidopsis thaliana*.

Since the last report I have been continuing work on the fate map of the *Arabidopsis* apex. The 35s-Ac-Rolc construct generates only rather small sectors and my experiments using EMS treatment suggest it is not a good mutagen for this work. In the last three months, I have been experimenting with x-rays and these look promising. Briefly, dry seeds heterozygous for *alb* 1 are treated with 15-20 krad of x-rays and sown. Approximately 1% of resulting plants have albino sectors. Using this procedure it should prove possible to get enough sectors to produce a good map. (I estimate I can generate and analyse 50 to 100 sectors per month).

Work on isolating more "apex mutants" has slowed down due to the unfilled research assistant position. I have readvertised and I hope to get someone appointed soon. I have started screening ems treated M2 families in soil and have just planted 400 families.

Renee Sung at U.C. Berkeley, California is carrying out a similar screen for apex mutants/seedling lethals. We have set up a collaboration and I have just returned from a visit to her laboratory. She has several interesting mutants and there is some overlap between the lines we are finding. We agreed to keep in close contact and exchange information and mutant lines. She has applied for funding to allow her or one of her people to come and work in my laboratory for a short period. I hope to expand this into an ongoing exchange.

While in Berkeley I did some benchwork. I took scanning electron microscope pictures of the fasciated mutants and two abnormal apex mutants. The fasciated mutants appeared to have enlarged disorganised meristems. This result confirms Ottoline Leyser's conclusion that fasciation is associated with and possibly results from enlargement of the meristem. At the Christmas Meeting I mentioned a dwarf mutant and speculated that it might be impaired in cell expansion. At Berkeley, I sectioned leaf tissue of this line and wild type sibs and found the mutant leaf has very small, densely-staining cells with no obvious vacuole. The wild type has poorly staining cells with large vacuoles. The results suggest that most of the size difference in the plants can be accounted for by cell size.

"Most of the size difference in the plants can be accounted for by cell size."

Paul Davison has been screening his apex c-DNA library with complex leaf and apex c-DNA probes. He has been looking for apex specific clones. The first screens gave a large number of promising clones. Unfortunately, rescreening these clones twice more has shown that most, if not all of these were false positives. He is currently rescreening the library under altered conditions to overcome this problem.

Ian J. Furner; Department of Genetics, University of Cambridge.

From Nic Harberd...

Irradiation mutagenesis.

With the help of Bernard Mulligan's group (University of Nottingham), we have been doing γ-irradiation mutagenesis of Landsberg *erecta* and of the dominant gibberellin insensitive dwarfing mutant *gai*. Some of the Landsberg material has been harvested in bulk, the rest (progeny of approximately 3,000 plants) has been harvested as single M1 families, thus enabling us to identify and recover mutations which are inviable/infertile when homozygous. We have just begun screening the M2 material, and preliminary observations have indicated the occurrence of yellow, yellow-green and albino individuals at frequencies higher than observed in untreated material. This suggests that the mutagenesis procedure was successful and that this material can now be used for the isolation of mutants of interest.

We have also treated plants homozygous for *gai* and for the linked marker mutation *tt1* (this mutation makes the seeds yellow rather than brown) with 92kR g-irradiation. This level of irradiation was probably higher than optimum for this particular batch of seeds since we observed a high death rate in the M1. Several of the surviving plants displayed either wild-type phenotype or were less severely dwarfed than expected for *gai* homozygous plants. These plants all had brown seeds and were eliminated from the investigation on the grounds that they were contaminants arising from cross pollination or from accidental seed mixing. Two further plants displayed interesting phenotypes. One possessed a region of tissue that was darker green and more dwarfed than in *gai* homozygotes. This suggests that a gene duplication event had occurred. The florets derived from this region were infertile, and despite attempts to rescue this event by pollination with Landsberg *erecta*, no seeds were obtained. The second plant had a stem branch with internodes considerably longer than expected for a *gai* homozygote, although other stems from this plant were of the expected internode length. Seeds obtained from self-pollination of the long branch were yellow, and have just been planted to determine if this potential *gai* derivative mutation is heritable. We have also harvested M2 seeds from the rest of the irradiated plants and will screen these for individuals which do not display *gai* phenotype. We have also bulked up more of the *gai/gai;tt1/tt1* starting material and will be doing further mutagenesis in the near future.

YAC cloning.

With the help of Renate Schmidt and others from Caroline Dean's group we have identified a number of YAC clones which contain DNA hybridizing to RFLP markers (from the Meyerowitz set) which map in the general vicinity of the *ga4-gai* region of chromosome 1. We are about to start chromosome walking by end labelling YAC clones containing the *phyA* gene.

Multiple *Ds* transformant lines.

Since *Ac* (and *Ds* derivatives) have a tendency to transpose to sites linked to their original insertion site we have been using construct 0282 (gift from Ian Bancroft and Caroline Dean) in transformation experiments. The aim is to increase the number of independent *Ds* transformants for future use in the two-component transposon-tagging system. After initial difficulties with the transformation method, we now have plants regenerating from approximately 14 different transformation events, and this number is still rising!

Phytochrome gene clones.

Using oligonucleotide probes we have isolated genomic clones containing the phyA and phyB genes from our λ genomic library (see previous newsletter). These clones have been restriction mapped and are now being used to make constructs for plant transformation. Our experimental aims in this are, firstly, to determine if the *phyB* gene can complement the *hy3* mutation, and secondly to assess the effect of *phyB* overexpression and antisense constructs on growth of transgenic plants in different light environments.

These latter experiments are part of a collaborative venture with Dr. Garry Whitelam of Leicester University.

John Cowl, Mary Holdsworth, Jing Rong Peng, Marion Rawlins, Paul Sinacola & Nicholas Harberd; J.I. Centre, Norwich, UK.

From Carole Harker...

Identification and exploitation of the interaction between a protein and host factors which control virus spread.

We have overcome our earlier problems of multimeric-CaMV instability by using a different isolate of CaMV. We now have a clone, pBCM1841, containing 1.2 copies of the CaMV genome in which there is only one copy of ORF1. This is the ORF encoding the protein implicated in cell to cell spread of CaMV.

The capacity of this construct to initiate an infection in *Arabidopsis* was shown after inoculation with naked DNA or using "agroinfection". The latter technique was significantly more efficient when *A.tumefaciens* C58 (pGV3850), rather than LBA 4404, was used to mediate CaMV introduction. This is the first time we have been able to deliver cloned CaMV DNA into *Arabidopsis*. This infectious clone will be used as the back bone for future mutagenesis of the cell to cell movement protein (P1). The analysis of P1 mutants requires an independent assessment of virus replication and spread. Verification that the spread function alone has been mutated will be shown by complementation in P1 transgenic *Arabidopsis*. The genomic analysis of these plants is continuing. We also plan to use P1- complementation of an ORF1 deletion mutant as an assay for P1 expression in transgenics. Confirmation that ORF1 mutants are still able to replicate in single cells will be obtained from the "agroinfection" of leaf discs where the multiplicity of the primary interaction between *Agrobacterium* and wounded cells is high. This facilitates the detection of replication-specific CaMV DNAs.

Carole Harker, Andy Maule & Christine Perbal; John Innes Institute, Norwich.
HARKER@UK.AC.AFRC.JII

From Nick Harris & Phil Gates...

Development of the silique of *Arabidopsis*.

Jacqui Spence has now largely completed the histological description of the development of the wild type silique and also looked, in detail, at the clv1 and clv2 mutants. A model is being devised that explains the bi-, tri- and quadrilocular interconversions which can occur even within a single silique. We have identified a number of 'markers' which are useful for identifying the differentiation of key tissues associated with both ovule development and the dehiscence mechanisms of the mature silique. In addition, we have produced, and are now examining, the development of parthenocarpic fruits. These fruits were produced by GA treatment of emasculated early flower buds.

Screening of EMS mutants has continued and we have isolated a number of potentially interesting lines with variations in, for example, endocarp layers and form. We are however still searching for a non-dehiscent mutant!

Lesley Edwards joined us from 1st February. She has just returned from US where she was working in Richard Dixon's lab. She will be doing molecular work to complement the microscopical efforts carried out so far.

Nick Harris & Phil Gates; Dept. of Biological Sciences, University of Durham.

From Pat Heslop-Harrison...

Localisation and characterisation of tandemly repeated DNA sequences in *Arabidopsis*.

A family of DNA clones known as pAL1 and pAS have been isolated from *Arabidopsis thaliana* by Martinez-Zapater *et al** (1986). The family constitutes more than 1% of the genome, and the molecular evidence suggests that it is present in tandemly repeated arrays of the basic 180 bp repeat unit.

We have used the sequence pAL1 (kindly provided by Chris Somerville) as a probe for *in situ* hybridisation. It hybridises to the paracentromeric region on all 5 pairs of chromosomes, and the sites of hybridisation co-localise with the centromeric heterochromatic bands on the chromosomes. Although not fully quantitative, visual analysis of the micrographs indicates that the size of all the tandem repeats is similar; Martinez-Zapater *et al.* reported that the sequence occurred in blocks greater than 50kb long, which agrees with these data.

