A-rap-a-bop-sis

The Seventh AFRC PMB Arabidopsis Newsletter November 1991

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How to Reach Us

PLEASE SEND ALL contributions to this newsletter by either, 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 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*. The deadline for the next newsletter is:

Monday, 3 February, 1992.

PLUS – attached:

***ARABIDOPSIS* CURRENT AWARENESS LIST** (Subscribers)	(4pp)
KÖLN *ARABIDOPSIS* DNA STOCK-CENTRE DEPOSIT INFORMATION SHEET	(1p)
PROTOCOL BOOK UPDATE (VERSION 1.2) (AFRC PMB *Arabidopsis* Grant Holders) OR **PROTOCOLS:**	
From Roger Innes... Update to Valvekens's transformation	(4pp)
From Keith Lindsey... Silver thiosulphate in transformation	(2pp)
From Keith Lindsey... Transient gene expression	(2pp)
From Bernard Mulligan... Addition to seed storage protocol	(1p)

A-rap-a-bop-sis: the seventh AFRC PMB *Arabidopsis* Newsletter, November 1991.

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Stock Centres

From Randy Scholl...

The *Arabidopsis* Biological Resource Center at Ohio State

Greetings to the AFRC! The *Arabidopsis* Biological Resource Center at Ohio State has just been established, with funding from the National Science Foundation. We will be working closely with our counterpart organisations at Nottingham, England and Köln, Germany to preserve and disseminate *Arabidopsis* resources. With the rapid increase of interesting new *Arabidopsis* mutants and the proliferation of diverse clones and libraries, the usage of Center services is expected to be high. We plan to co-ordinate all aspects of our operation with the other Centers and to consult the research community so that the best possible service can be provided.

Our Center differs from the other two in that the seed and DNA collections will be maintained at the same location. A database, containing detailed information about the stock collections and other aspects of the species, also is being developed. It is is planned that this system will include detailed information about our stocks as well as other data on *Arabidopsis*, such as a bibliography. The database will be accessible through remote, on-line means, and it will be possible to place orders directly through this system. Hence, the goal is to provide information and stocks to all investigators, in the most timely possible fashion.

All of these resource centers were established to aid the research efforts of the *Arabidopsis* community. Their creation was one of the first goals of the *Arabidopsis* Multinational Co-ordinated Genome Research Project . For the efforts of the Centers to be effective, the assistance of the entire community is required. Therefore, we welcome suggestions and will continue to seek advice on how best to serve you. We hope that contributions of biological resources and information will be given freely.

The Director, Dr. Randy Scholl, supervises the seed collection and, Dr. Keith Davis of the Ohio State Biotechnology Center serves as Associate Director and supervisor of the DNA collection. We plan to employ four technical assistants, having advanced (masters-level) degrees to manage the stocks, take orders and enter and retrieve database information. Dr. Sakti Pramanik of the Computer Science Department, Michigan State University will develop the computer database, with the assistance of several of his associates.

We are searching for individuals, experienced with *Arabidopsis* and/or molecular techniques, to fill the technical positions. While a move to Columbus, Ohio might represent a major relocation for much of the readership, all applications are welcome!

In the immediate future, we will be occupied with organisational matters, procurring the large seed and DNA collections, soliciting donations from the community and incorporating the donations into our collection.

Policy details of our Center have not been addressed in this article. This is because, at this writing, we have not met with our Advisory Committee. We have already discussed some of these matters with our Nottingham and Köln counterparts, and also will be consulting the *Araidopsis* community regarding Center policies. We will ensure that our policies are fully coordinated with the other two Centers.

In the future, we will be informing you of our progress through direct mail and the *Arabidopsis* electronic newsgroup. We will also be utilising these means to consult the community concerning policy issues pertaining to the Center. It is expected that we can begin to fill seed and DNA orders sometime after March 1992. We will keep the community appraised of our progress.

If you wish to contact us, our address is:

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From Albert Kranz...

Arabidopsis Information Service

FINAL INFORMATION

This is to inform you that the *Arabidopsis* Information Service (Frankfurt am Main) ISSN 0066-5657 will not be issued furthermore. We decided in agreement with the informal discussion of the *Special Group Meeting:*Arabidopsis *Resource Centres* of the Fourth International Conference on *Arabidopsis* Research, Vienna (Austria), June 2-5, 1990 and the EC-BRIDGE Program to stop our engagements of two decades in publishing the AIS-Newsletter with No. 27, 1990 (final volume).

Therefore, future contributions on *Arabidopsis* research will not be published under the responsibility of A.R. Kranz. We are looking forward that studies in this field will be published elsewhere. For publication of papers, I recommend to contact publishers of scientific journals.

Furthermore, we announce herewith that in a period of two years approx., all activities of AIS (gene bank, seed sample distribution, handling assistance, data transfer) will be stopped at Frankfurt am Main, FRG.

In the meantime, it's our intention to hand over the material (seeds, data files, etc.) and our experience to some other *Arabidopsis* centres in Great Britain, Germany and the U.S.A. After transfer and installation you will be informed by those institutions.

Best wishes, Yours sincerely,

Prof. Dr. Albert R. Kranz.

From Mary Anderson...

The Nottingham *Arabidopsis* Stock Centre

FIRST OF ALL, congratulations to Dr. Randy Scholl and Ohio State University for being awarded the NSF funding for the American Resource Centre. This is to be a joint Centre with Seed and DNA resources being held at the same site. We have already established a good working relationship with the US Centre and are in the process of determining the best database system to use, which will really establish the network between the Resource Centres.

Over the past three months, we have continued to canvass for support for the Centre. Some of you will have seen me publicising the Nottingham Stock Centre at the Canberra meeting, or Bernie and myself at our Information Booth, which we shared with the Ohio Centre, at the Tucson Conference. In general, the response and support for the Centre has been very encouraging. As a result of our publicity we have been kept very busy handling seed requests from all over the world. In the last two months I have distributed more seed lines than were distributed in the whole of last year! However, as far as donations are concerned the enthusiasm level has, perhaps predictably, been much lower. We are very grateful to the 16 International labs who have promised donations of *Arabidopsis* seed. Obviously, my request for seed in the last Newsletter fell on barren soil. I have had only one response, from Jon Clarke at Norwich (Ian Furner has said that he will also donate seed). I don't want to have to give a soap box diatribe about the needs and uses of the Stock Centre.

We have received our first lot of seed from Prof. Kranz. The first delivery has been the Form mutants. The characterisation of these lines will start immediately. Some of these lines should be available by the time the next newsletter reaches your desk.

If you require any help or advice about the Stock Centre collection, please do not hesitate to contact me.

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Arabidopsis Found on Moon!

NOW WE HAVE your attention: the ACM is disappointed to hear from Jeff Dangl, that so far, the Köln DNA Resource Center has received only six libraries: two from Köln, two from CalTech, one from Stanford and one from the U.K. This reflects a poor showing by all in Europe (it may not be 1992 yet, but this includes the U.K.), especially considering that the centre if funded by the EC as part of the BRIDGE project. To quote Jeff, "If it's going to work, it has to be investigator driven." In other words, use it or lose it. To help you to help Jeff to help yourselves, another copy of the Köln Deposit Information Sheet comes free with this newsletter.

From Ian Crute...

The Kent "MAP" Group

IT IS RUMOURED that the unhealthy condition of *Arabidopsis* populations in Kent (47% have been found to be suffering from one or both of the diseases downy mildew and white blister) has provided the stimulus for the formation of the Multinational *Arabidopsis* Pathology (MAP) Group. Jim Beynon's group at Wye College has joined the research effort of Ian Crute's group at HRI, East Malling to meet the following objectives:

Short term (6 months): identify the chromosome locations of several genes for resistance to *Peronospora parasitica* and Albugo candida.

Medium term (2 years): clone and sequence at least one gene for resistance to either fungus.

Long term (5 years plus): understand how resistance genes work at the molecular and population level and seek their practical exploitation in cultivated crucifer crops.

Present members of the Kent MAP Group include:

Wye	East Malling
Jim Beynon (UK)	Ian Crute (UK)
Edmar Brose (Brazil)	Eric Holub (USA)
Mahmut Tor (Turkey)	Karen Martin (UK)

The Kent MAP group collaborates with the research groups of Jeff Dangl (Cologne), Alan Slusarenko (Zurich), Mike Daniels & Jonathan Jones (Norwich) and John Lucas (Nottingham), and seeks close contact with other groups interested in the genetics of resistance to crucifer diseases. CRUTE@UK.AC.AFRC.EMRS

PMB Cell-Cycle Meeting

FOLLOWING ON from the success of the PMB Flower Development Workshop, held in March at the University of Leicester (which was well summarised by Gary Foster in the previous newsletter), a one-day meeting on "The Cell-Cycle" was held at the John Innes Centre on 18 October. The participants were the cell-cycle grant holders within the AFRC PMB Programme (*Arabidopsis* and general half). With P.I.s bringing post-docs and students, a total of 24 people attended the meeting; which was chaired by Felicity Watts. Presentations given by: Cliff Bray & John Rosamond, John Doonan, Felicity Watts, Paoli Sabelli & Nigel Halford, Jim Murray, Jeremy Hyams, and Mark Bell; were followed by a "round-table" discussion. Also attending were Programme Co-ordinators, Caroline Dean and Hugh Dickinson (for Chris Leaver), and Sue Riley from A.F.R.C. Central Office.

Arabidopsis Publications

PLEASE REMEMBER to acknowledge the source of funding in any papers that result from work supported by the AFRC PMB *Arabidopsis* Programme. Also, could all AFRC grant holders please supply the ACM with details of any publications arising, directly or indirectly, from their *Arabidopsis* grants? Reprints are particularly welcome.

Project Summaries

From Sue Albini...

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

Progress continues to be made. Working experimental procedures for (i) surface spreading *Arabidopsis* synaptonemal complexes (SCs) and ,(ii) *in situ* hybridisation (ISH) of DNA probes to plant SC preparations are now established. The accumulation of *Arabidopsis* SC preparations continues. The results of an analysis of pachytene nuclei will soon be submitted for publication. The first ISH experiments using *Arabidopsis* SCs as targets for DNA probes are probably happening as you read this. Success or otherwise will be reported in the next summary.

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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.

Tobacco leaf discs have been transformed with both our NEO and HYG ORI vectors, with and without the TGMV replicon. These are being taken on to transgenic plants for verification of the constructs and detection of extrachromosomal circles generated by intramolecular recombination. The plants we originally obtained, containing the HYG ORI vector and the TGMV replicon, have now been shown to contain an intact hygromycin phosphotransferase gene by PCR and Southern blotting. In theory, an intact gene can only be generated by homologous recombination, releasing a circle of DNA which will replicate if it contains an origin, in this case that of TGMV. The excised circles appear to be present in only a small proportion of the plant cells as expected. To confirm that the circles are replicating, we need to obtain hygromycin-resistant tissue. We have attempted unsuccessfully to do this by culturing protoplasts under hygromycin selection. However, we have now obtained hygromycin-resistant calli by slicing leaves from transformed plants into 1 to 2 mm strips and plating on growth media containing hygromycin. The tissue developed green spots in hygromycin-resistant areas and, when these were sliced through, they developed into calli. These will soon be large enough to extract DNA to test for the presence of replicating circles.

> We will be grateful if anybody could send clones for testing in our ORI vectors.

We have also transformed the NEO ORI vector, with and without the TGMV replicon, into *Arabidopsis.* This will tell us if *Arabidopsis* can act as a host at the single-cell level for TGMV. My bet is that it will. Wait for the next newsletter to find out!

As soon as we have confirmed that our vectors are performing as predicted we will be ready to clone into them *Arabidopsis* DNA fragments to try to identify replication origins. There is some evidence that origins may be associated with the upstream regions of genes. Therefore, if anybody has clones of *Arabidopsis* genes and their upstream regions we should be very grateful if they could send them to us for testing in our ORI vectors.

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From Jeremy Carmichael & Jim Murray...

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

I have used Northern blots to check the quality of the poly(A+) RNA that I had prepared from root and leaf callus for our cDNA library. As a homologous probe I managed to get a cloned cDNA from Christine Raines and Philip Horsnell (Essex) as well as an aliquot of their *Arabidopsis* cDNA library in lambda ZAP II from which I prepared two clones at random. One of the clones failed to give any signal and indeed it may not be expressed in callus tissue. The remaining two gave good signals and indicated that the RNA was undegraded. Interestingly, one of the clones hybridises to a sequence which is differentially expressed in callus derived from leaf or root, giving a stronger signal on blots of leaf callus RNA.

I have now prepared the first of our cDNA libraries in the plasmid vector pSport (BRL) using poly(A+) from root callus. All of the inserts should have directionality and should be in the correct orientation for expression. I have had problems preparing two other yeast expression vectors to accept directional inserts. The difficulty is mainly due to the fact that the cloning sites for insertion lie close on the engineered polylinker. I hope to be able to overcome this problem by inserting stuffer fragments into these plasmids which can then be cut more effectively reducing the background of non-recombinants in the library.

Experiments with redundant PCR have continued with attempts to clone cyclin-like genes using degenerate oligos. Two very short oligonucleotides (12 and 13 bases) were prepared based on the amino acid conservation of mitiotic cyclins from five different species. Genomic DNA failed to give amplification even when using very low annealing temperatures based on the low Tm of the primers. However, using a mixed oligonucleotide primed amplification of cDNA (MOPAC) reaction it was possible to synthesise cDNA using the antisense primer and reverse transcriptase before carrying out the PCR amplification. This experiment has successfully amplified four distinct sequences with sizes ranging from 0.1-0.6kb. I have begun screening John Mulligan's lambda YES cDNA library from whole *Arabidopsis* plants and so far have isolated six positives against one of the probes. The advantages of lambda YES are that the inserts can be looped out in the form of a plasmid using a system based on the cre-lox site specific recombinase, and these plasmids can be used directly as expression constructs in budding yeast.

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From Simon Covey...

CaMV infection of *Arabidopsis*.

We have isolated an *Arabidopsis* mutant with an altered response to cauliflower mosaic virus infection. The systemic symptoms on leaves of wild-type plants infected with CaMV characteristically show initially as vein banding (chlorosis adjacent to vascular tissue) developing into complete chlorosis of the leaf. This latter feature is accompanied by leaf wrinkling and stunting in which the centre of the rosette appears to be compacted.

"We have isolated an *Arabidopsis* mutant with an altered response to CaMV infection."

In the mutant, the vein banding is not apparent and the stunting effect is delayed relative to wild type plants. One obvious explanation as to why the vein banding effect is difficult to observe is that leaf lamina of the uninfected mutant is pale green and might not capable of expressing the chlorotic effects of infection. The leaf veins of most of the mutant plants are dark green although about one third of the progeny seed from the mutant germinates to produce severely chlorotic sometimes dwarf plants. Since the mutant plants presumably have reduced chlorophyll, it might be expected that they are "compromised" in some way resulting in greater susceptibility. However, this does not seem to be the case since the mutants appear less rather than more susceptible to infection by CaMV. This suggests that the mutation which produces the pale green phenotype also has a deleterious effect on virus multiplication or on symptom expression or both. We are currently looking at the levels of virus accumulation in mutant plants.

As a preliminary to identifying the biochemical lesion in the mutant producing the pale green phenotype, we have transferred some of the severely chlorotic mutants to media supplemented with nutrients in an attempt to complement the mutation. In the presence of supplemented vitamins, dwarf pale green mutants expressed three phenotypes. Some of the plants remained unaltered, some reverted to the pale green/dark vein phenotype whilst others showed a green phenotype. Although the leaves of these latter plants largely regained the green coloration of wild type *Arabidopsis*, they had vein banding effects typical of CaMV infection albeit in the absence of the virus. We are still trying to decide whether this is telling us something about the mutation or about what causes symptomatic effects in virus-infected plants!

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From Caroline Dean...

Transposon tagging.

Anuj Bhatt & Tania Page

We have analysed several putative transposon-tagged mutants at the molecular level for linkage to a transposed *Ac*. Tania had identified three different mutant phenotypes after screening progeny of 350 full green families. The mutants were christened: small & vitrified, pale cotyledons, and miniature. Unfortunately, after progeny testing and countless DNA preps there was no linkage between transposed *Ac* and the mutant phenotype in all three cases. The three full green families segregating these mutations were all descendants of the same transformant, 0194-17 (wild type *Ac*), which has multiple T-DNAs. We have concluded that these mutant phenotypes are not due to insertion of *Ac*, they are also unlikely to be due transformation as the T_2 progeny of 0194-17 did not segregate the mutation(s).

We have identified another mutant phenotype whilst screening full green families which were generated using Emily Lawson's *Nae1ΔAc* transformants. The mutant seedlings have pale green cotyledons which eventually bleach. Under Tania's loving care they have developed into small feeble plants with yellow-green leaves. They produced normal flowers, but failed to set seed. We are currently analysing whether *Ac* is linked to this mutant phenotype.

We (Anuj, Emily and Tania) are also generating another 1000 independent full greens from ten different transformants, five of which carry the wild type *Ac* and the others have the *Nae1ΔAc*. All the transformants have a single T-DNA locus and are diploid.

The *Arabidopsis* genome project.

Alma Balestrazzi, Gerda Cnops & Renate Schmidt

Since the last newsletter we have managed to use even more densely plated master filters. We are now plating 2,304 yeast colonies on the 7.5 x 11 cm nylon membrane. Thus, one small filter represents one YAC library. Even using duplicates, we can now screen the three available YAC libraries (EG, EW, ABI) on six filters.

Our walking efforts are concentrating in the moment on a 10 cM region of chromosome 4 (RFLP markers 210, 326, 580, 226). Currently, three contiguous regions of 795, 725 and 310kb lie within this region, involving 41 YAC clones from four different YAC libraries. The YAC clones from the fourth library were provided by Dr. Joe Ecker and his colleagues (University of Pennsylvania, Philadelphia), who had screened their new YAC library with RFLP markers 210, 326, 580 and 226. These YAC clones especially helped us to integrate the cosmid RFLP markers, pCITd23, 4108, 10086 and 4564.

In the region we so far have identified at least two chimaeric YAC clones. One of these clones (EG5D4) was found to hybridize to three RFLP markers in the region. But by using end-probes of this clone we demonstrated that it is chimaeric.

Alma Balestrazzi, Gerda Cnops & Renate Schmidt

"One small fillter now represents one YAC library."

The mechanism of vernalization.

John Chandler

An EMS-mutagenised population of the *fca* locus, (which confers a late flowering phenotype that is responsive to vernalization), continues to be screened, to isolate individuals partially or completely lacking the vernalization response. If the EMS-insduced mutation does not itself cause late flowering, then these ➵

mutants have a block in the perception and/or signal transduction pathway of the cold signal. Several individuals have been isolated so far, which are later flowering than *fca* after vernalization. Currently, M3 seed of all the putative mutants is being re-screened with and without vernalization, to confirm the heritability of the phenotype observed in the M_2.

The same population of EMS-mutagenised *fca* was also screened for suppressor mutants of *fca*, which flower at the same time as wild type Landsberg *erecta*. There is a lot of variation towards earliness in these families, but 17 of the earliest individuals were selected, from 140 M_2 families, representing at least 11 independent mutations. These plants were crossed to *fca* and *Ler*, to study the genetic nature of the induced mutations.

fca was also crossed to a range of growth regulator, starch, lipid and phytochrome mutants, to see how the phenotype of *fca* might be altered by the presence of the second mutation, and thereby possibly implicate a function for *fca*. So far, double mutants of *fca* with Δ*ga1*, *gai*, Abi1, *2* and *3*, *ein2-1*, two starchless mutants and a starch overproducer have been obtained. The F_2 populations of these crosses which involve *Ler* parents (some of the mutations are in Columbia background) are being scored for flowering time and leaf number. So far, data show that the presence of *aba*, Abi1, *abi3* and *gai* loci together with *fca*, does not cause a deviation from the expected 3 early:1 late segregation of *fca*.

Further characterisation of the photoperiodic response and growth rate of *fca* and its five alleles is being performed. Also, an analysis of which gibberellic acids can accelerate flowering in *fca* is being carried out, along with testing the hypothesis repoted by Metzger for *Thlaspi* (1990), that cold treatment may uncover a block in gibberellic acid synthesis following kaurenoic acid.

Metzger, J.D. (1990). Vernalization, gibberellins and thermoinductive stem growth in *Thlaspi arvense* L.
British society for Plant Growth Regulation, Monograph 20.

Still mapping *fca*!

Rob Ewing & Lore Westphal

The mapping of fca is still in progress. Having finished with Meyerowitz's lambda RFLP markers, we are now focussing on Goodman's cosmid RFLP markers and the relevant YAC end fragments. In order to identify more marker loci in the vicinity of fca, we are screening series of RAPD primers, utilising a bulked segregant analysis approach. We have begun to select recombinants from our hy4 fca cer2 (Landsberg)/ Columbia cross. We are also selecting more recombinants from our initial fca/ Columbia cross using a pooled DNA screen.

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From John Doonan...

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

Screening the Clontech lambda gt11 expression library with MAb350, a monoclonal antibody against mammalian nuclear pores has identified six positive clones. These all produce a fusion protein, which cross reacts with other antinuclear pore antibodies, suggesting that they represent plant nuclear pore genes. After some difficulty(*), the inserts from two clones (about 200 and 1800bp) were subcloned to pBC. We hope to obtain some sequence information in the very near future to confirm if these clones do encode nuclear pore genes.

Screening the same library with a bimG probe at low stringency has identified several clones. Four of these have been plaque purified and their inserts transferred to pBC. Three clones are related on the basis of restriction digests.

* Although the Clontech library seems to be a useful expression library, it appears to contain many inserts lacking functional EcoR1 cloning sites. While this is not a major problem, it is a nuisance!

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From David Evans...

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

Per Askerlund has continued refining the reconstitution system described previously for the plant calmodulin-stimulated calcium pump (July Newsletter) and has obtained data on nucleotide specificity, kinetics, pH optimum, erythrosin-b inhibition and other properties. He is currently refining the system further to improve purification and to begin a detailed study of regulation and (jointly with graduate student Freddie Theodoulou) effects of partial proteolysis.

Joy Boyce has continued cloning and sequencing; antibody screens of expression libraries have resulted in the detection of cDNA showing homology to mammalian calcium binding proteins, which we are following up. One shows homology with calreticulin, a protein which sequesters calcium in IP3-released calcium stores in mammals and may be the first suggestion of this important class of proteins in plants. This work is also be paralleled by LM and EM immuno-cytochemistry. Other efforts to detect plant Ca pump sequences are also continuing. David Coates is continuing to develop a PCR approach, while Joy Boyce is trying alternative libraries and probes (anybody got a really good plant cDNA library rich in rare messages for membrane proteins?). We are also micro-peptide sequencing more of the calmodulin-stimulated ATPase in order to get more information for probe design/ new antibodies.

"Has anybody got a really good plant cDNA library rich in rare messages for membrane proteins?"

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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.

This report is being composed exactly five hours before I fly to the States for the ISPMB conference in Tuscon, so you will have to forgive me if it appears a little short or rushed. I promise a more in-depth report for the next issue and a brief overview of the goings on in Tuscon.

The progress since the last newsletter has been good on some fronts and down right demoralising on others.

For those of you who have forgotten or who never knew, our project aims to stabilise *Ds* transposition by expressing the transposase, required for transposition, under a microspore specific promoter which, in simple terms, will allow *Ds* to jump in each separate pollen grain which will then go on to produce millions of plants with a *Ds* inserted into a different gene in each one. This *Ds* element will not move at any stage, as the seed grows up into a proper plant, until the microspores are formed again the promoter is switched on, producing transposase, and allowing *Ds* to jump again, and so on and so on.

In our way to achieving the above aims, we have also investigated some aspects of microspore development as a by-product of obtaining our microspore specific promoter.

We have now isolated and identified functions for three microspore-specific mRNAs. These include, a microspore specific oleosin (lipid body formation), a putative pollen-wall (structural protein), and another which has multiple roles of an alpha-amylase/protease inhibitor and a phospholipid transfer proteins. All three have thrown up some interesting results. Publications are on the way.

The microspore-specific promoter has been obtained from *Arabidopsis*, *via* a *Brassica. napus* cDNA to the structural protein mentioned above. The promoter has now been tested in tobacco and *Arabidopsis*. We have perfect expression in developing microspores in both plants, the promoter is active maximally prior to the microspore mitosis, and expression continues at a lower level in the maturing pollen grains. A 550bp fragment gives additional expression in the tapetum and stomium, whilst a 1500bp fragment directs additional expression in the entire anther wall, but not the internal connective tissue. This promoter is thus ideal for the proposed germinal transposition experiments, since transposition of *Ac* and *Ds* has been demonstrated to occur most commonly during DNA replication. No expression in vegetative tissues, which might cause somatic transposition, could be detected.

We have now fused this promoter to the transposase open reading frame as a transcriptional fusion which also incorporates the translational enhancer sequences of omega from TMV. After many months and several bottles of gin, we have finally (we think) transferred this construct into a binary. It has, we believe a life and a will of its own.

So now, well ahead of schedule we are now testing for germinal transposition using a microspore-specific transposase. Look out for further news in the next newsletter.

Congratulations to Mike Roberts, who has come to the end of his PhD on the *Ds* project, and has been appointed as a post-doc with Dr. Rod Scott on another AFRC project on the Identification of Microsporocyte-Specific Genes. The project welcomes two ESF Students, Samita and Anna, who will try and fill Mike Roberts's shoes over the next year.

Dr. Gary Foster, Dr. Rod Scott, Dr. John Draper, Mr. Mike Roberts, Mr. Rob Blundell; Botany Department, University of Leicester, Leicester LE1 7RH.
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From Ian Furner...

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

There were a couple of mistakes in the last abstract that I would like to correct. (I think at the time I wrote it I was suffering neurological damage from marking 720 Biology of Cells scripts). One third of the marked sectors appeared on the first leaf not one half on the first leaf apex and the cauliflower cDNA work was by June Medford not Carol Merith. Many apologies.

This summer I have been increasing the sample size of X-ray induced albino sectors affecting the leaves and flowers of the plants. I have now looked at 324 sectors affecting 512 structures (leaves and/or inflorescences). The overall picture has not changed much. The first leaf pair represent a large target (193 sectors) and of such sectors about 90% appeared only on these leaves.

"I have produced a fate map of the inflorescence meristem"

Sectors affecting later leaves were less frequent and often included more than one leaf. The latter property increased progressively with later leaves. For example, all 36 sectors affecting leaf 9 appeared on other structures. My impression is that the dry seed meristem contains a relatively large number of cells that contribute only to the first leaf pair surrounding a smaller number of cells which contribute the rest of the plant. These inner cells do not seem to have fixed roles and make rather variable contributions to leaves 3-9 and the flowers. This rather variable contribution may result from a particular marked cell remaining in the meristem and contributing to further structures or variable meristem size in the dry seed.

Vivian Irish has just published an interesting review of cell lineage in higher plants (Current Opinion in Genetics and Development (1991) 1: 169-173) in which she outlines a model for the vegetative meristem of *Arabidopsis*. Vivian and I are now collaborating on this work and we hope to submit manuscripts together on our respective models before the end of the year.

I have produced a fate map of the inflorescence meristem based on data from 43 chimaeric inflorescences. I used the frequencies of dissimilar sepal colour (albino or green) to generate an index of distance in terms of frequency. This works well and the final model ended up as a dome with elliptical flower primordia of ➵

decreasing size arranged in a spiral resembling the phyllotaxy. I am preparing a manuscript describing this work.

This summer Joanne Pumfrey and I have been screening EMS derived M2 families for seedlings showing abnormal apical development. This work was carried out in the greenhouse and 650 families of 50 seedlings each were screened. Many interesting phenotypes were observed. Where possible, seeds were recovered from the affected individuals. When this was not possible, as the affected individuals died or failed to set seed, the other members of the family were harvested. This method usually allows the recovery of seed from plants heterozygous for recessive deleterious mutations. We will plant out these families over the next two months and attempt to place them in broad phenotypic groups before trying complementation and conventional mapping studies.

Paul Davison has constructed another cDNA library this time from hand dissected shoots (the earlier one was from *in vitro* generated shoots). He has been screening with shoot and leaf cDNA looking for shoot specific clones. So far results have been discouraging with a large number of false positives. The problem may be with the quality of libraries or there may be relatively few differences between the two mRNA population. To test this and hopefully get this project moving he intends to try using the cDNA preparations to screen a genomic library.

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

From Nic Harberd...

Genetics and molecular biology of growth and development in *Arabidopsis thaliana*.

Genetics and molecular biology of *gai-ga4*.

Jing-Rong Peng, John Cowl, Paul Sinacola & Nicholas Harberd

The gibberellins (GAs) have been implicated in many developmental processes in plants. The *Arabidopsis* GA related mutants are classified according to their sensitivity to exogenously applied GA. Recessive, GA-sensitive dwarf mutants (*ga1, ga2, ga3, ga4* and *ga5*) are reduced in height and often display impaired seed germination and floral fertility. The phenotype of these mutants can be restored to normal by application of exogenous GA, and analysis of these mutants has identified genes controlling various steps in the metabolic pathways leading to the production of biologically active GAs. The semi-dominant GA-insensitive dwarf mutant (*gai*) has a phenotype closely resembling that of the recessive GA-sensitive mutants. However, this mutant is unresponsive to exogenously applied GA. Additionally, *gai* accumulates biologically active GAs at significantly higher levels than is found in wild-type controls. Two of these GA related loci, *gai* and *ga4*, map in close proximity (positions 20.9 and 21 on chromosome 1 respectively). We are focussing on the *gai-ga4* region since this should enable us to isolate and study two genes, one involved with GA biosynthesis (*ga4*), and the other with GA signal-transduction/sensitivity (*gai*).