"The probe can be used to determine chromosome number in interphase."

The probe hybridises well to interphase nuclei, and gives ten distinct sites of hybridisation in diploid nuclei. The probe can be used to determine chromosome number in interphase, including differentiated, nuclei since the number of sites can be counted easily. Because the sequence is now known to be located at all the centromeric regions, it can be used as a probe to isolate YACs from the library which may include the centromeric elements.

*Martinez-Zapater, J.M., Estelle, M.A. and Somerville, C.R., (1986) A highly repeated DNA sequence in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 204, 417-423.

Jola Maluszynska & J.S. (Pat) Heslop-Harrison, Karyobiology Group, JI Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, U.K.

From Eric Holub...

Identification and mapping of genes for resistance to fungal pathogens of *Arabidopsis*.

We have become budding breeders of *Arabidopsis thaliana* since the last newsletter as we have nearly completed a half-diallele cross of fourteen plants selected from lines including Columbia (gl-1), Landsberg

erecta, Cape Verde (Cvi0), USSR (RLD), Germany (Nd0), Norway (Oy0), USA (Kin0), Japan (Tsu0), Switzerland (from Weiningen provided by A. Slusarenko), and England (three plants from East Malling and two from Keswick). The plants were selected according to differential reactions to two isolates each of *Peronospora parasitica* and *Albugo candida* (see Newsletter No. 4). The crosses will be used to determine the number of host genes affecting resistance to each of the four isolates. Some ecotypes react the same phenotypically, such as Columbia, Landsberg *erecta* and RLD which are resistant to both *P. parasitica* isolates; but crosses between them should reveal whether they are also identical genotypically. To complement the genetic analysis, Jim Beynon (Wye College, Univ. of London) is going to assist us in comparing RFLPs in the various parental lines, thereby providing information that will allow us to decide which crosses from the diallele to utilise first in mapping host resistance genes. At the same time, we soon expect to receive from Jeff Dangl (Cologne) F3 families of a cross between Nd0 and Oy0. He is already engaged in examining segregation of RFLPs in this cross.

In the art of breeding *A. thaliana*, we have noticed how the flowering plants in a controlled environment can greatly affect the success of cross-pollinations. For instance, several parents were ideal (*e.g.*, Nd0, Kin0, and Tsu0) producing large buds, abundant pollen, and sufficient stems to perform all of the desired crosses on the same plant. Such plants produced each day 2-4 flower buds per stem which were receptive to cross-pollination. Consequently, we expected to produce on a given plant at least two pods per stem for each cross. However, Cvi0 was more difficult to pollinate producing only one receptive bud per stem each day. The receptivity of Cvi0 buds was poor, and pods that had set on Cvi0 often withered prematurely. We also found Columbia to be a more receptive female parent than Landsberg *erecta*. Maybe we need more practice. Nevertheless, we would appreciate comments from anyone with experience in breeding the same ecotypes.

Keep an eye out for *P. parasitica* growing on your plants. Jane Parker found downy mildew on some of her Columbia. Since neither of our *P. parasitica* isolates are virulent to Columbia, Jane's discovery adds to our search in the U.K. for new isolates with differential virulence. Contact us if you need help with disease diagnosis or if you have a sample to send us.

Eric Holub & Ian Crute; IHR, East Malling, Maidstone, Kent ME19 6BJ.
HOLUBE@UK.AC.AFRC.EMRS

"Keep an eye out for *Peronospora parasitica* growing on your plants."

From Gareth Jenkins...

Isolation and characterisation of photoregulatory signal transduction mutants in *Arabidopsis*.

Our main objective is to produce transgenic *Arabidopsis* expressing chimaeric constructs which we can subsequently use to screen for photoregulatory mutants. We are continuing to make and express constructs using several different promoters and reporter genes. We are at the stage where we have seed from two of our transgenic lines and will soon start mutagenesis and screening. In addition, we have started a conventional screen for mutants altered in photoregulation. So far this has not yielded any particularly interesting mutants of the types we want, but we have obtained some very strange looking weeds! One mutant has broad stems and appears to have an alteration in apical organisation. Unfortunately, flower development proceeds abnormally and the mutant so far appears to be sterile. We have an interest in genes expressed in apices and have cDNA clones of genes from *Brassica napus* which we would like to study in the mutant, so we are thinking of ways of persuading the mutant to reproduce. Does anyone have any ideas?

Gareth Jenkins, Karen Deeney & Jennie Jackson (and also Nigel Urwin); Departments of Biochemistry & Botany, University of Glasgow.

From Peter Jordan...

The genes encoding the early enzymes of the chlorophyll biosynthesis pathway in *Arabidopsis thaliana* and their regulation.

Oligonucleotide PCR probes have been synthesised based on highly conserved protein sequences derived from the *hemB*, *hemC* and *hemE* genes, or cDNA, from several different sources. The probes have been used successfully to generate DNA fragments from an *Arabidopsis thaliana* genomic library in λfix, provided by Dr. Deanna Raineri. PCR fragments from the *hemC* and *hemE* genes did not yield clear sequence information from direct sequencing and have now been cloned into restriction sites of M13mp18 and 19. Sequencing of these clones is currently underway. The clones identified on the basis of sequence similarity with *hemC* and *hemE* genes as well as oligonucleotide PCR probes will be used to screen a cDNA library in λzap, kindly provided by Dr. Philip Horsnell.

Parallel work is underway to complete the isolation and characterisation of the early enzymes in the chlorophyll biosynthesis pathway. The enzymes 5- aminolaevulinic acid dehydratase and porphobilinogen deaminase have been partially purified. Efforts are being made using FPLC to isolate the homogenous proteins and to carry out N-terminal protein sequence analysis. This will permit the start of the coding region of the mature enzymes to be identified. It has been necessary to synthesise two of the substrates, glutamate 1-semialdehyde and porphobilinigen used for the assay of glutamate 1-semialdehyde aminotransferase and porphobilinigen deaminase respectively. The former has been prepared chemically, whilst the latter has been prepared enzymically from 5-aminolaevulinic acid. The dual approaches using molecular biology and enzymology will complement one another.

P.M. Jordan; Biochemistry & Molecular Biology Laboratory, Queen Mary & Westfield College, University of London, Mile End Road, E1 4NS.

Project Summaries

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana.*

To remind you, the objective of the project is to generate functional tags of developmentally interesting genes by creating fusions between native promoters and a promoterless gusA coding region. Having shown in tobacco that active gene fusions occur at high frequency (in *ca.* 50% of all transformants), we have begun a detailed analysis of *Arabidopsis* transformed with the same promoterless gusA construct. We have used Southern blot/hybridisation analysis to confirm that kanamycin resistant plants contain the gusA coding region, and have used this information in concert with kanamycin resistance segregation data to determine the copy numbers of T-DNA inserts. As soon as we have identified single locus transformed lines that either exhibit developmentally regulated GUS activity which is transmissible or show stably aberrant phenotypes (which co-segregate with the T-DNA), we will isolate the sequences flanking the T-DNA using inverse PCR.

Until now our data for *Arabidopsis* have been slower in coming than for tobacco, because of early problems in achieving predictable and routine transformation and seed set (probably due to a large extent to the mutant npt-II gene in our Bin19-based vectors). Now that most of these problems have been resolved, we will study in detail GUS fusion expression in both T1 (primary transformants) and in T2 and beyond. Analysis of GUS expression in plants transformed with a construct containing the CaMV35S promoter linked to gusA is being carried out in parallel with studies of plants transformed with the promoterless gusA.

Keith Lindsey, Mike Clarke, Jennifer Topping & Wenbin Wei; Leicester Biocentre, University of Leicester.
DRL@UK.AC.LEICESTER

From Keith Mitchelson...

Identification and cloning of hypervariable loci from *Arabidopsis thaliana.*

Deborah Silcock has left the project. She will be replaced by Andrew Porter, who will start in April.

Keith Mitchelson; Dept. of Molecular & Cell Biology, University of Aberdeen.

From Bernie Mulligan...

Genetic male sterility in *Arabidopsis.*

Several aspects of our work are now running well. The γ-mutagenesis experiments are certainly producing mutants and for genomic subtraction purposes, we hope that some of these will represent deletions! We are screening for fertility mutants in our own experiments. Does anybody want us to keep an eye open for any other obvious developmental mutants? We seem to have overcome our problems with W100 (fertile) - we simply weren't giving enough thiamine. So, after scoring seedlings for bleached first leaves (thi$^-$), we now water our greenhouse grown plants 3 times a week with 1% thiamine to produce lush and fertile plants.

"The γ-mutagenesis experiments are certainly producing mutants."