Our strategy for the isolation of irradiation-induced derivative mutants from γ-irradiated plants homozygous for *gai* was described in a previous edition of this august organ (Rabido pp7-8). The reasons for isolating these derivatives are two-fold. Firstly, investigations of the genetic and physiological properties of such derivatives will increase our understanding of how *gai* (and its wild-type allele) modulates GA sensitivity. Secondly, irradiation-induced loss-of-function alleles of *gai* may be deletion mutants, thus facilitating the identification of the locus in gene cloning experiments. From a (very approximate) total of 100,000 *gai/gai; tt1/tt1* homozygotes (some treated with 90kR and some with 70kR γ-rays) we obtained 12 plants bearing stems/branches taller and/or with internodes longer than expected for a *gai/gai* homozygote. Seeds obtained through self-pollination of each of these branches were yellow (the progeny of plants homozygous for *tt1* and therefore unlikely to have arisen through accidental contamination of the initial *gai/gai; tt1/tt1* stock) and were planted to determine if these potential derivative mutations are heritable.

The 12 tall/long-internode branches fell into three classes on the basis of their progeny tests. Firstly, seeds from eight of these branches appeared to be uniformly homozygous for *gai*, and not to segregate for taller individuals. Thus the putative derivative mutation in these branches was not transmitted to the progeny. In the second class, seeds from another three branches were found to segregate for plants homozygous for *gai*, plants resembling *gai* heterozygotes and plants resembling wild-type (+/+) homozygotes, showing that in these cases the putative derivative mutation is heritable. We have provisionally designated these derivatives as *gai-d1, gai-d2* and *gai-d4*. In the third class, seeds from the final long-internode branch segregated *gai* homozygotes and plants resembling *gai* heterozygotes, but not plants resembling wild-type (+/+) homozygotes. This behaviour suggests that this derivative (*gai-d3*) may be homozygous inviable or fails to transmit either through pollen or egg.

Investigation of the inheritance of this derivative in reciprocal crosses (in which the effects of using the derivative as male or female are compared) may resolve this. Abnormal inheritance is a property characteristic of large scale deletion mutations. Consequently, *gai-d3* has been crossed to plants homozygous for the *ga4* mutation (0.1 cM from *gai*) to determine if this putative deletion mutation extends from *gai* to *ga4*.

Mutagenesis of *gai* homozygotes can give rise to individuals lacking *gai* phenotype in one of two ways. Firstly, the treatment may have induced intragenic (revertant) derivative alleles of the original *gai* mutation. On the assumption that *gai* is a gain-of-function mutation such intragenic revertants are most likely to be loss-of-function mutant alleles rather than alleles with restored wild-type gene function. Such intragenic revertants would be expected to map to the same locus as *gai* in recombination mapping experiments. Secondly, the loss of *gai* phenotype in these derivatives might be due to the

induction (at loci other than *gai* itself) of mutations causing suppression of *gai* phenotype. Such second-site suppressor mutations need not display genetic linkage with *gai*. We are testing the derivatives for recombination with *gai* in order to distinguish apparent intragenic revertants from unlinked second-site suppressors. (Note, however, that it is difficult to distinguish closely linked second-site suppressors from genuine intragenic revertants.)

We have attempted to determine accurately the position of *gai* with respect to the RFLP maps. *gai* was crossed to the Columbia and Niederzenz strains with the intention of scoring the F_2 (and/or F_3) generations for *gai* phenotype. In addition, the resultant (F_1) *gai* heterozygotes were backcrossed to Columbia or Niederzenz in the hope of observing segregation of *gai/+* heterozygotes and *+/+* homozygotes. However, we encountered great difficulty in scoring *gai* segregation in the progeny populations, finding it impossible to distinguish *+/+*, *gai/+* and *gai/gai* with any certainty. Since there is no difficulty in distinguishing these genotypes when *gai* is segregating on the progenitor Landsberg *erecta* background these observations suggest that the phenotype conferred by *gai* is susceptible to modification through interaction with the genetic background.

We have adopted two alternative strategies to establish an accurate RFLP map position for the *gai-ga4* region. Firstly, we have crossed *ga4* to Columbia and to Niederzenz and have isolated *ga4/ga4* homozygotes from the F2. In these experiments the frequency of detectable homozygotes is considerably less than 1/4th of the F2 progeny. However, we have identified approximately 100 *ga4/ga4* homozygotes with certainty, and are using these individuals to establish the position of *ga4* within the RFLP map. Secondly, in a previous edition of this edifying journal (For Thale or Went p8) Recknagel *et al.* described the identification of a T-DNA insertion in the vicinity of RFLP marker 322 and the *phyA* gene on the top arm of chromosome 1. We are using the resistance to kanamycin conferred by this T-DNA as a marker in test crosses to establish the genetic map position of this insertion with respect to *gai* and to other loci (*an*, *dis1*, *ga4*, *th1* and *tt1*) on chromosome 1.

The *Arabidopsis phyA* (phytochrome) and *gai* loci map to similar positions on chromosome 1 (positions 16.6 and 21.8 respectively, note these data are from two independent mapping experiments and that one of the aims of the mapping experiments outlined above is to get a more accurate estimate of the distance between these two loci). Intriguingly, there is a similar correspondence in genetic map position between the maize *phyA* gene and the *D8* mutation (a maize mutation sharing many phenotypic characteristics in common with *gai*). These observations suggest either that *gai* is a mutant *phyA* gene,

or that there is evolutionary conservation of gene order between dicots and monocots in a short segment of chromosome carrying *gai*(*D8*) and *phyA*, or that this apparent correlation in genetic map position is actually a chance coincidence. As a test of these ideas we are investigating the irradiation-induced *gai* derivative lines to determine if loss of *gai* phenotype is correlated with alterations in the structure and/or function of the *phyA* gene. The presence/absence of functional phyA protein in these lines is being assayed spectrophotometrically and immunologically (collaboration with Dr G C Whitelam, University of Leicester), and digests of DNA from these lines probed with the *phyA* gene to test for deletions of part or all of this locus. Additionally, we have chosen yeast artificial chromosome (YAC) clones containing the *phyA* gene as the start point in the establishment of a contig covering this region of chromosome 1. Clones from within this contig are also being used as probes in the search for DNA rearrangements in the *gai* derivative lines.

Genetics and molecular biology of phytochrome.

Marie Bradley, John Cowl, Marion Rawlins, Paul Sinacola & Nicholas Harberd

The photoreversible photoreceptor phytochrome plays a major rôle in mediating the control exerted by light on various aspects of plant growth and development. In our previous report, we described the isolation of clones containing the *phyA* and *phyB* genes from an *Arabidopsis* genomic DNA library. There is considerable unpublished evidence that mutants at the *hy3* locus (which have elongated hypocotyls and are unresponsive to light-mediated inhibition of hypocotyl elongation) contain mutant alleles of the *phyB* gene. This relationship is being tested in several ways. Firstly, we are identifying new *hy3* alleles in the progeny of plants mutagenised either with EMS or with γ-irradiation. *hy* mutants are identified on petri-plates by screening for individuals with long hypocotyls, these individuals are then crossed with the standard *hy3-1* mutant in order to identify new *hy3* alleles. These new alleles will then be characterised at the physiological and molecular levels. We are particularly interested in identifying alleles which specify either reduced levels of phyB polypeptide or alleles which specify normal levels of a phyB polypeptide with reduced, altered, or lost biological activity. Studies of such alleles will increase our understanding of the rôle played by phyB in plant growth and development. Secondly, we have made constructs in which the *phyB* gene (under the control of its own promoter) has been inserted into Ti-plasmid derived plant transformation vectors and are performing transformation experiments to determine if these constructs can complement *hy3* mutant phenotype. Finally, our *phyB* gene clone has been sent to Dr J Peters and Dr M Koornneef (University of Wageningen, The Netherlands) for use as a hybridization probe in the analysis of *phyB* gene structure in a new T-DNA tagged *hy3* allele (gift of Dr K Feldman, University of Arizona, USA)

Our molecular analysis of the phytochrome gene family of *Arabidopsis* has made use of PCR derived DNA probes kindly made available to us by Dr F Thummler (University of Munchen, Germany). The sequences of three of these probes correspond to the published sequences for *phyA*, *phyB* and *phyC*. The fourth is a ➸

novel phytochrome-related sequence, *phyD*. Using the *phyC* probe we have isolated a genomic DNA clone containing the *phyC* sequence and have shown by restriction mapping that this clone contains the entire gene plus flanking DNA on either side. We have also been using the PCR-derived phytochrome probes in low stringency DNA hybridisation experiments. Preliminary results from these experiments suggest the existence of several previously undescribed phytochrome related sequences. A long-term objective of these experiments is to assess the effects of overexpression and antisense constructs made from the different phytochrome gene sequences on the growth of transgenic plants in different light environments. These latter experiments are part of a collaborative venture with Dr G C Whitelam (University of Leicester).

Genetics and morphology of shoot apical meristem mutants.

Paul Sinacola & Nicholas Harberd

Plant leaves are distributed on the shoot stem in an ordered pattern. In many species (including *Arabidopsis*) the maturing leaves form a spiral (the generative spiral) ascending the stem with decreasing age. The leaf distribution pattern (phyllotaxis) is established in the shoot apex, where initiating and developing leaf primordia form a spiral around the central dome of the meristem. Since the behaviour of cells in the shoot apical meristem is responsible for determining the pattern of the mature shoot we reasoned that screening for mutants with altered leaf distribution pattern would be a method of identifying mutations affecting the behaviour of cells in the apex.

We have been screening for mutants with aberrant leaf distribution pattern by visual screening of seedlings at all stages of development up to the 6-8 leaf stage. During this period deviations from the normal pattern are clearly discernable, thus facilitating the isolation of mutant individuals. Plants grown from γ-irradiated seeds (Landsberg *erecta*, M_1 generation) were allowed to self-pollinate and were harvested individually (M_2 families). The M_2 families were then progeny screened in groups of ten for new mutants. Families from those groups displaying putative mutant individuals were then re-screened to confirm mutant segregation, and to isolate sibling heterozygotes to be used for the maintenance of any mutations causing reproductive inviability when homozygous. Three (out of 1,500) M_2 families segregated phyllotaxis mutants. An example of one of these mutants, *bushy*, is shown below. The apical meristems of plants homozygous for *bushy* initiate new leaves at a rate faster than wild-type, resulting in vegetative rosettes tightly packed with leaves. The phyllotaxy of this mutant appears patterned rather than disorganized, suggesting that this mutation confers a new pattern of behaviour on the meristem. The tightly packed rosettes of *bushy* mutants may result either from an increase in the frequency of turns of the generative spiral or from an increase in the number of leaf initiations per turn. Careful study of the relative positions of successive leaves in *bushy* mutants will resolve this problem. Two further independent mutants displaying a relatively disorganised phyllotaxis were also obtained (not shown).

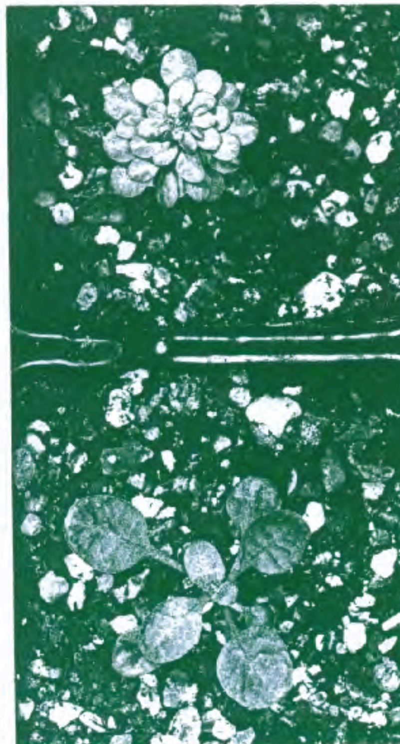

Figure 1. The vegetative rosette of wild-type (L. *erecta*, below) and *bushy* mutant (above) *A. thaliana*. The plants are of the same age. Note that the *bushy* rosette contains many more leaves than the wild-type control.

Multiple independent *Ds* transformant lines.

Marion Rawlins & Nicholas Harberd

One of our long term aims is to use transposon-tagging for the isolation of genes involved in controlling plant growth and development. For this reason, we have been making multiple independent transformants using construct 0282 (gift from Ian Bancroft & Caroline Dean). This construct contains a deletion derivative of *Ac* (functionally a *Ds* element) inserted into a gene conferring streptomycin resistance. The element has a hygromycin resistance gene inserted within it to enable identification of those individuals containing excised and reinserted *Ds* elements. Since *Ac* and *Ds* derivatives have a tendency to transpose to sites linked to their original insertion site, we are generating many independent transformants for use in the two-component transposon-tagging system. We have obtained seed from over 100 independently isolated regenerants. The progeny of these regenerants are being tested for segregation of kanamycin resistance. These tests eliminate untransformed lines and give an estimate of the number of independent T-DNA insertion loci in each tranformant. Plants from families showing a 3:1 segregation for kanamycin resistance (suggesting a single locus insertion) are self-pollinated in order to generate individuals homozygous for the T-DNA insertion. Plants from families displaying ratios suggestive of more than one locus of insertion are outcrossed (to Landsberg *erecta*) in attempts to separate the T-DNA insertion loci and generate families carrying single insertions. Additionally, we are determining the ploidy levels of transformant plants so as to eliminate individuals having more chromosomes than the 10 expected for diploid *Arabidopsis* plants. Once we have accumulated a number of diploid lines homozygous for single independent T-DNA insertions we will determine the genetic map positions of each insertion. Our aim is to develop a library of some 50-100 lines each carrying a single locus T-DNA insertion at defined sites in the *Arabidopsis* genome. These lines will

then be used for the isolation of genes of interest in the two-component transposon-tagging strategy.

Nicholas Harberd and group.; J.I. Centre, Norwich, UK.

From Nick Harris & Phil Gates...

Development of the silique of *Arabidopsis.*

Jackie Spence has been continuing her histological and cytochemical studies of silique development. These have included more immuno- and *in situ-* hybridisation work and the application of a variety of AGPs and lectin-gold conjugates. The probes (PAL, pectin methyl esterase, lectins, *etc.*) have been selected to give us information on the changes associated with wall differentiation / cell separations which are involved in the development of the tissues of the maturing / dehiscing fruit.

Jackie has also picked out a number of "interesting" samples from a screen of EMS-treated material. Of these a "flat-silique" and a "late-dehiscing" form are being examined in more detail. Of the established mutants, particular attention has been paid to clv1 which shows the most club-like fruit form and often contains a simple gynoecium-like structure within one of the four locules. The summation of the mutant and wild-type comparisons indicates that the overall fruit structure is apparently closely related with the form of the septum/septa and the degree of cell differentiation / cell separation in this tissue. Examination of the late-dehiscing fruit has indicated regions that are critical in the development of the abscission zone and the physical tensions which are associated with the normal dehiscence mechanism.

Lesley Edwards has isolated mRNA from both mature leaves and early (2-3mm) gynoecia. After a few pitfalls cDNA libraries from both have now been constructed in λ GEM4, which allows for orientation-specific cloning of the cDNAs between the T7 and SP6 promoters. Library DNA was isolated using Promega's Lambdasorb, and derived pGEM1 plasmids transformed into JM109 to produce plasmid libraries. cDNA insert size determinations are being carried out on a representative sample prior to subtractive hybridisation of the mature leaf from the developing gynoecium.

We hope that this will give us some probes, which are specific to the early gynoecium, for the next phase(s) of our programme.

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From Pat Heslop-Harrison...

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

Nuclei come in all shapes and sizes, but we are nevertheless amazed at the range of sizes of *Arabidopsis* nuclei and their chromosomes. Size depends not only endopolyploidy and endoreduplication, but on growth state and preparation technique. After our failed attempt to show you a picture of chromosomes in *Arab-adab-dopsis (I did warn them -- ACM)*, we will not illustrate the distinction, but we have preparations which range in diameter from 0.5 µm up to 12µm - and nuclear volume depends on the cube of these numbers. Since overall fluorescence intensities appear similar, reduplication of the DNA is unlikely to be the major cause of the difference. Pictures showing some of the range of sizes are shown in our paper in The Plant Journal, 1(2), 159-166, . Since then, we have found we can make chromosome preparations which show nearly ten-fold ranges in sizes for the same chromosome. If we could make the preparation techniques reliable for the large sized chromosomes, then morphological studies would be feasible.

> "...show nearly ten-fold ranges in sizes for the same chromosome."

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.*

The genetic model described in the last newsletter continues to be modified as we add new pieces to the puzzle. At least five matching gene pairs are now needed to explain the pattern of interactions observed between six genotypes of *Arabidopsis* and six isolates of *Peronospora parasitica* (see Table) and to accomodate the increasing data from the half-diallel analysis. New isolates have been included. So far, six isolates have been tested and each one has been a different pathotype. Isolate names designate the location from which they were collected (first two letters: EM -East Malling; CA -Canterbury; NO -Norwich; and WE -Weiningen, CH), and the susceptible host line used for routine maintenance of the isolate (third and fourth letters: OY-Oy0; LA-La-er; ND-Nd0; and CO-Col0). Isolates from the same source are distinguished by a number (*e.g.*, EMOY1 and EMOY2). Tsu0 was replaced by Wein (from Weiningen, CH) as the universal suscept when Alan Slusarenko (Zurich) informed us that Tsu0 was resistant to WELA.

Interaction phenotypes have hitherto been classified simply as resistant or susceptible on the basis of presence or absence of copious asexual sporulation. However, further detailed characterisation has shown that not all reactions classified as susceptible are the same. For example, Nd0 and La-er react differently to CALA. Nd0 is extremely susceptible with sporangiophores beginning to emerge three days after inoculation and no evidence of host cell collapse. By contrast, the reaction of La-er typically involves collapse of mesophyll cells and hyphae, and sporangiophores do not emerge until seven days after inoculation. Col0 expresses a similar phenotype following inoculation with EMOY2. It remains to be seen whether these effects are controlled by alleles at single loci or are an effect of the genetic background. If the former case is true, then the observations reflect incomplete expression of resistance genes which should also ➸

be incorporated into the model.

Phenotypes classified as resistant can also differ from one another. For example, Oy0 expresses a reaction to CALA characterised by discrete necrotic lesions involving 10 host cells; whereas, resistance of Col0 to the same isolate is characterised by larger necrotic lesions (visible with the naked eye) in which a few oospores sometimes form and beyond which hyphae still occur. From segregation studies, it appears that alleles at two independent loci regulate resistance of Oy0 to CALA. Data suggest that a phenotype similar to that of Col0 in reaction to CALA is observed when only one of the two alleles from Oy0 is present. As F_2 individuals are scored, plants from each phenotypic class, including susceptibles, have been saved for F_3 progeny tests. We also believe that microscopic evaluation of F_2 seedling populations will enable us to distinguish between plants which are heterozygous or homozygous at resistance loci.

New genes will probably need to be added to the model. The current hypothesis predicts that CALA and WELA are recognised by the same gene in Col0. However, preliminary work in collaboration with Alan Slusarenko suggests that this may not in fact be the case. Among 25 F_3 families of Col0 X La-er (supplied by Mike Daniels, Norwich) we have found several that respond differently to the two isolates. Repeat tests are required, but this could be explained by the existence of alleles at two loci in Col0. Other examples need verification where the current model predicts that host genotypes carry a common gene. This verification will come as we continue the analysis of F_2 families from our half-diallel cross.

So, what has happened to the fellow traveller *Albugo candida*? Edmar Brose, a PhD student in Jim Beynon's lab at Wye College, has been investigating the resistance of an *A. thaliana* collection from Keswick (Kes37) to a fungal isolate collected at East Malling. Segregation in the F_2 of Wein X Kes37 suggests the involvement of a single locus. Jim's group has also been looking for RFLPs among the parental genotypes of our half-diallel using a set of chromosome-diagnositc probes obtained from Elliot Meyerowitz. Our primary short term objective is to determine on which chromosome arm each of the putative resistance genes is located.

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		P. parasitica isolate					
		EMOY1	EMOY2	CALA	CAND	WELA	NOCO
	Avr-gene	1	1	*	*	1	*
		*	*	2	2	2	*
		*	*	3	*	*	3
		4	4	*	4	*	4
Host Line	R-gene	5	*	*	*	*	*
Wein	* * * * *	S	S	S	S	S	S
Nd0	1 * * * *	R	R[1]	S	S	R	S
Oy0	* 2 3 * *	S	S	R[2]	R	R	R
Col0	* 2 * * 5	R	S[3]	R[1]	R	R[1]	S
La er	* * * 4 *	R	R	S[3]	R	S	R
RLD	* 2 * 4 *	R	R	R	R	R[1]	R

Table 1. Hypothetical five-gene model for explaining the differential phenotypic reactions of six genotypes of *Arabidopsis* thaliana to six isolates of *Peronospora parasitica*. Lack of asexual sporulation by isolates on leaves of plants (7-10 days after inoculation) was interpreted as a resistant (R) reaction; whereas profuse sporulation was interpreted as a susceptible (S) reaction.

[1] A single gene for resistance is proposed based on preliminary analysis of F_2 segregation data.

[2] Two genes for resistance are proposed based on preliminary analysis of F_2 segregation data.

[3] Profuse sporulation is slow to appear and is typically associated with host cell collapse.

From Jennie Jackson...

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

At the time of the last newsletter, we had picked out some putative mutants from EMS-mutagenised M2 populations screened under blue light. Bobby Brown (a very diligent summer student) helped to rescreen and characterise these mutants and we now have eight lacking blue light-dependent hypocotyl elongation. We are currently crossing these to Hy4 and to each other to check for allelism. Readers of The Plant Cell (July 1991) will know that others have recently isolated similar mutants at three genetic loci. Hopefully, some of our mutants will be different from those already reported.

We are also screening mutagenised transgenic populations containing a ChS-GUS insert. Morgan Shaw, a new Ph.D. student, will be screening similar populations containing ssu-GUS.

The other work in the lab involves the characterisation of Hy4. Antje Ziemann (a visiting student from Germany) is currently studying the expression of various genes in hy4 and wild type *Arabidopsis*.

Gareth Jenkins, Bobby Brown, Karen Deeney, Jennie Jackson, Morgan Shaw & Antje Ziemann; Departments of Biochemistry and Botany, University of Glasgow.

From Kerrie Jones...

Ammonium toxicity in *Arabidopsis*.

Steady progress is being made with

characterisation of putative gdh clones and towards the construction of transgenic plants carrying the *E. coli* gdhA gene. We are also now using a shake flask culture method for growing material suitable for high quality enzyme and RNA purifications. This allows the analysis of gene expression at both the Northern and enzyme levels.

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From Peter Jordan...

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

Molecular biology. Work is continuing on the amplification of *Arabidopsis* gene/cDNA libraries for *hemA, L, B, C,* and *E* using PCR with oligonucleotide primers synthesised from a knowledge of conserved protein sequences. A 300bp fragment obtained using *hemL*-directed oligonucleotide primers has been identified using this approach. Secondary PCR on the purified fragment with an additional primer indicates that it also has an internal conserved sequence of the predicted size. Subcloning and sequencing of one of the large PCR fragments indicates a derived amino acid sequence which shows 53% and 49% similarity to the *Salmonella typhimurium* and barley *hemL* sequences respectively. The *hemL* gene encodes glutamate 1-semialdehyde transaminase, the enzyme responsible for the transformation of glutamate 1-semialdehyde into 5-aminolaevulinic acid. Work is in progress to sequence the complete cDNA and the gene using a combination of PCR and hybridisation techniques. Similar studies with *hemB* are also showing positive results. Sequencing cloned PCR fragments of predicted size from oligonucleotides for both *hemC* and *hemE*, despite extremely encouraging preliminary indications, has shown that the DNA sequences do not translate to any amino acid sequence resembling strongly those found in other species. From these studies, it appears that proteins found in very low amounts, such as porphobilinogen deaminase and uroporphyrinogen decarboxylase, encoded by *hemC* and *hemE*, have correspondingly low levels of message and are thus difficult to isolate by the PCR technique. Methods are being devised to increase the levels of the message specifying chlorophyll biosynthesis enzymes by other means.

Protein purification. As with the summer of 1990, the summer of 1991 was to hot for growing *Arabidopsis* (*The ACM overcomes a similar problem with his French Beans by whitewashing the greenhouse.*) and little progress has been made with the further purifications of enzymes from this source since June, although work has now recommenced in earnest. In view of the relative ease of obtaining other plants in quantity, some studies are also underway to isolate early chlorophyll biosynthesis enzymes from brassica and spinach and to use their protein sequences for the synthesis of suitable oligonucleotides for probing the *Arabidopsis* libraries.

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

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

The aim of this work is to develop a gene tagging system based on the activation of the *gus* reporter gene by native gene regulatory sequences, following random T-DNA integration. Some of our preliminary results have now been published (*Development* 112, 1009-1019), and demonstrate that the activation of promoterless and weak promoter reporters occurs at a surprisingly high frequency. These data are consistent with those emerging from other labs (Csaba Koncz, Marc van Montagu). This raises some interesting questions: are we seeing preferential insertion into chromatin which is, or potentially can be, transcriptionally active? Are there 'pseudo-promoters' scattered around the plant genome? It is clear that this approach can be used to identify *bona fide* promoters /enhancers, but we are addressing these other questions to obtain some more information on the mechanism of activation.

'Are we seeing preferential insertion ...or 'pseudo-promoters'

We now have approx. 2000 *Arabidopsis* transformants containing our promoterless *gus* 'interposon', and we are gradually working our way through the population (screening for GUS activity and aberrant phenotypes, collecting seed). We have found expression, to a greater or lesser extent, in diverse floral organs, siliques, seeds, stem, leaf and root, in a range of different cell types and at different developmental stages. Every transgenic line is different. Our interests have led us to concentrate on the screening of (a) flowers, (b) embryos, (c) roots and (d) vascular tissue. We currently are carrying out a more detailed molecular characterisation of lines expressing in (1) the stigma surface, (2) late pollen, and (3) vascular tissue (the latter aided by further financial support from AFRC, employing Hayley McArdle as a research assistant as from October 1991). We have started sequencing some PCR-amplified genomic sequences, but have only limited information to date. We are also trying to define the extent of transcriptional fusions in selected lines. There's a lot of interesting work ahead.

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From Andy Maule...

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

In July, Carole and Colwyn produced an F1 male called "Seth", congratulations to them. Carole is currently doing experiments at home optimising the conditions for maximum growth of the progeny but will be back with us at the end of November.

We have made progress in our analysis of the "spread protein" transgenics and using 3' race analysis have verified that most of our homozygous plants are expressing the inserted CaMV gene. However, we have not detected the protein, possibly indicating that the expressed P1 protein is relatively unstable. The protein does accumulate during natural infection of *Arabidopsis* with CaMV. The plants are phenotypically normal and electron microscopy has revealed no ultrastructural changes. A functional analysis for the expressed protein through complementation of a virus deletion mutant is in progress. Transgenics expressing the TMV spread protein are being similarly analysed.

P1 protein expressed in *E. coli* (clones obtained from Vitaly Citovsky, UC Berkeley) has been used to probe for specific interactions with host receptor proteins in a filter binding assay. P1 protein appears to bind to a host protein from infected or healthy tissue, but further characterisation needs to be done before we would be bold enough to call it a "receptor."

Andy Maule, Christine Perbal, Carole Harker & Seth; John Innes Institute, Norwich.
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From Keith Mitchelson...

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

Since our last report, Beata Luczak has returned to Posnan in Poland where she has a research position in the laboratory of Halina Augustyniak.... "Bye-bye all, it was nice to be in Scotland!"

After continuing hard work testing different hybridisation conditions and different restriction enzymes, Beata has developed the conditions for detection of hypervariable RFLPs using a number of different sequence probes. Results continue to look very promising. Difference signals are detectable between six *Arabidopsis* ecotypes with four of the six probes so far tested. Identification of the endogenous *Arabidopsis* sequences which hybridise to the probes is the next task.

Andy and Beata have continued the screening of YAC clones (EG -- Colombia library) with several of the positive probes. Putative positive clones appear to be present in YAC chromosomes from an initial screening of pooled cultures. About half the library has now been examined for one probe. These positive clones produce a stronger autoradiographic signal than the weak signal seen with all yeast host chromosomes. Screening of individual YAC clones has confirmed the presence of both strong and weak hybridising elements, consistent with the picture seen with pooled YAC clones. *Arabidopsis* hypervariable elements which hybridise weakly with these probes will therefore not be easily identifiable if whole YAC clones are sub-cloned into phage *lambda*.

We are now commencing a screen of an *Arabidopsis* library in *lambda* (from Denis Murphy) with the same probes which gave positives in the YAC screen. Putative positive *lambda* clones have been isolated and are now being dissected further. An honours student (Charlotte Oi) will commence next week to subclone hybridising elements directly from selected YA's fractionated by pulse-field from yeast host chromosomes.

Keith Mitchelson, Beata Luczak & Andy Porter; Dept. of Molecular & Cell Biology, University of Aberdeen.

From Bernie Mulligan...

Genetic male sterility in *Arabidopsis.*

We, that is Janet Dawson (née Fuller), have been refining our analysis of the ms mutants we talked about last time. Our msH mutant does not dehisce properly. The problem seems to be that anthers of this mutant start the dehiscence process and anther locules fuse to form a bi-lobed structure, but then cleavage of the stomium is not completed. Another possibility we are considering is that the anther walls do not retract (spring back?) after dehiscence; perhaps some elastic property of the anther wall is affected. The pollen in this mutant is fertile and when manually extruded from mature anthers can be used to pollinate and induce seed set on msH flowers. In contrast, the pollen of mutant msZ appears normal until a very late stage and then becomes abnormally vacuolated; this pollen is infertile. This mutant is interesting because elongation of stamen filaments seems to be inhibited during the later stages of flower development. We think this may mean that in the last stages of pollen grain formation, nutrient supply to the anther through the filament is blocked or reduced.