We have now screened the Somerville YAC library using a range of RFLP probes which have been mapped close to the *ms*-1 gene. Positive colonies have been identified for all the probes that are hopefully very close to *ms*-1. These YACs have been characterised both by sizing using pulsed field gel electrophoresis and by restriction enzyme digestion and probing with the original starting probe. This establishes small contigs for each region of RFLP probe. End-probes, using YACs which hybridized to RFLP probes 4560 and 21503, are currently being produced by IPCR and used to re-screen the YAC library. These RFLP probes are known to be close to *ms*-1 by RFLP mapping using recombinants with breakpoints close to *ms*-1 (pers. comm. Mandy Walker). We have also managed to strip and then reprobe the colony blots used for YAC library screening by 0.1% SDS at 65°C for 20 min. This will hopefully overcome the requirement for frequent filter production.

We are also exploring the potential of using genomic subtraction for gene isolation from *Arabidopsis.* A range of control studies have been carried out, the most recent being re-association experiments. This work is looking promising and we hope to begin a true test of genomic subtraction in the near future.

Zoe Wilson, Janet Fuller, Jane Russell, Mark Aarts, Greg Briarty & Bern Mulligan; Dept. of Botany, Nottingham University. Mac and modem available. Tel. 0602-484848 (ext 3467).
PBZBM@UK.AC.NOTTINGHAM.CCC.VAX

From Jim Murray...

Molecular identification and analysis of genes involved in plant development and growth control.

Progress this quarter has been interrupted by the move to our new lab in the new Institute building on Tennis Court Road. We moved the lab just before Christmas, but have spent most of January organising the space and the services. The lab has been up and running now for about 4 weeks.

We were encouraged by some preliminary DNA sequence data for the *Chlamydomonas* PCR product, amplified using primers against cdc2, that predicted a conserved amino acid sequence. This sequence data was obtained using one of the PCR primers as a sequencing primer directly on the amplified DNA (denatured using NaOH). The two *Arabidopsis* PCR products, which were amplified using the same primers, have been cloned, with some difficulty, after polishing the ends of the amplifed DNA with Klenow and T4 DNA polymerase. We have some sequence information for each, *Arabidopsis* but have not yet had access to the network computers (still being connected) to see what sequences they predict.

Jeremy Carmichael & Jim Murray; Institute of Biotechnology, University

of Cambridge. Tel 0223 334754.
JAHM@UK.AC.CAMBRIDGE.PHOENIX

From Steven Neill...

Identification of water stresses and ABA regulated genes using wilty mutants of *Arabidopsis thaliana.*

An even shorter report than last time! We have confirmed that the wilty mutants do indeed fail to synthesise ABA in response to water stress. Wild type leaves increased their ABA content from 37 to 150 ngg-1 f.wt following 8h stress. While the ABA content of *aba*-1 leaves remained at *c.* 25 ngg-1 f.wt. We have also demonstrated that stress-induced ABA production has a requirement for *de novo* transcription and translation. Pre-treatment of leaves with cordycepin or cycloheximide led to reductions in the rate of stress-induced ABA synthesis from 25 to 2 and 0 ngg-1h-1 respectively.

We have isolated mRNA from different tissues after various treatments and analysed the mRNA by *in vitro* translation and one-dimensional SDS-PAGE. These analyses have demonstrated that in wild type leaves at least one mRNA is induced by both stress and ABA treatment and at least one mRNA by stress *per se.*

In conjunction with Mike Bulman, a SERC-supported RA, we have started to analyse a cDNA library prepared from stressed wild type leaves, using cDNA probes prepared from mRNA isolated from turgid and wilted leaves. Furthermore, we have begun to construct a subtractive cDNA library, enriched for stress-inducible sequences.

Steve Neill & Jackie Williams; Bristol Polytechnic.

From Helen North...

Cell cycle control genes in *Arabidopsis.*

RNA has been prepared from carrot suspension culture cells and cauliflower meristematic tissue. Poly (A+) RNA has been purified from both preparations and we are now in the process of producing cDNA libraries in a *S. pombe* expression vector. We have succeeded in efficiently transforming *S. pombe* with a test vector and are now repeating this using a *S. pombe* genomic library, prior to an attempt to rescue *S. pombe* cell cycle mutants with our plant libraries.

Helen North & Jeremy Hyams; Dept. of Biology, UCL.

From Jane Parker...

Infection of *Arabidopsis thaliana* with *Xanthomonas campestris* pathovar *campestris*: The search for resistance genes.

We seem to have made some progress! The plants are growing well and uniformly in controlled environment cabinets under high light intensity and short day length. About 25 ecotypes have been tested for their response to *Xanthomonas campestris* pv *campestris* strain 8004 and 8004 containing the putative avirulence gene(s) derived from strain 1067, denoted 8004(avr/*Xca*). Most ecotypes gave the same response as Columbia (*i.e.*, susceptible to 8004 and resistant to 8004(avr/*Xca*)). However, one candidate was susceptible to 8004(avr/*Xca*). This is being propagated through a single seed and the next generation will be retested and used for growth studies. Two other ecotypes appeared to be resistant to 8004 and these are also being taken further. In addition, we have started an extensive screening of ecotypes Columbia, Landsberg *erecta*, Niedersenz and the local strain, JI-1 for variation in their responses to 45 different natural isolates of *Xcc.*

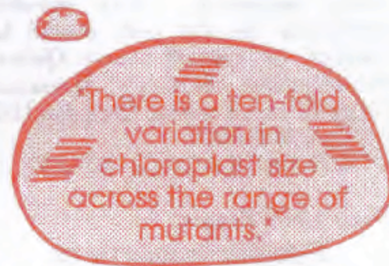
Tn5-mutagenesis of the 25 kb fragment of 1067 DNA containing the putative avirulence gene(s) followed by testing the mutants on Columbia plants has defined a discrete region of approximately 1.5 kb which contains the avirulence function. This suggests that the activity is indeed conferred by only one gene. A suitable fragment is being subcloned for pathogenicity tests and sequencing. In addition, selected mutants are being marker-exchanged into strain 1067 to test if these become pathogenic on normally resistant *Arabidopsis* plants.

Jane Parker, Christine Barber & Michael Daniels; The Sainsbury Laboratory, John Innes Institute, Norwich.

From Kevin Pyke...

An analysis of leaf development and chloroplast division in *Arabidopsis thaliana.*

We have now completed primary screening of the 37 putative chloroplast division mutants selected from an initial screen of 3337 seedlings of an M2 population. Of these 37 mutants, 18 were selected in the M3 generation which had shown stable inheritance. Of those rejected; some had poor seed set, some lost the mutant phenotype and some died before flowering. On the basis of phenotype, these 18 mutants fall into several phenotypic groups. Mutants with more chloroplasts than wild type for a given cell size have smaller chloroplasts than wild type. Whereas those mutants with fewer chloroplasts than wild type have larger chloroplasts.



There is a ten-fold variation in chloroplast size across the range of mutants examined. Thus it appears that division and expansion of chloroplasts are mutually compensating processes yielding a relatively constant coverage characteristic for the cells. We are currently backcrossing M4 and M5 plants from a selection of these mutants to establish whether they are single genes and also performing crosses between mutant lines to determine how many allelic groups we have. In addition we are analysing in more detail the mutant phenotypes through development.

Kevin Pyke (RA) and Rachel Leech (PI), Department of Biology, University of York, Heslington, York,Y01 5DD UK.
KAP2@UK.AC.YORK.VAXA

(See Kevin's leaf cell fixation & separation protocol – ACM.)

From Mandy Walker...

Trichome differentiation in *Arabidopsis*: molecular characterisation of the TTG locus.

In order to clone the gene TTG involved in both leaf hair development and anthocyanin production in the plant body and in the testa, we have been aligning the RFLP markers of Hauge and Goodman with the genetic markers, ttg and ms1. ttg is on the top of chromosome 5, 6 map units proximal of ms1.

We can conclude from Southern analysis of recombinants between ttg and ms1 that the RFLP markers 6843 and 4556 come from the region close to, or proximal to ttg; while 4560 is located close to, or distal to ms1. A new RFLP 21503 maps between ttg and ms1. Further Southern analysis will show the exact map position of 21503 relative to ttg and ms1.

Mandy Walker & John Gray; Botany School, Cambridge University.
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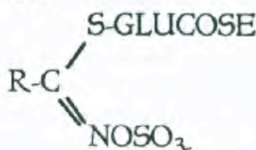
GUEST SUMMARIES

From Fatima Bano...

Glucosinolates in *Arabidopsis thaliana*

I am a third year post-graduate student, working on this project with Dr. John Turner at the University of East Anglia and Dr. Roger Fenwick at the Institute of Food Research, Norwich Laboratory.

Glucosinolates are a uniform class of secondary metabolites. More than 100 glucosinolates are known, all have the following common basic skeleton, but differ only in the nature of the side chain R:



They are extensively found in the family Cruciferae, especially in the brassicas, including green and root vegetables and rapeseed. The exact functions of glucosinolates are not known, but they or their breakdown products may be involved in flavour and aroma, bitterness, toxicity, goitrogenicity, insect attraction and deterrence, carcinogenicity, anti-carcinogenicity and disease resistance.