> "We think we are close to ms1."

Mutants msW and MsY seem to be affected at or around meiosis. The exact timing of ms gene action is not clear. However, at the first division of meiosis characteristic abnormalities are apparent for both mutants. Though further meiotic division and cell differentiation may occur, in both cases the pattern of anther and pollen development becomes progressively more abnormal. Tapetal abnormalities are also apparent post-meiosis. These mutation may thus represent loci with rather general functions in anther formation.

The walking side of the project is going well. We have now reliably mastered the production and subsequent use of end probes from the YACs. We are routinely using two approaches: (i) end rescue of the left arm fragments and, (ii) vectorette PCR (we have found this better than IPCR). We are now walking out in both directions around ms1, and are using the large battery of recombinants that we, in combination with Mandy Walker at Cambridge, have around ms1. From these data we think we are close to ms1...watch this space!

We have recently been joined by Igor Vizir, an EC funded post-doc, from Kishinev in the Soviet Union and Kriton Kalantidis from Greece, who will be doing a PhD with us.

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From Steven Neill...

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

Back to the lab after the distractions of the summer -- especially as it's now

safe again after the deliberations of the (Inquis...) progress review panel!

At last we can grow the wilty *aba1* mutant in a reasonably healthy state. The key thing is to use fresh seeds, as those of this mutant lose both viability and vigour fairly rapidly during storage.

We've done some more *in vitro* translations using both [^{35}- S] - methionine and [^{3}-H] - leucine and RNA from wild type and mutant tissues subjected to various treatments. We hope to write up this work fairly soon, along with the [ABA] and inhibitor studies. A poster on this and the cDNA work was presented at the IPGSA Conference in Amsterdam at the end of July.

We've screened our cDNA library with probes representing turgid and stressed tissue. The primary screen turned up a number of potential clones, but most of these subsequently turned out not to be stress inducible. However, we're still looking at some others.

We *think* we've made good progress on our subtractive cDNA library(!). Using PCR to generate sufficient cDNA for the subtractions seems to have been successful; we've now biotinylated this and are about to do the subtractions à la Straus.

On this point, it's worth emphasising the added value to be gained from coordinated research programmes. We're basing our subtraction procedures on the extremely useful protocol provided by Donald Straus *via* the *Arabidopsis* Newsletter and have also benefitted from discussions and advice from members of the PMB programme, particularly Mark Leech at the JII and Andy Philips at Long Ashton.

Mike Bulman, Jackie Williams & Steve Neill; Bristol Polytechnic.

From Helen North...

Cell cycle control genes in *Arabidopsis*.

Since the last report, we have succeeded in constructing a cDNA library from cauliflower meristematic tissue using the Stratagene Lambda ZAP cDNA kit. The library will now be circularised into the Bluescript vector and then subcloned into a yeast expression vector so that it can be used to complement yeast cell-cycle mutant functions. To confirm that the library is enriched for cell-cycle transcripts we are currently screening with a highly conserved region of the *S. pombe* cdc25 gene.

We have also been carrying out a test yeast transformation using the *S.pombe cdc7* cytokinesis mutant so that we know how many rescued mutants are required to identify both the gene and various groups of suppressors. From 71 plasmids that rescued with a wild type morphological phenotype, we have identified at least two different classes of rescuing plasmid and these are being restriction mapped and remaining plasmids classified.

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.

Sequencing of the avirulence gene from *X. campestris* strain 1067 has been completed by Chris Barber and an open reading frame of approximately 1.7kb has been defined. Its position on the genomic clone of 23kb is (thankfully) exactly as predicted by the Tn5-transposon insertions described in an earlier report. So far, no significant homologies have been found with other known bacterial *avr* sequences (Brian Staskawicz, who is at the Sainsbury lab for an energetic year's sabbatical, has provided us with the relevant gene sequences) or to anything in the gene data banks. An internal fragment was used to probe genomic DNA from a variety of plant-pathogenic bacteria and a couple of non-pathogens. Interestingly, it hybridises with all dicot-infecting strains of *X. campestris* tested, but not to *X.c. graminis* or *X.c. holcicola*, both of which attack monocots. Also, no hybridising DNA was detected in strains of *Pseudomonas* or *Erwinia*. It does have an allele in *X.c. campestris* strain 8004, the virulent recipient strain into which the 1067 DNA was transconjugated. The 8004 homologous DNA does not have an internal BamH1 site and therefore shows as an RFLP on a Southern blot. However, by the criterion of 8004 virulence and growth on Col-0 and Ler plants, this gene is inactive.

I think we've come to the end of our laborious search for an *A. thaliana* ecotype which is susceptible to 8004(*avr*Xca). Only in Kas-1 does the transconjugant produce symptoms and multiply by 2 orders of magnitude after 5 days. However, its growth rate and the severity of symptoms are not as great as strain 8004 alone. Two other ecotypes (Aa-0 and Pr-0) gave mild symptoms upon inoculation with the transconjugant, but the bugs failed to grow to any reasonable extent in these plants. The different levels of resistance (or tolerance) to 8004(*avr*Xca) in these ecotypes suggest underlying 'horizontal' resistance effects contributing to the plant phenotype. Our best susceptible candidate is therefore Kas-1 and analysis of resistance to 8004(*avr*Xca) in the F_1 and F_2 progeny of a cross between Kas-1 and Col-0 will reveal if it segregates in a simple manner or not.

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

From Jo Putterill...

Isolation of the flowering-time gene *fg*.

Our strategy is to chromosome walk to *fg*, which is located on the upper arm of chromosome 5. To achieve this, we are building a YAC contig around RFLP markers in the *fg* region. At the same time, *fg* is being mapped on the YAC contig using plant recombinants with chromosome breakpoints close to *fg*. YACs that contain *fg* will be subcloned into a binary vector. The *fg* mutant will be transformed with these clones to find a complementing clone which restores normal flowering time.

In order to build YAC contigs in the vicinity of *fg* we screened three *Arabidopsis* YAC libraries for clones in the *fg* region using RFLP markers; CHS/*tt-4*, 6833, 5962 and pCIT1243. Positively hybridising ➻

colonies were picked and three single colonies of each were rescreened by colony blot hybridisation. YACs were sized by pulse field gel electrophoresis with Southern analysis using a YAC vector fragment as probe. Colony blot results were confirmed by Southern hybridisation analysis of digested YAC DNA using the RFLP markers as probes. Note that much improved YAC growth in liquid and solid selective media has been seen on addition of 50-100mg/L adenine + 11g/L casamino acids (Difco, vit assay).

"Two YAC contigs have been now been built in the *fg* region."

Overlapping YACs are being detected in the YAC libraries by use of YAC end probes and other cosmid probes. End probes are generated by inverse PCR and YAC left end rescue techniques. The E. Ward YACs have lost the cloning site making extraction of end probes, free of vector, more difficult. To counter this problem, we have been using a PCR reamplification protocol from the Goodman lab, which introduces a new Fok 1 site very near the cloning site of Ward YACs, allowing removal of vector. These PCR primers, in combination with a primer that anneals near the Nde 1 site in the left end of the vector can also be used to amplify small inserts in left end rescue plasmids derived from Ward YACs.

To position *fg* on the YAC contig, we have crossed a doubly marked *tt-4, fg* Landsberg *erecta* strain to Niedersenz and in the F2, selected 7 recombinants which contain crossovers between *tt-4* and *fg*. Restriction enzymes that yield RFLPs with the markers and some YAC end probes have been found. Southern hybridisation analysis has been carried out on digested parental and recombinant DNA. According to the RFLP patterns, the probes have been placed to the left (towards CHS2/*tt-4*) or to the right of the breakpoints (towards *fg*). We have found it important to establish that the RFLPs that we are following are really linked to the *fg* region. (We probe eight F2 *fg tt4* homozygotes and expect to see only Landsberg RFLPs if the probe is closely linked to *fg*.) Work is underway to try and identify closely linked recombinants proximal to *fg* (to the right of *fg*).

Two YAC contigs have been now been built in the *fg* region. One around CHS/*tt-4*, 6833 is about 700kb and the other formed from the newly-joined (hooray!) 5962/pCIT1243 contigs is about one megabase. The 6833 component of the CHS contig orientates towards *fg*. This latest order contradicts that of the last newsletter as we were previously misled by RFLPs that were not linked to the *fg* region. Both 5962 and pCIT1243 are to the right of the recombinants that have been analysed to date, but this contig has not yet been orientated. Hence, the order of markers in this region appears to be CHS/*tt-4*, 6833 and then either 5962 or pCIT1243. To determine whether the current contigs already contain *fg* and to locate *fg* with respect to 5962/pCIT1243, requires analysis by more markers on recombinants both distal and proximal to *fg*. Long range mapping techniques will also be employed to monitor the walk.

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

From Kevin Pyke...

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

We have started to analyse the genetic basis of five of our 18 chloroplast accumulation mutants by reciprocal backcrossing to wild-type. The five mutants were chosen for further analysis to represent the full range of mutant cell phenotypes. In each cross, all F_1 progeny had a wild-type leaf cell phenotype. In the F_2 populations, mutants segregated in a ratio not significantly different from 3:1 in each half of the backcross in all cases. These results suggest that these five mutants are each conferred by a single recessive nuclear locus which we have termed *arc* loci, i.e., "accumulation and replication of chloroplasts" loci. Allelic crosses between these mutants indicate that at least four different loci are represented. Now that we possess backcrossed seeds for these mutants we can begin a more detailed biochemical analysis of the mutant phenotype.

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From Peter Quinn...

Thermal tolerance of fatty acid desaturase mutants of *Arabidopsis*.

Nelly Tsvetkova from the Central Laboratory of Biophysics in Sofia has worked diligently on the project over the summer supported by a FEBS fellowship. She has been persuaded to take up the post-doctoral fellowship vacated by Srinivas Volety earlier this year and the grant of a work permit is anxiously awaited. The experimental problems in isolating quantitative amounts of chloroplast lipid by HPLC have been considerable. We are presently deciding whether to press on with HPLC or resort to Argentium tlc with its attendant risks of oxidation. Our attempts to perform dynamic X-ray diffraction studies on samples already prepared have also been frustrated as no beam appeared at the Synchrotron Radiation Source during our last shift schedule. We're hoping for better luck next time.

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From Christine Raines...

Genetic analysis of regulatory factors determining the development of the photosynthetic apparatus of plants.

Progress through the summer has been slow due to the illness of my postdoc, Philip Horsnell. Despite this we have got some interesting results to report. Philip and a project student of mine successfully cloned and

sequenced a cDNA encoding *Arabidopsis* carbonic anhydrase. We have now used this probe to investigate the effects of elevated carbon dioxide on CA mRNA levels; growing *Arabidopsis* in x2 carbon dioxide resulted in a 50% increase in steady state message levels. This result was both unexpected and interesting as the data in the literature indicated that we might expect a decrease in the levels of the message for this enzyme.

Our mainline work has also moved forward, we have now completed the sequencing of the upstream regions of all three of the Calvin cycle genes. Philip has worked out his strategy for producing deletions for insertion into transgenic *Arabidopsis*. In addition fragments are being prepared for use in gel-shift assays in conjunction with our wheat work. Nicola Willingham is currently producing constructs using the upstream region of the SBPase gene and will hopefully have some interesting transgenes in the near future.

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From Steve Slocombe...

Expression of oil synthesis genes in *Brassica napus* and *Arabidopsis*.

In the developing *Brassica napus* embryo, oil synthesis rate is greatest 4-6 weeks after anthesis and precedes the peak in accumulation rate for storage protein deposition and oleosin synthesis. In relation to oil synthesis, the sequencing of two cDNAs for the stearoyl-ACP desaturase from a developing rape embryo library were described in the last report. These have subsequently been used to monitor transcript levels and to obtain genomic clones. Transcript levels for this oil synthesis enzyme correlate with oil synthesis rate during embryogenesis and peak before those for cruciferin and oleosin in rape. Isolation of the promoter from a genomic clone corresponding to this desaturase is underway. It is hoped to use the promoter both in anti-sense studies and for the expression of foreign oil synthesis genes with the aim of adjusting storage lipid synthesis in a seed-specific manner and thus produce transgenic oilseeds with novel fatty acid profiles.

Steve Slocombe & Denis Murphy; John Innes Centre, Norwich.

From Alison Smith...

Investigation of the gene for hydroxymethylbilane synthase from *Arabidopsis* in transgenic tobacco plants.

We have rescreened the pea cDNA library with our HMBS clone and pulled out three more positives, one of which (P1) was larger than the original clone. Sequencing of P1 at the 5' end revealed two in-frame ATG codons, so hopefully it is full-length. We will test this by seeing whether the precursor it encodes is competent for import into pea chloroplasts. Initial northern analysis using a probe internal to the mature protein showed a single transcript of about 1.8 kb, but when a longer probe covering almost the entire cDNA was used, a second larger transcript was also detected although with a much weaker signal. At present, we are in the process of doing tissue specific and developmental northern analysis to see if the two transcripts represent different forms of the enzyme, for instance for photosynthetic versus non-photosynthetic cells. Huguette Albrecht presented this work at the ISPMB meeting in Tucson.

As I explained in my last report, I spent three months working in Paris, and I am pleased to say that, apart from being very enjoyable, my trip was also successful. After a week of transforming a yeast mutant for the last enzyme of haem biosynthesis (ferrochelatase) with an *Arabidopsis* cDNA library in a yeast expression vector, I had about 60,000 colonies. On screening these for the restoration of the wild-type phenotype, one colony was obtained, which also had the correct genotype of the original mutant (five other putative positives turned out to be wild-type contaminants of the original transformation plates). The rescued strains had measurable ferrochelatase activity and all the respiratory cytochromes, which were entirely absent from the original mutant. Furthermore, the plasmid extracted from the rescued colony and retransformed into the mutant, again gave functional complementation. This indicated that the plasmid carried the cDNA encoding ferrochelatase from *Arabidopsis*. I am currently sequencing the clone and have located regions of sequence similarity to the enzymes from human and yeast. Since the enzyme in yeast is mitochondrial, it is likely that we have found the clone for the plant mitochondrial enzyme. We will therefore use the clone to screen our libraries to locate the chloroplast enzyme. I am also intending in the near future to start screening two other haem-biosynthesis mutants of yeast with the same library. I am extremely grateful to my hosts in Paris, Rosine Labbe-Bois and her husband Pierre Labbe, and to EMBO for awarding me a Short Term Fellowship for three months.

"Transforming a yeast mutant for the last enzyme of haem biosynthesis with an cDNA library resulted in fuctional complementation."

Alison Smith, Huguette Albrecht, Michael Witty & Ashley Cook; Department of Plant Sciences, University of Cambridge.
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From Mandy Walker...