The subject of my research is to study the general biology of glucosinolates, their regulation and molecular genetics in *Arabidopsis*. It is anticipated that the knowledge gained will lead to a better understanding of glucosinolates in other economically important plants (for example rapeseed), which are not as genetically amenable as *Arabidopsis*.

My studies carried out on the *Arabidopsis* ecotype, Landsberg *erecta* show that tissue distribution of glucosinolates varies considerably. Glucosinolate content varies with the age of the plant and is affected by sulphate and nitrate nutrition. Glucosinolate induction occurs on wounding, infection and infestation of the plant. Screening of lines (wild types) of *Arabidopsis* has revealed both quantitative and qualitative variations in these compounds. A cross between Landsberg *erecta* and Columbia has shown the emergence of at least one glucosinolate not found in the parents.

Work is in progress to screen further lines and species of *Arabidopsis* for glucosinolate variations and selection of suitable parental types for more crosses.

One major problem encountered is the absence of internal standards for some of the glucosinolates present and the non-availability of LC.MS facilities. As a consequence, I have to isolate the unknown glucosinolates, which entails not only about two weeks full-time work, but also the collection of at least 30 grams of *Arabidopsis* seeds. For analysis, too, much larger quantities of leaf material is required than can normally be expected from such a small weed. So, I have to raise more plants, harvest more seeds and this coupled with tedious and time consuming extractions, purifications, quantitations and identifications almost make me want to shout: "*Arabidopsis* is not a suitable choice for biochemical work!"

Fatima Bano, School Of Biological Sciences, University of East Anglia.

From Axel Brennicke...

A mitochondrial genome of *Arabidopsis thaliana* is being sequenced - progress, but no end yet.

Cosmids containing mitochondrial DNA fragments from *Arabidopsis thaliana* were selected from the total DNA cosmid library of Ulla Halfter and Lothar Willmitzer with heterologous probes of mitochondrial genes. So far, 350 kb of unique sequence of the mitochondrial genome of *Arabidopsis* have been identified in numerous cosmids.

> "350 kb of unique sequence of the mitochondrial genome of *Arabidopsis* have been identified in numerous cosmids."

These cosmids could be arranged in several linkage groups, but the definite structure of the mitochondrial genome in *Arabidopsis* still has to be established. The mitochondrial genome of *Arabidopsis* by the way is by no means the smallest of the plant mitochondrial DNAs so far analysed (unfortunately not). To be able to differentiate between chloroplast sequences integrated in the mitochondrial genome and genuine chloroplast DNA, the entire map and clone library of the chloroplast genome has also been established by Wolfgang Schuster. Several cosmids of the mitochondrial genome were selected to start sequencing (Wolfgang Schuster). A combination of several approaches was used, completely random sequencing of sonicated total cosmids, terminal sequencing of BamHI subclones and of Sau3A or TaqI subclones. So far, a little over 160 kb of unique sequence have been determined, but are still only stored as numerous small linkage groups between 200 and 8,000 nucleotides in length. Several tRNA genes and parts of protein coding regions could be identified, but have to be investigated further and completed. Analysis of cDNA clones and sequences (Volker Knoop) is essential in the context of RNA editing in ➛

plant mitochondria. Genomic sequencing and logistics are in the hands of Michael Unseld and Petra Gronger, assisted by 1½ able technical assistants and student labour. Structure, organization and transcription of several *Arabidopsis* genes have been analysed in comparison to other plant species by Bernd Wissinger, Wolfgang Schuster, Stefan Binder and Anita Marchfelder. The sequencing project is currently supported by the Bundesministerium für Forschung und Technologie as a national affair. It will be a while yet until the entire basic sequence information can be put together for the mitochondrial genome of *Arabidopsis*, but we are working on it.

Michael Unseld, Petra Gröngeer & Prof. Dr. Axel Brennicke; Institut für Genbiologische Forschung Berlin GmbH, Ihnestraße 63, 1000 Berlin 33, Germany.

From Vicky Buchanan-Wollaston...

Isolation and characterisation of genes involved in leaf senescence in *Arabidopsis*.

I have just started at Wye college on a lectureship funded by the UFC Biotechnology directorate. My research group at this moment consists of a research technician, Ailsa Chambers and a graduate research student from Pakistan, Raziuddin. Hopefully, external funding will be obtained to expand this group. I am planning to study the molecular genetics of leaf senescence in *Arabidopsis* and *Brassica napus* with the aim of increasing the understanding of the regulation and processes that are involved at this stage of plant development.

The project consists of two main parts. We will isolate and characterise cDNA clones of genes that are induced during leaf senescence. Currently, we have RNA of both species isolated from leaves at various stages of senescence. The quality of this RNA is being assessed and then cDNA libraries will be constructed. Subtraction techniques may be used to isolate genes that are specifically induced during senescence.

The second part of the project involves the isolation and characterisation of *Arabidopsis* mutants that are defective in some stage of leaf senescence. Currently, we are screening Lehle mutagenised M2 seeds of Landsberg *erecta*. Several potential mutant phenotypes are being assayed in each plant. We have identified growth conditions in which the level of N supplied to the plants is minimal. Under these conditions a mutant that cannot utilise all integral nutrients should show very poor seed development in the absence of extra N. We plan to produce gamma irradiated seed with the help of Bernie Mulligan's group and collect M2 families from this that can then be screened for such potentially lethal phenotypes. If deletion mutants can be obtained by this method the isolation of the mutated gene is possible by the genomic subtraction method.

From Robin Chapple...

The *Arabidopsis* genome project - Australian input.

We have begun work on the genome mapping project in the region of chromosome 1 around the RFLP markers 281, 213 and 280, where we hope to order a dozen or so YACs from the Somerville library. So far, we have been establishing conditions for colony hybridisation using riboprobes, basing our methods on the protocols used by Renate Schmidt, but with modified hydridisation conditions. We are developing some of the techniques we need in the region of ms-1, which is also of interest to us. We have successfully pulled out two clusters of YACs so far, and are working on ordering and aligning them. We have begun to clone the YAC ends, and using yeast genetics, we hope to determine the extent of overlap between YACs, as well as using YAC ends to screen the library for further contiguous YACs. This work is a collaboration between Liz Dennis, Abdul Chaudhury, Robin Chapple, & Jim Peacock at CSIRO Division of Plant Industry, and Richard Williamson, Jacek Plazinski & Brian Gunning, The Australian National University, Canberra. It is supported by the Dept. of Industry, Technology and Commerce.

BURN@AU.OZ.PICAN

From Jeff Dangl...

Isolation of *Arabidopsis* genes for resistance against phytopathogenic bacteria.

Firstly, let me say "Fröhliches Neues Jahr" to our friends and colleagues in Norwich and elsewhere in England. We'd like to contribute an update on our progress in identifying and isolating *Arabidopsis* genes necessary for a successful resistance response against phytopathogenic bacteria.

> "In one case, the resistant phenotype is dominant, and segregates as a single locus."

We started by screening several ecotypes (18) with a collection of *Pseudomonas syringae* isolates from various *Brassica* species (12). Both ecotypes and bacterial isolates were chosen for maximal geographic diversity. After tinkering with plant growth and leaf inoculation conditions, we were able to optimise our experimental regime. In these experiments, we look for visible evidence of resistance (a hypersensitive response) or disease (wet lesions which spread from the initial inoculation site, often accompanied by chlorosis, but finally also become necrotic). We also decided early on that analysis of *in planta* growth kinetics was crucial for proper interpretation. This is true because some virulent bacterial strains cause little or no symptoms until around 7 days post-inoculation, and thus would be misclassified in a fast screen relying only on phenotype. We have identified a fairly large number of reproducible differential reactions, and have begun genetic analysis of ecotype crosses. In one case, the resistant phenotype is dominant, and segregates as a single locus. RFLP mapping has been used to localise this locus, and we are continuing to "fine-map" its position as a prelude to either chromosome walking, or YAC/cosmid "isolation by telephone". In a second cross, resistance to the same bacterial isolate

does not segregate simply. To further this analysis, we have cloned one, and possibly two *avr* genes from the relevant bacterial strain.

We now will screen this second cross with a coned *avr* gene, which we assume will simplify segregation analysis of a corresponding R gene.

We have also screened 17 isolates of *P. cichorii* and found, under our conditions, that all were avirulent on *Arabidopsis* Col-O. Using one, we screened an EMS-mutagenised population, looking for any alteration of phenotype. Several strong candidates survived 3-5 more selfings and re-screens, and genetic analysis is in progress.