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

As you will all remember, we are attempting to clone the ttg locus by chromosome walking. We know that only one RFLP maps between ttg and ms1, though several map proximal to ttg. I have isolated a number of recombinants between ttg and ga3 so that we can map these RFLPs to decide which to use as the starting point of the chromosome walk. Nigel Kilby from Ian Furner's lab in Genetics will be joining us on October 1st and Rebecca Wilson will also be helping us out, so I hope we will have lots of results to report next time.

Mandy Walker & John Gray; Botany School, University of Cambridge.
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Guest Summaries

From Felicity Watts...

Cell cycle control genes in *Arabidopsis*.

We are continuing our characterisation of the *Arabidopsis* homologue to the *S. cerevisiae* RAD6 gene, which we have now named UBCa. DNA sequencing is now almost complete and indicates the presence of five putative introns. Only three had been identified by the time of the last newsletter, but we think we have now identified all of them. This will be confirmed by sequencing of the corresponding cDNA.

We had a chance to talk to Dr. Jentsch, who works on the *S. cerevisiae* ubiquitin carrier gene family, at the yeast meeting at Cold Spring Harbor this year. It appears that nine members of the gene family have been isolated from *S. cerevisiae* and that individual members are proposed to have a variety of different functions, *e.g.*, cell cycle and DNA repair. Although our UBCa gene has homology to the published sequence, it appears that the UBCa sequence may be a plant specific member of the gen family. As yet, the role of the *Arabidopsis* UBCa gene product remains unknown.

The UBCa gene appears to be expressed at low levels (*i.e.*, a transcript is difficult to detect in total RNA from a variety of different tissues, *e.g.*, roots, leaves , seeds, *etc.*). We are now analysing the function of the UBCa promoter by cloning it upstream of the GUS gene and preparing transgenic *Arabidopsis*. So far, a couple of transformation experiments have been carried out using *Agrobacterium* infection of *Arabidopsis* roots, and we await the production of shoots from our callus. The analysis of other members of the *Arabidopsis* UBC gene family is continuing.

We are combining an interest in promoter sequences with our cell cycle project and are isolating *Arabidopsis* homologues to *S. pombe* cdc22 and suc22 (which encode the small and large subunits respectively, of ribonucleotide reductase). In other organisms, these genes have promoters induced either in a cell cycle dependent manner or in response to UV or DNA damage. So far, several putative positives have been isolated from an *Arabidopsis* cDNA library using the *S. pombe* genes as hybridisation probes, and sequencing is now underway. once we identify *Arabidopsis* homologues from this cDNA library, we will use them to probe genomic libraries to allow isolation of the respective promoters for analysis in transgenic plants.

Isolation of genes via PCR and antibody screening of expression libraries is continuing.

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Guest Summaries

From Mark Aarts...

Enhancer trapping with the *En-I* transposable element system.

Since the last newsletter, the whole molecular biology department of our institute has moved to a new building, leading to a forced delay of activities. Fortunately, before this hectic period, some transformations had been done. The resulting plants of which were flowering at the time. Seeds of these plants have been collected and have been sown on selective medium to determine if they contain T-DNA with an antibiotic resistance marker and also to estimate the number of T-DNA loci if present. The T_2 progeny tested so far show a 3:1 segregation for antibiotic resistance. This means that only one active T-DNA copy is present. Most of the constructs integrated and tested so far contain defective transposable elements inserted in the untranslated leader of a marker gene and also a marker gene inserted within the transposable element. To activate this transposable element, the plants have to be crossed to transgenic plants containing an active, *i.e.*, transposase producing, but immobile transposable element. Before doing so. the T-DNA loci have to be present in a homozygous state to ensure that the F1s of the crosses contain both elements. Each antibiotic-resistant plant will therefore be allowed to self-fertilise and its progeny tested again for antibiotic resistance.

One of the constructs with which transgenic plants are obtained, contains an *I*-element inserted in the 5' coding sequence of an NPTII gene with, on the same T-DNA, an *En* element without termini, under control of the CaMV 35S promoter. Apart from testing the progeny for hygromycin resistance, which was the marker for transformation , the plants will be tested for kanamycin resistance that excision of the *I*-element will lead to in one in three cases.

Finally, I am having quite some problems in getting *in vitro* plants to set seed. Now I am using Magenta pots of which I have raised the lids a little, but after having survived a major infection, I am a bit cautious in doing so. If there is anyone who knows a better way of getting seeds on *in vitro* I would be glad to hear about it.

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From Abdul Chaudhury...

Late flowering and light.

We are using *Arabidopsis* genetically to dissect the developmental steps leading to male-fertility. Such a dissection will not only illuminate an important biological process, but should also provide strategies for the control of self-pollination in economically important crops, thus facilitating the generation of hybrid seeds. There are two parts of this project: developmental and molecular genetic. In the developmental biology part of the project, we have isolated more than twenty male-sterile mutants of *Arabidopsis*. The mutants cover the whole range of the developmental steps leading to male-fertility. They include a mutant devoid of any anthers called antherless, seven mutations that affect the stages in microsporogenesis, and one mutation that alters the surface of the pollen. We are also interested in the isolation of these genes. Attempts are being made to

clone the gene ms1 by chromosome walking. Three yeast artificial chromosome libraries containing *Arabidopsis* DNA are being screened to isolate DNA fragments with the help of RFLP probes that are linked to ms1, as well the end probes made from these YACs. Fine RFLP mapping of the ms1 region is being used to delineate the ms1 gene between two RFLP markers. Finally, genetic complementation of the ms1 phenotype will be used to isolate the gene.

The mutants include one devoid of anthers

Abdul M. Chaudhury, Kathi Bloemer. Leigh Farrell, Robin Chapple, Bjorg Sherman, Stuart Craig & Liz Dennis; CSIRO Division of Plant Industry P.O. Box: 1600 Canberra, ACT 2601, Australia.
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From Darroch Hall...

The genetics of fungal resistance in *Arabidopsis*.

Successful infection of *A. thaliana* by an ascomycete pathogen of *Brassica napus* has been accomplished recently here at UEA. This pathogen has been used to screen fifty ecotypes of *A. thaliana*, plus several other species of *Arabidopsis*, for resistance. Initial tests have identified three resistant ecotypes from geographically diverse origins. A genetic analysis of these three ecotypes has just begun to determine the number and nature of resistance genes involved in this interaction. Ultimately, the chromosomal location of these resistance genes will then be determined by observing their co-segregation with known RFLP markers.

Darroch Hall, John Turner & Richard Oliver; School of Biological Sciences, UEA, Norwich.

From Mike Jones...

Arabidopsis as a suitable host for plant-parasitic nematodes.

Murdoch University, in Perth, Western Australia, has been designated the State Centre for Agricultural Biotechnology. Western Australia, which constitutes about one-third the land mass of Australia, has vast agricultural and economic potential. One endemic pest is the root-knot nematode (*Meloidogyne* spp), and a research programme is underway to study and develop strategies for resistance to this nematode. To aid this work, we have found that various lines of *Arabidopsis* are good hosts for root-knot nematodes endemic to Western Australia, and that infections cultured *in vitro* are an excellent source of experimental material. I am also updating lectures in Plant Sciences, and would be happy to receive any practical class experiments with *Arabidopsis* that anyone else has developed and would be prepared to share. Thanks, (and regards to former colleagues in the AFRC).

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From Natalya Klueva...

Arabidopsis in the USSR.

It turned out to be not a trivial task to give a summary of *Arabidopsis* studies in the USSR. This noble plant still remains a rare object to be found in Soviet greenhouses, but to tell the truth, a couple of years ago you could have found none at all. The progress is obvious and the necessity for co-operation now arises among the Soviet *Arabidopsis* people. That is why I am really glad to present you what I have learnt about in search for my *Arabidopsis* colleagues here.

Three groups of studies are developing now in the USSR using our favourite weed. The ecology-genetical branch concentrates on the structure of *Arabidopsis* populations in extreme conditions, *i.e.*, high altitudes and, especially, high radioactive backgrounds resulting from the Chernobyl atomic-station explosion (O. Fedorenko in Petrozavodsk and V. Shevtshenko in Moscow); high intensity of UV-irradiation (P. Usmanov and his group in Dushanbe). Studies of mutagen effects on *Arabidopsis* are included in the International Program of Chemical Security with three labs participating: the Institute of Chemical Physics in Moscow, the lab from Dushanbe, and the Institute of Genetics and Selection in Baku. T. Gichner and J. Veleminsky from Praha, Czechoslovakia are also taking part, as are A. Mechandjiev, C. Chankova and C. Petkova from Sofia, Bulgaria. Molecular genetic studies have several points of interest. E. Mushammedshin (Institute of General Genetics in Moscow) with his group concentrates on the mutagenic effects of Ti-plasmid incorporation into *Arabidopsis* genome. Alexander Pemov (Plant Physiology Institute, Moscow) studies histone families. He is the one most likely to report in the next issue with his latest results.

As for myself, I am plant physiologist and for the last three years I have been busy with supermolecular organisation of heat shock proteins and their possible functioning in *Arabidopsis*. I love three things about my subject: full sterility of plant material, the fact that the whole plant temperature response can be studied; and the ease of purification and fractionation of proteins. My latest finding is heat shock protein(s)-containing a high molecular weight (700 kDa) structure which accumulates to significant quantities in tissues after heat shock. After a 2-week vacation, I will return to do immunoblotting with an HSP 70 probe from tissue culture of tomato, and possibly with a groEl probe, in order to elucidate the nature of HSP included. I hope to have the chance to report my progress in the next issue.

With warm regards from Russian Arabidopsis People!

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From Maarten Koornneef...

Some old mutants revisited.

In 1962 AD, McKelvie published (Radiation Botany vol. 1 233-241) an impressive list of 'mutants.' In addition to a short description, a gene symbol was given to each mutant ➵

despite the fact that a segregation analysis was probably not performed in most cases. The mutants were induced by chemicals and irradiation in Estland or Limburg background and in a few cases a photograph was published.

Going through his list one comes across many intriguing descriptions of mutants that would be of interest for further study with modern technology. Unfortunately, these mutants are lost (that is the story). However, in the old days, some of these mutants were distributed and kept alive by *e.g.*, Prof. Napp-Zinn in Cologne and Prof. Goto in Japan. We have been able to compare these mutants with similar mutants isolated in Wageningen and report about their genetic relations.

"The C24 ecotype used and distributed by the van Montagu lab is glabrous."

A very interesting mutant seemed axillaris with flowers growing from the base of the pods. This is a characteristic of the ap1 mutant described in detail by Irish and Sussex (Plant Cell 2, 741-753, 1990). However, McKelvie did not mention reduced petals for ax. When we grew the mutant obtained *via* Napp-Zinn, it looked like a leaky ap1 and indeed we confirmed this with a complementation test. Some years ago we had also shown that McKelvie's ap is allelic to ap1. Clavata mutants are flower morphology mutants and therefore of interest. I named our mutants with club-like pods, clavata because they resembled the picture in McKelvie's paper. We tested his clavata (probably his clv1) with our clv1 and clv2 mutants and found McKelvie's clavata a clear allele of clv1. A phenotypic difference with our Ler clv alleles is that McKelvie's clavata showed a much more pronounced stem fasciation.

The lepida (le) mutant, which is a dwarf with rather dark brown seeds, is allelic to the pa mutant described by Hirono and Redei (Genetics 51, 519-526). This mutant is apparently present several times in the AIS collection, because we found that F127 and F132 are le mutants too.

The ax mutant which we received from Napp-Zinn segregated for a glabra mutation. We confirmed this to be gl1.

The C24 ecotype used and distributed by the van Montagu lab is glabrous, which appeared to be due to a gl1 mutation. I hope that this type of information will be of some use for people working with the types of mutants mentioned above and that it will stimulate others to do part of genetic ordering of mutants.

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From Dianne van der Kop...

About auxins and gene targetting.

Since our project report in the last (July) issue we had good summer holidays and also made some progress with our experiments concerning the auxin signal transduction (Eric van der Graaff and Dianne van der Kop) and gene targetting projects (Stephan Ohl and Annette Vergunst).

Annette is still working on *Agrobacterium*-mediated DNA transfer to protoplasts. Experiments to get protoplasts dividing in liquid culture have not yielded good results so far. A problem with the *Arabidopsis* protoplasts is that they need to be embedded in alginate after isolation for a good regeneration. The alginate matrix, however, forms a barrier for the agros to do their job. She is trying now to tackle this problem. Regeneration and PEG transformation are now working under our growth conditions.

Stephan completed the cloning of the first ArrpII constructs, which he plans to use for gene targeting. He has also started working on protoplast transformation.

Eric finished the construct containing a strong plant promoter/enhancer. He started *Agrobacterium*-mediated transformation experiments of tobacco protoplasts, since an *Agrobacterium*-mediated transformation system is not yet available for protoplasts of *Arabidopsis* (Annette). A high number of transformants, mainly single copy inserts are desired, which he will screen for phenotypes related to overexpression or disruption of phytohormone or morphogenesis-related genes. At the moment he is testing different auxins and auxin concentrations for the selection of the seeds of his primary transformants. Dianne is analysing the transgenic *Arabidopsis* plants, which are transformed with an auxin inducible (tobacco) promoter hooked up to GUS. The blue staining which is observed mainly in the root tip has the same pattern as the staining observed in transgenic tobacco. She plans to make crosses between her transformants and different auxin related mutants. She is also starting up cell suspension cultures from which she hopes to isolate auxin inducible genes. The construct she made to study the upregulation of the auxin inducible promoter is completed. Root transformation was done and she is now waiting for the first transformed seeds.

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From Ottoline Leyser...

More about auxins.

Many exciting things are happening in the Estelle lab, so here are just the news head lines.

AXR1: Cindy Lincoln, Jocelyn Turner and I have now isolated a series of overlapping clones in a T-DNA vector which extends from a RFLP probe known to be distal of *AXR1* to a probe known to be proximal to the gene. We are currently transforming these into *axr1* mutants to identify a clone which rescues the mutant phenotype. Doug Lammer is using the clones to screen a cDNA library.

Cindy Lincoln has isolated a second site suppressor of *axr1* auxin resistance. Plants homozygous for the suppressor have an interesting phenotype of their own including early bolting and short stature.

AXR2: Candace Timpte in collaboration with Pam Green's lab has shown that the auxin induced SAUR genes (Gee *et al.* Plant Cell 3 419-430) do not respond to auxin in *axr2* mutant plants. Instead they show an interesting pattern of expression; being off in roots, hypocotyls and inflorescences, but constitutively on in leaves.

AXR3: I have found that *axr3* root elongation is stimulated by cytokinin concentrations which severely inhibit root elongation in wild-type plants.

AUX1: Bryan Pickett and Lawrence Hobbie have identified an RFLP that maps less than 1 map unit from AUX1.

Auxin transport: Lawrence Hobbie has an auxin transport assay up and running based on that of Okada *et al.* (Plant Cell 3, 677-684). We are testing all our mutants for defects in auxin transport and Lawrence Hobbie and Max Ruegger have isolated some mutants altered in response to auxin transport inhibitors which are now being characterised.

Ottoline Leyser, Mark Estelle, *et al*; Dept. of Biology, Jordan Hall 142, Bloomington, Indiana, 47405, U.S.A.