Besides this plant work, we are also investigating bacterial genes that are necessary for generation of an HR on *Arabidopsis*. As mentioned, we've isolated a couple of *avr* genes *via* classic methods. As well, we've identified two *P. cichorii* genes encoding membrane or periplasmic proteins, which when mutated, allow *P. cichorii* to grow *in planta*, and remain there, undetected, for up to two weeks. These mutants are still, however, unable to cause symptoms. Further structural and functional analysis of those bacteria proteins is in progress. Hiltrud Lehnackers, Thomas Debener and Maren Gerwin are the major "plant locus identification" group, Martin Arnold cloned the *avr* genes, and Christoph Rupprecht and Corinna Clemens have identified the bacterial membrane protein gene mutants.

Jeff Dangl; Max-Delbrück-labor in der MPG, Carl-von-Linné-Weg 10, D-5000, Köln 30, Germany.

From Louise de Villiers...

Senescence in *Arabidopsis*.

The objective of this project is to isolate senescence-specific genes from *Arabidopsis* with the aim of studying their regulation and ultimately the manipulation of senescence in *Arabidopsis* and other species. A mutagenesis programme has been initiated and 2000 plants have already been screened for advanced or delayed senescence, but as yet nothing interesting has been found. Preliminary physiological characterisation of protein and chlorophyll degradation has been carried out in *Arabidopsis* induced to senesce in the dark. Gene expression has also been studied by examining the cell-free translation products of mRNA isolated from senescing tissue. No new major species of mRNA appear to have been transcribed or to have become translatable during dark-treatment. A cDNA library in λgt10, containing 500,000 pfu, has been prepared from nonsenescing leaves of *Arabidopsis*. This is in readiness for differential screening with both a cDNA library prepared from leaf tissue of a senescence mutant and a cDNA library prepared from senescing leaf tissue of the wild-type, with the aim of isolating senescence-specific sequences.

Louise de Villiers, WPBS, IGER.
GAYA@UK.AC.AFRC.WPBS

From Michel Delseny...

Seed formation and germination.

Work in Perpignan on *Arabidopsis* is a continuation of our previous programmes on seed formation and maturation in radish. Two main projects are being developed in our group. The first consists of characterising the structure and regulation of genes expressed during seed maturation as an example of ABA regulated genes. Several cDNA clones have been previously characterised from a library made from radish dry seed mRNA. Most of these clones hybridise to *Arabidopsis* genes in stringent conditions. Two different genes coding for an Em-like protein have been isolated and sequenced by Monique Raynal, Pascale Gaubier and Gordon Huestis. Gillian Hull is preparing chimeric constructions to study the promoter. Several other radish cDNA clones are being used to select additional *Arabidopsis* genomic clones, including one homologous to the RAB 17 or dehydrin genes.

"Our group is willing to exchange information and material with the British group."

The second project is a collaborative one between Patrick Gallois and Keith Lindsey and is aimed at tagging genes expressed during embryogenesis and germination with T-DNA insertions.

First transformations have now been made in Perpignan and the project will develop with the arrival of Martine Devic in our group, coming from the Sainsbury lab in Norwich.

Our group is willing to exchange information and material with the British group. Michel Delseny, with Jerome Giraudat in Gif and Michel Caboche in Versailles, is co-ordinator of the *Arabidopsis* club in FRANCE and he will be happy to help in circulating information between the two countries.

Michel Delseny; Laboratoire de Physiologie et Biologie Moleculaire Vegetale, Universite de Perpignan, 66860 Perpignan, France. Tel: 33.68.66.88.48 Fax: 33.68.66.84.99
DELSENY@FRPERP51.BITNET

From Darroch Hall...

Antifungal compounds in *Arabidopsis thaliana*.

This project is a biochemical and genetical study of antimicrobial compounds involved in the response of *Arabidopsis thaliana* to infection by fungi. I hope to find variation in the susceptibility of different ecotypes of *Arabidopsis* to at least one of the several fungal pathogens that are being evaluated. This variation will then be characterised in terms of glucosinolates, phytoalexins and other possible resistance mechanisms. In addition to this, I hope to map any resistance genes by a system of crossing and RFLP mapping. With this in mind, the initial stages of the project have concentrated on optimising the infection conditions for a variety of pathogenic fungi on Landsberg *erecta* to enable rapid screening of the other ecotypes at a later date. So far, I am pleased to report that I have discovered one fungal pathogen of *Arabidopsis* and I will shortly be screening to identify resistant ecotypes.

Darroch Hall; John Turner & Richard Oliver, School of Biological Sciences, University of East Anglia.

From David Hanke...

Screening for inositol auxotrophs.

As part of a CASE-collaboration with MSD's Neuroscience Research Centre, our aim is to sequence a plant gene for inositol 1-phosphate phosphatase. Our strategy is to isolate inositol auxotrophs of *Arabidopsis* by screening for inositol-responsive dwarves amongst seedlings grown from M2 seed. We would be interested to hear from anyone attempting to isolate auxotrophs.

Harry "Bidopsis" Charlton & David Hanke; Botany Dept., Cambridge University. Tel: 0223-333913; FAX: 0223-333953.

From Malcolm Hawkesford...

Isolation of transport-protein mutants in *Arabidopsis*.

We are involved in a programme which will hopefully lead to the identification of transport proteins for anions at the plasma membrane of plant cells. One of the several approaches we are taking is to isolate *Arabidopsis* mutants with altered transport characteristics with a view to restoring transport competence by phenotypic complementation. Thus far, we have isolated a number of lines which show marked resistance to a toxic screen of selenate. Preliminary analysis encourages the view that such lines have a restriction placed on sulphate influx via a high affinity transport system. Wild-type and mutant lines did not differ in the K_m or V_{max} of sulphate influx in plants where sulphate supply was optimal (K_m 40 mM, V_{max} 78 nmol $SO2^-_4$ g^{-1} root h^{-1}), but when the transport system was derepressed by S-limitation, the capacity of the transport system in the mutants responded far less than the wild-type (V_{max} of the wild-type was 697 nmol $SO2^-_4{}^{-1}$ root h^{-1}, V_{max} of line 2c was 217 nmol.$SO2^-_4$ g^{-1} root h^{-1}). While quantitative variation of this kind may not directly help us, it encourages the view that the mutants we seek may be found using selenate tolerance. Similar protocols are being developed using vanadate and arsenate to select for individuals with impaired high affinity phosphate transport systems.

Malcolm J. Hawkesford & David T. Clarkson; Long Ashton Research Station, Long Ashton, Bristol, BS18 9AF.

From Roger Innes...

Resistance of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* pv. tomato.

Work continues on the interaction between *Arabidopsis* and the bacterial pathogen *P. syringae* pv. tomato (Pst). A single bacterial gene, avrRpt2, that converts a virulent Pst strain to avirulence on ecotype Col-0 has been identified (see the January 1991 *Plant Cell*). Sequence analysis of this gene has been remarkably non-informative.

"*Arabidopsis* may contain disease resistance genes that are also found in agriculturally relevant plants."

We found no similarity to previously identified avirulence genes, or to anything in the data bases. However, the promoter region does have a 8bp sequence that seems to be well conserved in several other avirulence genes and in the so-called "hrp" genes, which are required for induction of resistant reactions and for pathogenesis. The avirulence gene avrRpt2 also induces resistant reactions on soybean and common bean, suggesting that *Arabidopsis* may contain disease resistance genes that are also found in agriculturally relevant plants.

Mapping of the resistance gene in *Arabidopsis* Col-0 that corresponds to avrRpt2 is progressing. We identified a susceptible ecotype, Po-1, and have crossed Col-0 and Po-1. Segregation of resistance in the F2 suggests that Po-1 is lacking 2 genes that are required for expression of resistance (*i.e.*, approximately a 9:7 ratio of resistant:susceptible progeny). We are currently scoring F3 progeny to confirm our F2 scores.

We are also screening mutants of ecotype Col-0 that are susceptible to Pst strains expressing avrRpt2. To date, we have screened about 3,000 plants from a DEB-mutagenised population kindly provided by Joe Ecker. We have one "putant" that we are quite excited about. The M3 progeny were just planted the last week of January and will be tested the first week of March for transmission of the susceptible phenotype. Stay tuned.

Roger Innes, Barbara Kunkel, & Andrew Bent, (Brian Staskawicz's lab.), Plant Pathology Department, Univ. of California, Berkeley, CA 94720, U.S.A. (after April 1, 1991, Roger Innes will be at Department of Biology, Indiana University, Bloomington, IN 47405)
LIAM1@EDU.BERKELEY.VIOLET

From Graham King...

Compositional compartmentalisation in the *Arabidopsis* and *Brassica* genomes.

We have started to compare the genome architecture of *Arabidopsis* and *Brassica* nuclear genomes as a preliminary to studying evolution of linkage groups in cruciferae. Our first objective has been to survey variation in base composition in these two genomes at different levels of organisation. Total base composition is being determined using several methods. Sequence analysis of all published cruciferae sequences has been carried out to look at base-composition variation, dinucleotide frequencies and its effect on codon usage. Experiments are underway to fractionate genomes according to base composition to determine whether they are organised according to the 'isochore' model of Bernardi *et al.* Characterised clones will be hybridised to genomic fractions to determine their distribution, and provide information on relationships between base composition and genetic map location for different classes of genes.

In future, it is hoped that mapped lambda, cosmid and YAC inserts will be analysed for base composition heterogeneity to build up a picture of interactions in sequence variation at different levels of organisation.

G.J. King; Breeding & Genetics, H.R.I., Wellesbourne, Warwick CV35 9EF.
GJKING@UK.AC.AFRC.NVRS

From Marteen Koorneef...

Arabidopsis in the low countries.

Although not supported by the AFRC, we have an interest in *Arabidopsis* and your exciting newsletter. For this reason, it is fair that I should let you know that there is still some *Arabidopsis* growing on the other side of the North Sea.

Our present research programme involves two projects, which from this year on will be supported by the EC Bridge Programme. The first project deals with the genetics of seed maturation in *Arabidopsis*. This process takes place after embryogenesis and is clearly affected by abscisic acid. We already have isolated new mutants and will characterise these in collaboration with groups in France, Spain and the Netherlands. The second project is in collaboration with Caroline Dean, George Coupland and José Martinez--Zapater, and deals with the genetics of flowering time. In addition to the 11 loci which we identified among our own late flowering mutants, more loci exist and are identified by mutants isolated in other labs.

By describing the genetic analysis of Rédei's *ld* mutant (Genetics, 1962), I want to illustrate the complications of using mutants in other genetic background, especially when they control quantitative traits such as flowering time.

"The difference between *ld* and Ler involves two recessive late-flowering alleles, which have an additive effect in the double mutant."

As a routine procedure, we backcross all mutants with our wild type Landsberg *erecta* (Ler). The first complication was that the very late *ld* mutant was homozygous for a mutation leading to a paler green colour in the first weeks of growth, which apparently reduced growth (and delayed flowering). By counting leaf number (bypassing the effect of growth reduction on flowering time), we found that only approximately 1/16 of the F2 generation had the same high leaf number as the original *ld*. Apparently, the difference between *ld* and Ler involves two recessive late-flowering alleles, which have an additive effect in the double mutant. By isolating non-segregating F3 lines from middle-late F2 plants and intercrossing these lines, we could identify genotypes with only one (flowering time) gene difference from Ler, without the chlorophyll mutant and recessive for *er*. One of these genes has already been mapped on the top of the (UK) chromosome 5. We will further backcross these mutants with Ler to be able to make a fair physiological comparison with the other Ler mutants.

From Ottoline Leyser...

Having (finally) finished my Ph.D. in Ian Furner's lab., I am now working in Mark Estelle's lab in Bloomington, Indiana. I naturally wanted to continue to receive my exciting copy of the *Arabidopsis* Newsletter, but David said he'd only send it if I contributed, so here is the news from Indiana.

Mark's lab is working on auxin resistant mutants of *Arabidopsis*, concentrating on four loci: axr1, axr2, axr3, and aux1. Apart from auxin resistance, all the mutants have at least one pleiotropic phenotype suggestive of auxin related defects. For example, all the mutants show root agravitropisms. All known alleles at axr1 and aux1 are recessive, whilst the axr2 and axr3 mutations are dominant. We are studying the genetics and physiology of the mutants and some of this work has already been published or is in press. A major lab effort is now directed towards cloning these genes using YAC walking from the most closely linked RFLP. Closely linked RFLPs have been identified for axr1, axr2 and axr3. YACs homologous to these RFLPs have also been isolated. The hottest news is that we believe we have isolated a YAC which hybridises to RFLPs that flank the axr1 locus.

"We believe we have isolated a YAC which hybridises to RFLPs that flank the axr1 locus."

Apart from these projects, the lab is also working on a pollen mutagenesis protocol, mutant revertant screens, and new screens for auxin resistant mutants in M2 populations generated with agents known to cause small deletions, such as DEB.

(Ottoline sent this for the previous newsletter, but it unfortunately did not arrive in time! -- ACM .)

From Peter Sijmons...

Zandraket in Leiden.

A cynical coincidence in this period of time; the common name for *Arabidopsis* in The Netherlands is zandraket (which translates in English as sand rocket) and in Leiden, we just started to use this little but harmless rocket in our research programs. This research is a collaboration between the Molbas group at the University of Leiden and Mogen, the plant biotech company in Leiden. As most of this work has just started, we will now only outline our objectives in general terms and mention the names involved. In the upcoming newsletters, we hope to fill you in with more details.

Paul Hooijkaas and Dianne van der Kop focus on auxin receptors and auxin- related signal transduction. Stephan Ohl (who just moved from Chris Lamb's lab), Erik van der Graaf and Annette Vergunst work on homologous recombination. Peter Sijmons is using *Arabidopsis* in a programme on plant-pathogen interactions.

The first 5 names can be reached at: Moleculaire Plantkunde, Wassenaarseweg 64, 2333 AL, LEIDEN. Fax: +31-71-274999;
MPKRODENB@RULGL.LeidenUniv.NL

PS is at: Mogen, Einsteinweg 97, 2333 CB, LEIDEN. Fax: +31-71-221471;
MPKDEKKE@RULGL.LeidenUniv.NL

Guest Summaries

From Brian Thomas & Brian Jordan...

Studies on photoperiodic induction and the effect of ultraviolet-B radiation on *Arabidopsis thaliana.*

Greetings from sunny Littlehampton. This our first contribution to the newsletter and any feedback or contact from other members of the club will be very welcome. Although not grantholders in the programme we have two AFRC-supported projects in the department that use *Arabidopsis* as a model. One of us (BT) with the aid of AFRC student, Dave Mozley is looking at photoperiodic induction of flowering and the other (BJ) is looking at responses to UV-B. Summaries of the state of play in these projects are as follows.

In photoperiodic induction, daylength perception and induction occur in the leaf. This, in turn, regulates the choice between alternative vegetative or reproductive routes of apical development. We are interested in genes involved in the early stages of this regulatory system, *i.e.*, daylength perception and induction. We have a dual strategy. Firstly to analyse the photoperiodic responses of existing flowering and photoreceptor mutants. Secondly, to generate new mutants with altered photoperiodic responses. The first stage of the project has been to define closely the photobiological and chronobiological requirements for induction in the wild type strains Landsberg *erecta* and Columbia. This is nearing completion and we can define the earliest stage at which seedlings can distinguish between long and short days, the critical daylength, and minimum number of inductive days. We expect shortly to have defined the full window of photoperiodic control and light quality requirements. We are using this information to define our screening strategy and will begin to screen EMS-mutagenised seed in the the next couple of weeks. We'll let you know how it goes.

With the depletion of the stratospheric ozone layer, it is envisaged that the earth's surface will be exposed to increased solar UV-B radiation, with potentially deleterious consequences for crop productivity. Characteristic changes in gene expression associated with UV-B stress are the rapid "down-regulation" of gene expression for chloroplast proteins and the increased biosynthesis of protective flavonoid compounds. These responses are similar to those caused by plant/pathogen interactions and therefore elucidating the molecular mechanisms involved is particularly important. We are now studying the response of *Arabidopsis* to UV-B supplements to normal irradiance and preliminary results indicate that *Arabidopsis* shows characteristic symptoms of UV-B stress. After characterisation of changes in gene expression we will construct cDNA libraries in λ Zap II to isolate genes involved in the UV-B response.

B. Thomas, D. Mozley, B.R. Jordan, P. James & R. Anthony; Dept of Molecular Biology, Horticulture Research International, Littlehampton, W. Sussex, BN17 6LP. Tel: 0903 716123 FAX: 0903 726780
THOMASB@UK.AC.AFRC.GCRI

From Richard Williamson et al...

Cell shape and root morphogenesis.

We are looking at root growth and development in *Arabidopsis* and in particular at events involving the wall and cytoskeleton that determine cell shape. We have spent a lot of time establishing methods for immunofluorescence that we can now carry out on sections, whole roots and apical cells released by partial wall digestion. These have given us the arrangement of microtubules through the cell cycle and through and beyond the elongation zone. We are having some success in looking at wall architecture, but would be interested to hear from anyone who has replica or other methods working to reveal cellulose microfibril orientations.

We are quantifying cell sizes and root growth rates from which we can calculate the rate of cell expansion at various points in the root and, when the work is completed, the rate at which new cells are added to the distal ends of cell files. This is all by way of giving us the information with which we can analyse the mutants that we have selected as having abnormal root morphology. We screened microscopically (too laborious to recommend to others) and, by using a temperature shift from 18 to 33°C, obtained several

"These have given us the arrangement of microtubules through the cell cycle and through and beyond the elongation zone."

mutants whose phenotypes are strongly temperature-sensitive. This is important in trying to get mutations disrupting cytoskeletal components whose deletion may well be lethal. At the moment, we are mapping a number of the most interesting ones with a view to carrying out chromosome walks. Our temperature-sensitive mutant collection includes changes to cell shape, root hair initiation, complete but reversible block to root development and so on. Most but not all are apparently root-specific in their effects.