From Nicola von Mende...

Development of nematodes on hormone mutants of *Arabidopsis*.

It has been shown frequently that plant hormones are involved in the development of plant-parasitic nematodes. We have tested the development and reproduction of the sedentary nematodes, *Heterodera schachtii* and *Meloidogyne incognita*, on *Arabidopsis* mutants which are defective in the biosynthesis or receptors of auxin (axr-1, aux-1), abscisic acid (aba, abi-1 ,2 & 3), gibberillic acid (ga-1, 2, 3, 4, 5 and gai) or ethylene (etr). Both nematode species reproduced well on all tested mutants. There were slight differences in the time of invasion and the production of lateral roots at the feeding sites. The mutants had been obtained from seed banks (Nottingham and Frankfurt) or from other research workers. We are interested in testing more hormone mutants, particularly those which are defective in auxin or cytokinin biosynthesis or receptors. If you have any available please contact: Nicola von Mende, Rothamsted Experimental Station, Harpenden, AL5 2JQ

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"We are interested in testing more hormone mutants..."

From Javed Mirza...

Polyamines and *Arabidopsis*.

The polyamines (putrescine, spermidine and spermine) occur ubiquitously in plants, animals, and prokaryotes. While required for normal growth and development, their precise physiological functions remain obscure. At present, there is a debate whether to consider polyamines as a new class of plant hormones or not. Polyamine mutants might help reach a decision. So far no systematic approach has been adopted to induce and isolate such mutants in plants primarily because of the occurrence of more than one biosynthetic pathway of polyamines in plants. It is well recognised that in plants, putrescine can be formed directly through decarboxylation of ornithine by ornithine decarboxylase (ODC) or indirectly from arginine by arginine decarboxylase (ADC). It is hypothesised that blocking one pathway (for example, by induced mutation) would not deplete the plant of polyamines.

These mutants suggest a role of endogenous polyamines... comparable to hormones.

In an attempt to isolate polyamine mutants of *Arabidopsis thaliana*, we screened EMS treated M_2 seed (the same lot was used for screening NAA-resistant mutants) against growth inhibitory concentrations of putrescine and spermine. This amazing plant has given us several mutants which are either resistant or sensitive to putrescine or spermine. Isolation of these mutants probably indicate a preference of one biosynthetic pathway or occurrence of only one pathway for polyamine biosynthesis in *Arabidopsis thaliana*. Some of these polyamine mutants exhibit developmental abnormalities which are very different from simple mineral deficiency symptoms. These mutants rather suggest a role of endogenous polyamines in plant development comparable to other well-recognized hormones.

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From Peter Sijmons...

Worms on weeds!

An increasing number of nematologists are becoming interested in *Arabidopsis*. Our laboratory protocol for monoxenic culture of plant-parasitic nematodes was requested by, and sent to, several labs in Europe and the US; so we may be looking forward to some interesting new results in plant-pathogen interaction. I have had several reports from other labs who were successful in infecting *Arabidopsis* roots with *Meloigogyne incognita* in non-sterile sand cultures. This opens up possibilities to screen large numbers of individual plants. Between conferences and holidays I have primarily been occupied with *Arabidopsis* root transformation which seems to work well now. Hopefully, more news next time.

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From Mark Young...

Studies on the *in vivo* activity of ribozymes directed against *Arabidopsis* ADH.

We continue to make progress to optimise ribozyme design for *in vivo* activity using the inactivation of ADH as a model system. Homozygous T3 seedlings expressing various ribozyme designs and expression strategies are being screened on allyl alcohol. We will hopefully have initial results in the next month or so.

A new series of ribozymes are currently being constructed that target ADH cleavage sites selected by computer analysis. The computer program uses an algorithmm that identifies local stem/loop structures in an RNA molecule. We have modelled the structure of ADH exon IV to determine potential accessible loops for ribozyme mediated cleavage.

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Conference Reports

From Richard Williamson...

Arabidopsis in Canberra

Over 200 participants attended the Robertson Symposium on *Arabidopsis* research at the Australian National University on 30 September and 1 October. It was a big week for plant science in Canberra, with the prime minister opening the new Plant Science Centre (ANU/CSIRO/Biocem) and meetings on "High CO_2", "The Genetics of Plant Mineral Nutrition" and the annual meeting the Australian Society of Plant Physiologists all competing for audiences. Sir Rutherford Robertson opened the meeting to dispel (as he put it) the common assumption that death invariably precedes having something named in one's honour and recalled that in the 1950's he examined John Langridge's thesis on the biochemical genetics of *Arabidopsis*. Dick Brock (CSIRO Canberra) outlined Langridge's work which, together with Brock's own work on the inheritance of quantitative traits, were major parts of the first era of *Arabidopsis* research in Canberra.

The first morning was given over to reproductive matters and was opened by Maarten Koornneef (Wageningen) considering the transition to flowering. The assignment of genes to interacting constitutive and environmentally sensitive pathways was discussed from the responsiveness of various mutants to environmental factors and from the properties of double mutants. David Bagnall (CSIRO Canberra) showed that a deficiency of 730 nm radiation (as commonly found with fluorescent tubes) delays the onset of flowering. The parallel responsiveness of various late flowering mutants to light quality and vernalization suggest some commonality in control pathways for the two stimuli. Joanne Burn (CSIRO Canberra) presented evidence consistent with the involvement of methylation in vernalization: 5-azacytidine reduces flowering time only in vernalization-sensitive lines among the late flowering mutants and ecotypes tested.

Elliott Meyerowitz (Caltech) moved the focus to genes specifying organ identity once the transition to flowering has occurred. In a masterly presentation, he further supported the genetically deduced combinatorial model for the control of organ identity with *in situ* hybridisations showing gene expression in the predicted regions of wild type and mutant floral meristems. The cloned organ identity genes belong to a 20 to 30 member gene family that probably share homologous DNA-binding and other regions. Impressive progress in identifying cadastral genes that determine the expression pattern of the organ identity genes was reported together with data on hetochronic genes that regulate meristem identity. Plants carrying strong alleles of leafy, for example, are trapped in the cauline leaf stage. David Smyth (Monash Uni) and Ry Meeks-Wagner (Oregon) gave somewhat different emphases in their views of the inflorescence meristem and of the rôle of the terminal flower gene in maintaining indeterminate growth of the inflorescence meristem. The gene keeps the central dome of the inflorescence meristem from differentiating, but does not apparently affect floral and vegetative meristems.

The morning's final group of speakers dealt with the development of the male and female parts of the flower. Mary Webb (ANU) described development through to the proembyo with particular emphasis on the microtubular cytoskeleton, while Abdul Chaudhury (CSIRO Canberra) and Zoe Wilson (Nottingham) described pollen development. Both of the latter speakers had obtained many new mutants conferring male sterility with structural abnormalities apparent at characteristic stages of development and both had initiated chromosome walks to the ms1 locus described some years ago.

The afternoon session focussed mainly on metabolism, with John Browse (Pullman) describing the value of mutational analysis in dissecting the complexities of lipid metabolism. Most mutants were obtained by "brute force" screening for altered lipid profiles and have no obvious visual phenotype under normal growth conditions. Reassuringly for the prevalent view of cold tolerance, however, several mutants are severely affected at low temperatures. Rudi Dolferus (CSIRO Canberra) described work in progress to dissect the promoter function of the alcohol dehydrogenase gene to see whether diverse environmental stimuli induce expression by acting through the same regions. Chris Cobbett (Melbourne) described a mutant with 10% of the wild type arabinose kinase activity that is specifically sensitive to L- arabinose. Suppressor analysis sugggests that the gene encodes both kinase and transport activities. Nigel Crawford (La Jolla) described the genetic analysis of nitrate assimilation using chlorate resistance mutants first reported some years ago. Six genes are required to produce the molybdenum cofactor and two genes encode the nitrate reductase polypeptide. Surprisingly, nitrate reductase activity can be reduced by 90% in mutants without overt stress symptoms.

The Tuesday morning session on development was led off by Gerd Jurgens's description of what may be a fairly complete set of genes specifying embryo pattern. These were identified from a screen of M_2 seed collected from individual M_1 parents so that mutations causing major pattern defects (duplications, deletions, transformations and multiplications) that are lethal in homozygotes are propagated as heterozygotes. Attention turned to the root with papers from Kyotake Okada (Okazaki) and Tobias Baskin (ANU Canberra). Okada analysed root responses to physical stimuli (light, gravity, obstacles) and argued that the auxin polar transport system is probably involved in all these processes: transport inhibitors phenocopy several mutants and some mutants are allelic to previously isolated auxin-resistant mutants. Baskin illustrated three strongly temperature-sensitive mutants whose roots are wild type at 18°C, but undergo pronounced radial swelling at 31°C. The defect probably lies downstream of the ethylene response system that causes less marked swelling: silver salts inhibit the ethylene response, but not expression of the mutant phenotype. Rich Meagher (Georgia) described the 11 member actin-gene family and identified functional,

developmental and phylogenetic constraints that could lead to the conservation in divergent plants of various classes of actin. The complex phenotype of ttg mutants was described by Amanda Walker (Cambridge) who speculated that the best bet is that it encodes a transcription factor.

Photomorphogenesis was discussed by Ronald Susek (San Diego) and Xing-Wang Deng (Albany). Susek considered the mechanisms that coordinate the expression of nuclear and chloroplast genomes. He described mutants in which nuclear genes encoding chloroplast proteins continue to express even after chloroplasts have been photo-oxidatively damaged. Deng described seven mutants that show some or all of the features of light-grown plants even when grown in the dark. The first of these mutants at least is epistatic to five phytochrome-defective mutants and none are allelic to the det mutants of the San Diego group. Jerome Giraudat (Gif-sur-Yvette) described some physiological features of three abi mutants insensitive to abscisic acid. According to the particular mutant, there may be changes in seed dormancy, water relations or inhibition of the production under water stress of short, swollen lateral roots (drought rhizogenesis). Tony Bleecker (Madison) described aspects of leaf and floral meristem senescence and the first results of a mutant screen to identify beat-the-reaper (btr) mutants with impaired senescence!

The meeting concluded with three talks dealing with cloning strategies that highlighted points made in a number of earlier talks. Renate Schmidt (Norwich) summarised progress in building an ordered library of YACs. While coverage is uneven, over 30% of the genome is probably covered by YACs that hybridise to RFLP markers, but she cautioned that filling in the gaps by walking was a much slower process. Larger inserts, such as those in the YAC library of Ecker, are attractive because they reduce the number of such steps. A similar note of caution regarding walking had been sounded by the previous two speakers in noting that they had yet to clone the genes involved in abscisic acid and ethylene responses on which they were working. Giraudat described the fine mapping that accurately defines the region around the abi locus and identifies in that region a transmembrane Ser/Thr protein kinase that unfortunately does not seem to be the product of the abi locus! Schmidt and, in the following talk, Pascual Perez (Aubiere) described transposon tagging using the maize *Ac* element. Clipped versions and constructs driven by a strong promoter increase the frequency of excision. Both reported some obvious visual phenotypes among the plants and were looking to confirm that these were due to the transposed element. Ken Feldmann (Tucson) described

> "A high proportion of the genes cloned in the near future from mutants will probably come from this strategy."

insertional mutagenesis by T-DNA and the high probability that the gene is properly tagged if the kanamycin resistance marker and mutant phenotype show Mendelian segregation. The chances of true taggging vary, however, between different phenotypes -- good for dwarfs, bad for ethylene-related. A slide identifying a small army of collaborators screening for most obvious categories of mutants suggest that a high proportion of the genes cloned in the near future from mutants will probably come from this strategy. Feldmann, however, did not believe that saturation was within reach (certainly not by his efforts!), but felt that, where many genes were involved in a pathway, T-DNA tagging would efficiently clone some of them. Mary Skotnicki (ANU Canberra) described efforts to obtain virus tolerant mutants from her own seed transformation programme.

In the period since the Vienna meeting some 15 months ago, genetic analysis has clearly proceeded rapidly in many areas and temperature-sensitive mutants and screening seed from individual M1 parents represent viable (if labour-intensive) solutions to mutating essential genes without lethality. Large groups of genes are being identified in many areas and used to generate hypotheses about the structure of control pathways and the interaction between different stimuli. The emerging picture regarding flowering and embryonic pattern formation in particular clearly show the potential of available methods. The air of expectancy regarding chromosome walking that prevailed in Vienna as a number of groups closed on their mutant loci appeared to have dissipated somewhat by Canberra; many walkers have set out, but no one has yet arrived. The next *Arabidopsis* meeting will hopefully provide reassurance that these walks will be rewarded and give a better idea of the likely impact of T-DNA and transposon tagging.
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American Phytopathological Society Meeting: St. Louis

From Eric B. Holub...

"Gateway to the Future"

A QUESTION which must linger in the back of all our minds is: how do scientists and other members of the public view the attention being paid by so many biologists to research of a wild crucifer? It's not easy to find an answer, but my recent trip to the States, including the American Phytopathological Society meeting in St. Louis, provided an opportunity to sample current sentiment.

As usual, the meeting was large and well-attended. Numerous concurrent sessions were held throughout the four-day meeting. However, there was not a cohesive theme apart from "Gateway to the future." Topics ranged from a colloquium on signal-transduction to discussions on how global change may affect plant health. By far the most thought provoking session I attended dealt with the effects of pathogens in natural forests. Attention focused on a tremendous challenge which exists in determining what rôle humans should play in managing whole ecosystems; a major difficulty stems from disparity in what a "whole ecosystem and natural forest" is in the minds of the public.

Conference Reports

Arabidopsis pathology had an official debut on the final day in a symposium devoted to the subject. Alan Slusarenko, a man whom I have looked forward to meeting for over a year, led the presentations with a talk about fungal pathogens and he highlighted the growing prospects of research using *Peronospora*. Five of the eight speakers described the steady progress being made with bacterial systems. There were few surprises: much of what was presented has either been or soon will be published. However, there was sufficient evidence from bacterial systems to alert plant pathologist to potential breakthroughs in elucidation of the molecular basis of host/pathogens interactions. Unfortunately, the symposium failed to attract a diverse audience. Most attendees already knew and accepted the virtues of *Arabidopsis* and many were probably practitioners.

Why did the heralded "model plant" fail to draw a more diverse crowd? *Arabidopsis* by no means lacks appeal to a general audience including most plant pathologists. The poster I constructed for the meeting proved indispensable as a tool to test the water. It featured a 68 cm enlargement of a 10-day-old seedling colonised from the infecting oospore of *Peronospora* on the tap root to asexual sporulation on the cotyledons; a SEM cross-section of a leaf and pustule of *Albugo*; and a 50 cm enlargement of a leaf packed from petiole to leaf tip with oospores of both fungi. Before and after the meeting, I showed the poster to teachers, professionals with limited training in biology, and assorted friends and relatives. Without prompting, they began asking sophisticated questions about parasitism in plants, chromosomes and genes, and breeding plants for disease resistance (now my parents understand why I moved to the U.K.). Plant pathologists gave the same response at the meeting. Many of the people I spoke with had already heard about *Arabidopsis*, but did not appreciate it's importance. I suspect that what a wider audience needs are well chosen illustrations of biological research to capture their imaginations. Words alone will not suffice.