Richard Williamson. Tobias Baskin, Andreas Betzner, Jacek Plazinski & Ann Docherty, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia.
WILLIAMSON@RSBS0.ANU.EDU.AU

From John Wray...

Isolation of *Arabidopsis* mutants defective in sulphate assimilation.

The initial aims of the project are to isolate whole plant mutants of *Arabidopsis* carrying defects in the sulphate assimilation pathway. We hope to identify such mutants by screening for individuals within M9 populations which are resistant to the toxic sulphate analogue, selenate. Current studies are directed towards optimising the screening conditions.

John L. Wray, Amanda F. Gilkes, Plant Molecular Genetics Unit, Department of Biochemistry and Microbiology, Sir Harold Mitchell Building, University of St. Andrews, St. Andrews KY16 9TH. Tel: 0334-76161 (Ext. 7253).

Please send your next summary to the newsletter by Monday, 10th June, at the very latest.

The Arabidopsis Team

1. Who has the smallest genome?
2. Who has the largest genome?
3. Who contributes the most to science?
4. Who gets paid the least from AFRC?
5. Who gets paid the most from AFRC?

From John A. M. Brown...

ARABIDOPSIS REVISITED

MY RESEARCH with *Arabidopsis* was principally with race "Estland", in the 60's and 70's. Some readers might be interested to know that I'm alive and well and living in Cornwall! One morning in October 1989, I read with an amazed sense of déjà vu, a newspaper report about this little plant and £14 million of grants to support research into its genetics!

I wished such support had been available in 1971, the dawning of the age of "genetic engineering", when I really needed it, and I wondered what had happened to catalyse this "second spring" of *Arabidopsis* research?

A visit to Exeter University started me on the trail, and on a steep learning curve, helped by an old colleague, Dr. Ray McKee, into this "third generation, contemporary Arabidopsology", where RFLP's, PCR's, YAC libraries, and chromosome walks are new, and genome analysis seems as much a goal as any applied aim of genetic engineering. I am still learning, so please send me your reprints! "Arabidopsology" commenced in the 1940's, when Laibach and his students in Germany described and collected ecological races from the Baltic to North Africa.

The post-war period resulted in knowledge of this wartime research being disseminated throughout Europe and U.K., to the U.S.A. and U.S.S.R. Studies in biology including research on both radiation and chemically induced mutagenesis received government support, and *Arabidopsis* was an experimental tool used for these purposes on both sides of the Iron Curtain.

In the U.S.A in the 1960s, I worked at Brookhaven National Laboratory, where *Arabidopsis thaliana*, due to its small chromosomal volume and low DNA content, was found very resistant to ionising radiation. The nature of the minimum change in the eukaryote chromosome, required to cause mutant expression, was being explored, comparing ionisation with chemical mutagenesis. As part of this research, I found that some competitive halogen analogues of thymidine caused both morphogenic and mutagenic effects in *Arabidopsis*. Further investigation of these phenomena, with other research and teaching, occupied me for some years, aided by travel and research grants, but funding was difficult.

However, in W. Germany, Gerhard Röbbelen enthusiastically developed research with *Arabidopsis* mutants affecting chloroplast development, and in the Agronomy Dept. of University of Missouri, George Rédei, who had come from Hungary, studied the genetics of flowering. Röbbelen set up the *Arabidopsis* Information Service and also organised the First International Symposium on *Arabidopsis* Research, held in April 1965 at Goettingen University, at which I presented an account of our base analogue research on behalf of the Brookhaven Lab. group. Prof. Laibach appeared and was honoured, and John Langridge was present, but by then was working at the Institut Pasteur with *E.coli*! The proceedings and discussions were published in detail, but not commercially.

In the U.S.A. George Rédei, at Columbia Mo., and myself at Notre Dame, were the only "players" who had attended the symposium. The National Academy of Sciences/National Research Council awarded me a Smithsonian Associateship. So on a sabbatical in Washington D.C., with Bill Klein, I was able to study the wavelength and intensity dependence of floral morphogenesis in *Arabidopsis*.

Lucien Ledoux, after a seminar at Brookhaven, visited me at Notre Dame to discuss his research on the introduction of *Micrococcus lysodeikticus* DNA into *Arabidopsis*, and its apparent persistence and integration, as interpreted from equilibrium density gradient experiments where *Arabidopsis* DNA (d-1.698), was distinguished from *M. lysodeikticus* DNA (d-1.731). This visit led to an invitation to work in Belgium with the Ledoux group at CEN/SCK Mol. where I was able to carry out a confirmatory experiment before leaving for Australia. In 1974, I returned to Belgium to attend the NATO Symposium on Genetic Manipulation with Plants, organised by Ledoux, and stayed on in the U.K., where I was able to demonstrate a protocol for phage DNA uptake by *Arabidopsis*, working with Ray McKee at Sutton Bonnington.

Today, the advent of more powerful, precise, yet less expensive, DNA technologies has allowed more people to become involved in plant molecular biology. In 30 years *Arabidopsis* research has progressed from the description of ecotypes to genome analysis, and a new generation of researchers has found *Arabidopsis* a convenient plant to use. Perhaps I was somewhat ahead of my time, but no matter, my own work has been confirmed, I also have two wheat varieties ascribed to me, so not working with *Arabidopsis* has also been fruitful! To present-day researchers, I urge you to sort out the facts from the artifacts, I wish you luck, go for it!

John A.M. Brown, 8 Queen Mary Court, Falmouth, Cornwall, TR11 4SX, U.K.

➛ *continued from page 2...*
gadget is more effective than the "inverted funnel in a tube"-type collector and it is the next best thing to a non-shattering mutant (anybody found it yet?). If you are interested, mail me your address and I will send you the instructions with a drawing. Requests accompanied by a self-addressed stamped envelope would be nice. Happy harvest.

Luca Comai, Botany KB-15, University of Washington, Seattle, WA 98195, U.S.A.
COMAI@EDU.WASHINGTON.U.MILTON

From Bernie Mulligan...

Jobs

THE JOBS described below have recently become available at the Dept. of Botany, University of Nottingham, UK.

POST-DOCTORAL MOLECULAR BIOLOGIST

Applications are invited for a post-doctoral research assistantship to join a group working on the molecular biology of male sterility in *Arabidopsis*. Current attention is being placed on gene cloning by chromosome walking and genomic subtraction. The post is funded by the AFRC for a period of up to 18 months from 1 April 1991 with a salary of approximately £12792 pa.

RESEARCH TECHNICIAN

Applications are invited for this post to work in a research group involved in various aspects of the genetics and molecular biology of pollen development in *Arabidopsis*.

Applicants for this full or part time post should be prepared to participate in laboratory and greenhouse work. Some scientific background, preferably with an interest in plant biology and genetics, is desirable. The post is funded by the AFRC for up to 18 months.

Applications with CV should be sent as soon as possible to Dr B Mulligan, Department of Botany, University of Nottingham, University Park, Nottingham, UK, NG7 2RD. Informal enquiries are welcome on 0602 484848 Ext 3467. Enquiries can also be made through e-mail to: PBXZW@UK.AC.NOTT.CCC.VAX

What does "Little Weed" call herself these days?

Sclobber-dopsis !

CLARE LISTER

WATCH WITH AFRC

Stanzas for Scientists

DOES ANYONE apart from Keith Roberts read this section? If so, how about sending in a suggestion for a poem? Until someone does, it will have to be one of the ACM's favourites again. This one seems appropriate for time of year:

e.e. cummings
(1894-1963)

In Just - spring

in Just -
spring when the world is mud -
luscious the little
lame balloonman

whistles far and wee

and eddieandbill come
runnning from marbles and
piracies and it's
spring

when the world is puddle-wonderful

the queer
old balloonman whistles
far and wee
and bettyandisbel come dancing

from hop-scotch and jump-rope

it's
spring
and
the
goat-footed
balloonMan whistles
far
and
wee

1923

T-shirt Competition

THE T-SHIRT competition was a great success. Of the several designs received, Eric Holub's tripartite entry received a highly commended from the judges, but Ian Furner's design, "Arabidopsis Angels: Born for fun, loyal to none" was chosen as the winner (shown on the left above). As this was thought to appeal more to the anarchists of

Arabidopsis research, a more "corporate logo" T-shirt was also printed. This was drawn by the ACM from an original idea by Jonathan Jones, with the motto; "Join the extended family: AFRC PMB *Arabidopsis* Programme" (shown on the right above). Ian and Jonathan each received a free T-shirt for their efforts.

"Angels" was printed as a black design on a red T-shirt, whilst "Family" was printed with green (what else?) *Arabidopsis* plants on a white T-shirt with black lettering and one mutant plant picked out in red. Nearly all of the 75 T-shirts printed were sold at the Nottingham conference at a bargain price of £5.75 each. Another print run will be considered, however, if there is sufficient demand. Please let the ACM know if you would be interested in buying a second edition of either of the above. Don't send any money yet, as there has to be sufficient demand -- at least 25 per design -- before it is worth ordering another run.