Arabidopsis research is at a crossroads. It has outgrown its "buzz word" status, and it now needs to settle in to mainstream research. Along with this transition, I hope in future planning of large professional meetings that research on *Arabidopsis* is integrated into regularly scheduled symposia, instead of a special session focused exclusively on *Arabidopsis*. If given a choice between a symposium about an inconspicuous weed and one about their own specialised interests, scientists usually choose the latter. However, when the fruits of our labour begin to blend in, it will shatter any false perception that remains about *Arabidopsis* as simply an alternative for those attracted to a cute plant and high-powered technology.

From Jane Parker...

In August, I attended the American Phytopathological Society meeting in St. Louis, Missouri where 1800 conferees converged to present and discuss a diverse range of plant pathological topics. A large portion of the conference was devoted to applied and commercially-based projects, but there were several very interesting sessions dealing with pathogen, in particular bacterial, molecular biology, plant signalling mechanisms in relation to resistance and susceptibility, and novel approaches to cloning plant resistance genes. The highlight for weed devotees was a workshop on *Arabidopsis* pathology organised by Keith Davis of Ohio State University. Disappointingly, it was poorly attended and one sensed that the *Arabidopsis* 'WORD' had not quite spread to the more traditional corners of plant pathology. However, it was an extremely useful forum for discussion among the converted and I felt that tremendous progress had been made in establishing model pathosystems in *Arabidopsis*-fungal and -bacterial interactions. Also, Anne Simon of the University of Massachusetts, Amherst, introduced us to resistance in *Arabidopsis* to turnip crinkle virus.

> "Tremendous progress had been made in establishing model pathosystems in *Arabidopsis*-fungal and -bacterial interactions."

Developments in *Pseudomonas-Arabidopsis* interactions were described by Roger Innes (formally in Brian Staskawicz's lab, Berkeley, and now at Indiana State University, Bloomington) and by Mike Mindrinos (of Fred Ausubel's lab, Mass. Gen. Hosp., Boston). A major thrust of their approach to analysing pathogen recognition is to generate plant mutants which are altered in their response to particular defined bacterial avirulence genes (see Roger's contribution to the last newsletter). In this way it is hoped to dissect our genes operating in common signalling pathways as well as the specific genes governing resistance. In this respect, the use of deletion mutagenesis was discussed and Mike Mindrinos is working to improve a protocol for genomic DNA subtraction to isolate unique sequences in *Arabidopsis* and in closely related bacterial strains. Improvements in the efficiency of screening for plant mutants that no longer recognise bacterial avirulence genes were also described. For example, large numbers of plants can be quite rapidly inoculated with leaf-spotting bacteria by controlled vacuum infiltration of seedlings or by dipping plants into a bacterial suspension containing a small amount of surfactant. These advances are essential to screen sufficient numbers of plants in deletion-mutagenised populations.

Finally, Ray Hammerschmidt from Michigan State University at East Lansing described the structural analysis of purified arabilexin, a phytoalexin compound which accumulates in the leaf in response to attack by an avirulent bacterial strain. It has an indole ring attached to a thiozole ring and is very similar to the previously characterized brassilexins isolated from *Brassica* plants.

Altogether an interesting, productive and not too humid trip.

AFRC PMB Meetings

The general (non-*Arabidopsis*) "half" of the AFRC PMB Programme will hold their final meeting at St. Catherine's College, Oxford from 20-23 September, 1992. A reminder also that the equivalent meeting for the *Arabidopsis* part of the PMB will be Programme will be held at UEA, Norwich from 13-15 July.

From Richard Price...

How Can We Get More Young People Interested In Plants?

THE IMPORTANCE of plants in the global economy is increasingly being realised by politicians and other decision makers, and we face difficult plant science problems today. These include the effect of global warming on plants especially on crops, the conservation of habitats and of germplasm, the problem of how to feed a projected world population of 10 billion by the year 2100 and decisions about the release of genetically engineered plants into the environment.

Science education has a vital role to play in cultivating a positive approach to these problems. However, many young people think that plants are boring. Few of them understand the importance of plants and only a very small number consider a career in plant science.

The Science and Plants for Schools (SAPS) programme has been set up in the UK to work with school teachers on the development of exciting practical work in plant science. SAPS is funded at present by the Gatsby Charitable Foundation, which is one of the Sainsbury family charitable trusts.

The SAPS programme:

- Is working with science teachers to develop practical investigations in plant science for the National Curriculum and at 'A' level.
- Runs workshops for teachers which show, through hands-on practical work, how teachers can use plants to support exciting science teaching.
- Offers secondment to suitably qualified and experienced teachers so that they can help to develop curricular material for plant science. The seconded teachers also help to organise and run workshops.
- Has developed an educational kit which schools can use to grow a rapid cycling variety of *Brassica campesfris* (syn. *rapa) L.* These remarkable plants, which were produced by Professor Paul Williams at the University of Wisconsin-Madison in the USA, complete their life-cycle from seed to seed in just five weeks. The plants are suitable for studies of plant reproduction, genetics, growth and development, nutrition, evolution and ecology and are being grown in well over one hundred schools in the UK.
- Sponsors selected schools which, in exchange for a grant towards the cost of building a light bank and a free kit for growing rapid-cycling *B. campestris*, are asked to work on specific areas of interest in plant science and to provide feed back to SAPS.
- Publishes a regular Newsletter containing ideas for interesting practical work in plant science, a forum for the exchange of ideas. A forthcoming issue contains an interview with Dr Mary Anderson, Director of the Nottingham *Arabidopsis* Stock Centre.
- Is forging links with universities, colleges and other plant science research institutions in order to provide a channel through which science teachers can have access to information on the very latest discoveries and techniques in plant science.

One junior school teacher writes, "The brassicas have captured the imagination of decidedly mixed groups of ten and eleven year old juniors in a way I had not thought possible. By popular request the children are repeating the work this term with their own self-harvested seeds in order that they can try further investigations of their own devising. Mustard and cress have never had this impact!" A high school teacher says, "The bright lights and yellow flowers were marvellous publicity. The biology staff were particularly impressed and are using 'fast plants' for the Applied Genetics option. The outcome is considerable enthusiasm for plants and a number of students are using the plants for their projects."

> "An *Arabidopsis* mutant with larger flowers would...open the way to some practical genetics in schools."

SAPS links with universities and research institution include, of course, the exciting research currently under way with *Arabidopsis* and we are looking at ways in which schools and colleges might undertake work with this plant. However, there are some problems, the flowers and especially the seeds, are very small. An *Arabidopsis* mutant with larger flowers would make emasculation and pollination easier and open the way to some practical genetics in schools.

In the meantime, if you have any ideas for interesting practical work in plant science which schools might undertake, please get in touch with me. But do not forget your suggestions have got to be cheap and reliable. In 1990, the Education Committee of the Royal Society carried out a study on the equipment resources needed by secondary schools to teach science to all their students, aged 11-16 years. Based on a total of 180 pupils per year over 5 years, they estimated that schools would need £8.86 per pupil per year, just for science equipment. Many school science departments get much less than this.

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Are you sure you've chosen the correct direction?

From Elucius...

Arabidian Kitchen

(third in an occasional series)

WELL POP-PICKERS, winter approacheth and Elucius has been cooking up a delicate soup for your enjoyment.

Arabidopsis soup.

Three good handfuls of *Arabidopsis* leaves; one onion; 1 lb. potatoes; 1 oz butter; 1½ pints of stock; cream.

Heat butter in pan and add finely chopped onion. Cook for five minutes and add ⅔ of *Arabidopsis* leaves finely chopped and potatoes well sliced. Stir for two minutes and add stock. Season well and simmer for 15 minutes until potatoes are soft. Add rest of *Arabidopsis* (coarsely chopped) for last 2 minutes. Liquidise and reheat carefully. Add cream just before serving. Decorate with an array of mutant flowers! Happy cooking Elucius.

This Issue's Quote

THE PREVIOUS issue's quote: "Few organisms have yielded such a wealth of scientific information as has come from this plebeian weed." was recognised by Richard Williamson, from Canberra, who in a brief break from organising the highly successful Robertson Symposium (see his report on this meeting on page 22 of this newsletter) had time to check through rare sources to correctly reveal that the plant in question was, *Datura*. Once he lets the ACM know which Beano Annual he wants, his prize will be sent to him. No competition this time, but a quote from Socrates.

"There are two birds in the tree of life. One eats. The other watches."

Thanks to...

- Barrie Allen for offset-litho printing this newsletter.
- Mary Anderson for the Nottingham update.
- David Baulcombe for his suggestion of the poem.
- Jon Clarke for help with for photocopying and collating.
- Jeff Dangl for the Köln update.
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- Elucius for the cookery column.
- Joan Green for the Current Awareness List.
- Eric Holub for his conference report.
- Natalya Klueva for the cartoon.
- Jane Parker for her conference report.
- Richard Price for the SAPS article.
- Black Rot for the crossword.
- Renate Schmidt for suggestions & proof-reading.
- Randy Scholl for the Ohio State ad.
- Richard Williamson for his conference report.

T-shirt Photo Competition

LAST YEAR, many of you had the good sense to purchase A.F.R.C. P.M.B. *Arabidopsis* Programme T-shirts. Chris Somerville recently sent Caroline Dean (whose executive penthouse office lies very near to the Newsletter's suite of offices) pictures of him and Shauna Somerville wearing these sacred items in the glorious setting of the South Seas.

This arrived soon after Pat Heslop-Harrison had sugges to the ACM that he runs a "photo of someone wearing an A.F.R.C. *Arabidopsis* T-Shirt in the most unusual place" competition. Can anyone beat Tahiti? A prize for the sender of the photograph showing said T-shirt whilst being worn in the most bizarre location.

The ACM reserves the right to publish poor-quality photocopies of the winning photo in this newsletter.

Stanzas for Scientists

The assault, reported in the previous newsletter, launched by the current leader of the Sainsbury lab on the ACM's earlier choice of a poem by e.e. cummings so incensed a member of that lab that this person -- who wishes to remain anonymous for fear of reprisals -- suggested the following choice, taken from cummings's *selected poems 1923-1958* published by Faber and Faber:

O sweet spontaneous
earth how often have
the
doting

fingers of
purient philosophers pinched
and
poked

thee
, has the naughty thumb
of science prodded
thy

beauty . how
often have religions taken
thee upon their scraggy knees
squeezing and

buffeting thee that thou mightest conceive
gods
(but
true

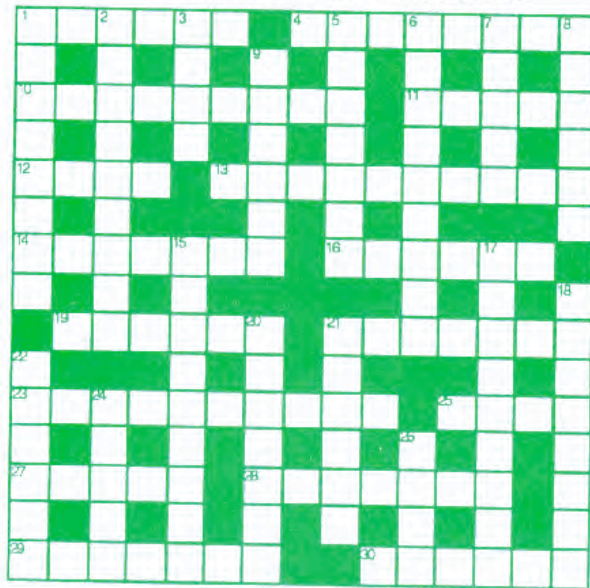
of the incomparable
couch of death thy
rhythmic
lover

thou answerest

them only with

spring)

Arabidopsis Prize Crossword



Clues Across

1. Awful low sin in 1974 (6)
4. Detective inspector surrounds middle east in 1868 (8)
10. Steeple-bearer unwell in 1951 (9)
11,16. Curious to see logger yodel in 1915 (5,6)
12. Paradise in 1955 (4)
13. Primitive insect may well follow (6,4)
14. Board breaks net before fall ? (7)
16. See 11 Across
19. Larval form immured against army ants ? (6)
21. Fishing right: I spy car (anag) (7)
23. Thy tongue, tripe perchance in 1804 (3,7,4)
25. Continent (4)
27. Heath (not Edward!) (5)
28. ____ _ __ count the hours... (Shak. sonnet) (4,1,2)
29. Unstable teetotaller about to toe the line ? (7)
30. Red light area ? (2,4)

Clues Down

1. Bad error in osiered form sounds evil (8)
2. Inhibitor puts pep into broken lutine (9)
3. Killer whale (4)
5. Opposite of outlying (7)
6. Pious, devout (9)
7. Girl, alone, is crazy (5)
8. Chemical compound – one lid (anag) (6)
9. ...Decoration holds tie (3,3)
15. Instrument to bolster AA (anag) (9)
17. The bagman in 1868 (9)
18. Acquaintance of 11,16 Across (2,6)
20. Scram (3,4)
21. Bobby in 1834 ? (6)
22. Coronation, Ramsay or Downing (6)
24. Throw out (5)
26. See 23 Across

At Number Ten

by Black Rot

A grand total of no less than eight correct entries were received for the previous crossword. These included an entry from each of the famous Alison Smiths (the Cambridge Alison is a regular supplier of correct entries and a former winner, the other Alison is a newsletter neophyte from J.I.) and two from AFRC Central Office. After many promises, this is the first time that C.O. has come up with the goods. To recognise this, Sue Riley, who is the new Secretary to the Plants and Environment Board (Penny Maplestone's former position), performed the grand draw on a recent visit to J.I.. Despite her best efforts to ensure a win for her boss, the winner of the Newsletter £5 book-token was **Sue Bunnewell** from J.I. The John Innes site prize, of a bottle of last year's Beaujolais Nouveau, was picked up by **Jim Smith** of the Cambridge Lab., also at the John Innes Centre. Congratulations to the winners and also to the runners up: Mary Anderson (Nottingham), Penny Maplestone & Malcolm Carpenter (C.O.), Jo Ross (Cambridge lab., J.I.C.), Alison Smith (Cambridge), Alison Smith *et al.* (lab. 205, J.I.), and Michèle, Kevin, Donald & Norman (C.O.).

In order to try to keep up the high number of entries, Black Rot has prepared another entertaining, but still relatively simple crossword. The clues, 1, 4, 10, 11, 12, 15, & 23 Across and 17, 21, & 26 Down are all connected by a common theme related to the title. In order to make things even simpler, there are several really easy clues scattered around (The Newsletter Office's Cat even got one) and *some* of the anagrams are labelled as such.

A £5 book-token for the first correct entry out of the draw.

For the few readers of this page that did not finish the crossword, here are the answers to *Sun Special,* the crossword in *For Thale or Went:*

DON'T FORGET!

The deadline for the next newsletter is:
Monday, 3rd February, 1992.