From Elucius...

Arabidian Kitchen

(first of an occasional series)

COGITATING one day on the more applied potential for our wonderous weed, I started thinking about its culinary attributes. In these days of poverty and the threat of war, why not eat all those fresh green *Arabidopsis* plants that you'd otherwise throw away and do yourself a nutritional favour. So here are a couple of protocols for your delictation:

Arabidopsis souffle.

25 g butter, 25 g flour, 1/2 pint warm milk, 50 g grated Cheddar cheese, 1/2 tsp mustard, 4 egg yolks, 4 egg whites, salt and pepper, 2 handfuls of *Arabidopsis* leaves.

Preheat oven to 200°C, gas mark 6. Stir the flour into the melted butter and cook for 2-3 minutes. Add the warm milk and keep stirring. Bring to boil and simmer for 3-5 minutes. Remove from heat and add cheese and mustard. Cool a little and add egg yolks and season. Chop the *Arabidopsis* leaves finely and add to the sauce. Beat the egg whites to the soft peak stage and carefully fold into the sauce. Immediately pour into a greased souffle dish and cook in centre of oven for 25 minutes. Don't look 'til it's done and eat straight away.

Arabidopsis blue cheese dip (take care with this one).

142 ml soured cream, 1 crushed garlic clove, 175 g Blue Stilton cheese, juice of 1 lemon, one handful of *Arabidopsis* leaves.

Combine everything in a small bowl and beat together well. Chill and add whole leaves or flowers as decoration before serving. Best with savoury biscuits.

The potential for screening for tastier mutants is of course enormous and any feedback via ACM would be useful. Happy cooking, Elucius.

This Issue's Quote

AN APROPOS quote from Bertolt Brecht:

"War is like love; it always finds a way."

Agony Column

Dear Aunty Raby,

I am 24 days old and really desperate. I still haven't flowered and my rosette leaves are beginning to die. Do you think it's my appearance? Why do maize plants have all the fun? Please help me before I introgress myself.

Erica Erecta

Dear Erica,

Don't worry! See a specialist. I recommend genetic counselling. You may have an inherited mutation. Why not try 10µm gibberellic acid, it's a wonderful pick-me-up. I always finds it helps.

Aunty Raby

Thanks to...

- Barrie Allen for help with printing of both the nameplate and the graphics.
- Jon Clarke for photocopying and collating this, as well as the previous, edition.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying some of the graphics.
- Elucius for the cookery column.
- Gary Foster for his cartoon.
- Joan Green for the Current Awareness List
- Mary Holdsworth for her suggestions & proof-reading.
- Clare Lister for her cartoon.
- Denis Murphy for his cartoon.
- Black Rot for the crossword.
- Renate Schmidt for her suggestions & proof-reading.

How to Reach Us

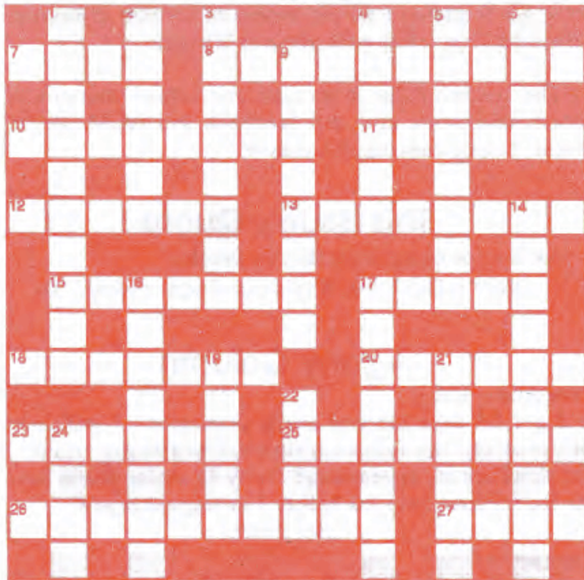
Wherever possible, please send all contributions to this newsletter, by e-mail to:

ARABIDOPSIS@UK.AC.AFRC.JII

(non-U.K. e-mailers may need to reverse the order of the components of the sitename), or failing that, on disk. Mac disks are ideal, but we can import MS-DOS (IBM) too. With IBM output, please send the file on either a 3½" (preferably) or 5¼" disk with the file in word processor format *and* as a text-only (ASCII) file. Whatever the disk, please enclose a printed copy and *ensure* that the disk and originating machine are virus-free. Disks will, of course, be returned. Further details about communicating via computer are given in the second newsletter (*Arabidian Notes*). File transfer by modem is also available for the *cognoscenti*. Our full address, telephone and FAX numbers are given on the front page.

The BackCross Page

Arabidopsis Prize Crossword



Out of molehills

by Black Rot

'Erewego! turned out to be our most successful crossword to date. Eight correct answers were received. However, none came from Central Office, despite the ACM being cajouled into revealing the answer to 19 across at the Nottingham meeting. In a draw performed by Mary Holdsworth (who the ACM can exclusively reveal is soon to start in Nottingham as supremo of the Mulligan Genetic Bank, which is being set up there largely thanks to AFRC funds) the lucky winner of the £5 book token was **Brian Forde** from Rothamsted. Congratulations to him and also to the runners up: Barrie Allen (Cambridge lab, J.I.), Clive Lloyd (J.I.), Joanne Morrison (York), Helen Ougham (WPBS), Jo Ross (Cambridge lab, J.I.), and Alison Smith (J.I.). In a draw carried out by Rosi Allen, secretary to Our Man In Brussels, Mike Bevan, the winner of the John Innes site prize of a bottle of wine was the joint entry from Cathie Martin & Justin Goodrich. As these two are die-hard *Antirrhinum aficionados*, it just goes to prove that we in the *Arabidopsis* Community bear no grudges against those not yet converted to the noble weed. (It is interesting to note, however, that it takes two snapdragons to do the usual work of one thale cress -- is this is a scientific metaphor?)

For this edition's puzzle, Black Rot's tip is that clues 7, 8, 10, 11, 12, 13, 18, 20, 23, 25 & 26 across and 4 down are all linked in a common theme, which is expressed in the title: *Out of molehills*. As a further help, the ACM suggests that an atlas might prove useful. Another book-token for the first correct entry out of the draw.

Don't Forget!

Your next project summary is due by **10 June**.

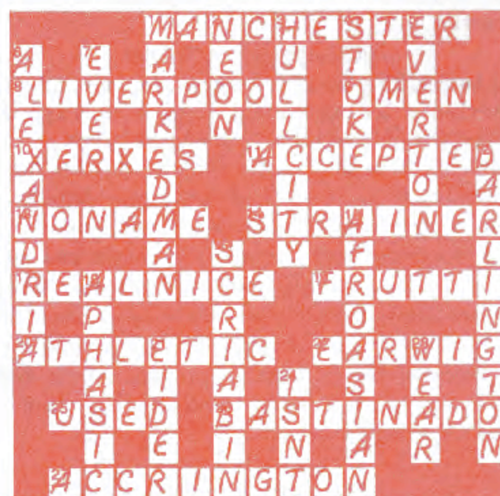
Clues Across

7. Italian returns ante (4)
8. Pakistani makes mum brasher (10)
10. Japanese may be in a jam if you lose nothing (8)
11. Nepali has half chosen to confuse you (3,3)
12. Nepali's warm inside London college (6)
13. Scot arranges for Noel Presley to follow Tony (3,5)
15. Accountant declares independence in a summit (7)
17. Rig to remove poster from location (5)
18. Lakelander gets beaten over foreign article (7)
20. Nepali man, also known as Leo, uses initials (6)
23. Explosive Javan appears partly to stammer a piece (6)
25. Shropshire eminence reputed to have extensive brainpower (4,4)
26. Lakelander in part of Anne's house? (5,5)
27. Blue flower - or white? (4)

Clues Down

1. Will Dutch courage provided by Guinness win fair lady ? (5,5)
2. Brutal fellow unhappy at Diss (6)
3. Native impressionist introduces himself around the North (8)
4. Welshman at crossing in Brecon ? (6)
5. Former pupil has only note to show he's past it (8)
6. ...hearing a minor in the dock (4)
9. I protect blackleg poet (8)
14. Cheekily replace nylon tiles (10)
16. Detectives spoke when upset over gallery key (8)
17. Mad about books - deemed unsound (8)
19. Grail upset in Scotland (5)
21. On 10, in this shivering? No I'm OK (6)
22. Beat iron suit (4)
24. Heroine I removed from eagle's nest (4)

For those of you with a struggling knowledge of football clubs, here are the answers to *'ere we go!* the crossword in *Araba-Daba-Dopsis!*:



Rules of the *Arabidopsis* Prize Crossword:

This competition is open to all readers of this newsletter. The answers as well as the winner's name will be in the next newsletter.