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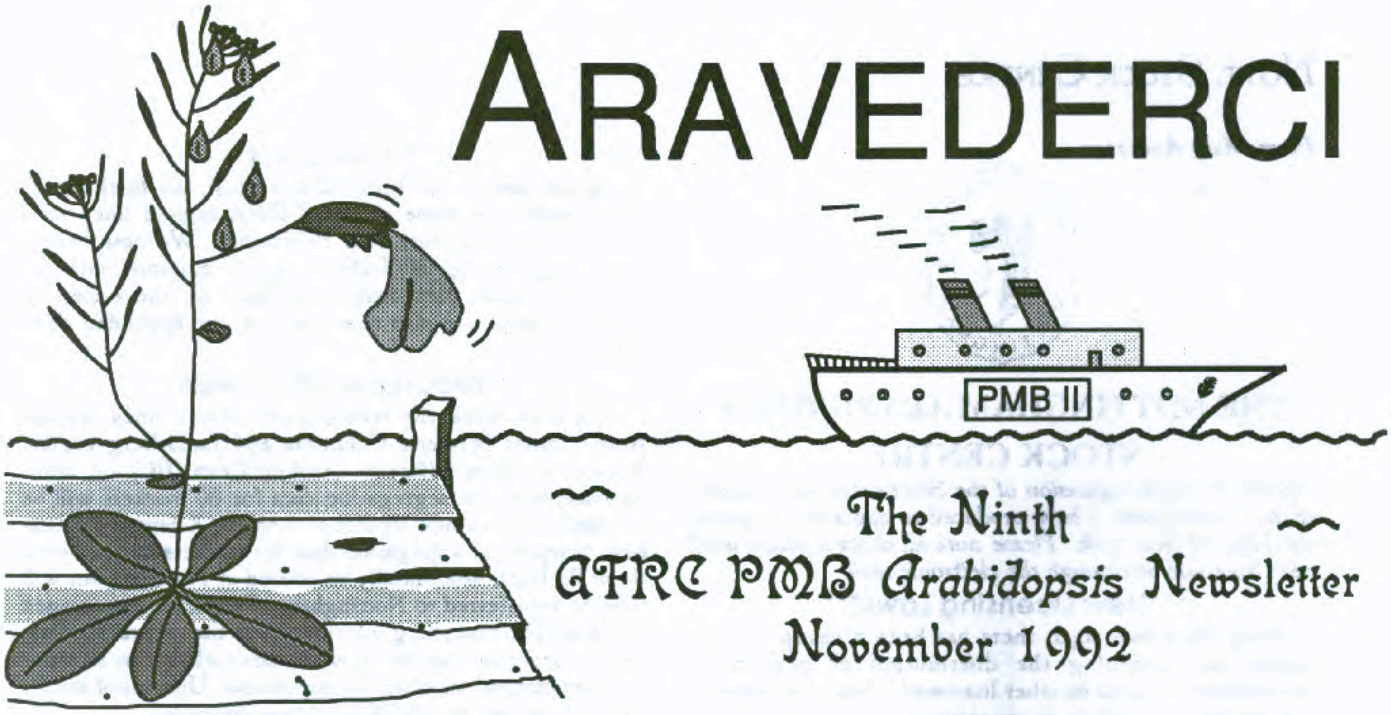

The Ninth AFRC PMB *Arabidopsis* Newsletter November 1992

In This Issue:

This is the final Newsletter of the AFRC *Arabidopsis* Programme.

See Page 3 for details.

Plus – attached:

Aravederci: the ninth AFRC PMB *Arabidopsis* Newsletter, November 1992.
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NOTT. STOCK CENTRE

From Mary Anderson...

THE NOTTINGHAM *ARABIDOPSIS* STOCK CENTRE

Due to the rapid expansion of the Stocks that are available at the Stock Centre, I have produced an appendix to update the June 92 Seed List. Please note all other updates until next June will be through the electronic mail.

New Licensing Laws

FROM SEPTEMBER 1992, there has been a change in the legislation governing the distribution of genetically manipulated material to other licensees. "Licensed material may be sent to persons or organisations who hold current MAFF Licences to obtain and keep such material. The licensee shall send a list of all names and addresses to which licensed material has been sent during the validity of the license. Licensed material may also be sent to persons overseas who have authority from their national Plant Health Service to receive such materials."

In the past we have stated that it is the responsibility of the recipent of this material to have the correct domestic licence. Due to this change in legislation we cannot distribute transgenic material prior to the receipt of a photocopy of your MAFF Licence or, for requests outside the UK, appropriate domestic licence details.

The Kranz Collection

Over the Summer we have continued to characterise the AIS Collection. We now have completed the colour mutants. Most of these are now available for distribution (see the appended Seed List). The remainder of this part of the collection will be available once we have bulked the seed from lines that gave low yields of seed first time round.

Our next task is to go through the ecotypes. These are going to be harvested in two different ways. We shall bulk harvest the majority of plants, but will also take a plant through several single seed generations. The idea being, that for general analysis, *i.e.*, looking for resistance *etc.*, the bulk lines shall be used. Single seeded lines can be used for RFLP analysis.

The final categories of lines to be characterised will be the marker lines and biochemical mutants.

We anticipate that all these lines will be available in the Summer of 1993. Watch the e-mail for updates.

The catalogue of photographs of the mutant stocks is now taking shape. We are investigating the cost of having high-quality colour photocopies made of the catalogue that will be available at cost. We need to assess the demand for this so that we can estimate how many to print. Obviously, the more we have done the cheaper the production costs. I estimate there will be a reasonable demand for photocopies of individual lines. Please let me know if you anticipate that you may require any of these services.

T-DNA Lines

As you saw in the June '92 Seed List, we stock the T-DNA pools and some of the T-DNA tagged lines with visible mutations from Ken Feldmann. We now have a much larger range of T-DNA tagged mutants with the arrival of seeds previously advertised on the e-mail by ABRC. These are also described on our appended Seed List.

Recombinant Inbreds

We now stock the recombinant inbred lines derived from a cross between Columbia and Landsberg *erecta*, donated by Clare Lister & Caroline Dean (JIC). A mini-database with the segregation data for 60 markers will be available with the next update of AAtDB. A central RI database, carrying all segregation data for markers mapped onto these RI lines, will initially be housed at Norwich, but will soon be transferred to Nottingham. People are encouraged to submit their mapping information to the central RI database in order to map the new markers relative to all other markers mapped onto this RI population. Updates of the RI map will be sent to AAtDB at regular intervals.

Nottingham Links with Ohio

ABRC have now started to characterise the American collection of George Redei. In time this will be transferred to us. The Ohio team also continue to establish the *Arabidopsis* Information Management System (AIMS). We shall be putting on a joint presentation at the Genome 1 meeting in San Diego Nov 9 -11 1992.

New Donations

Many thanks to the people who have donated seed to the Stock Centre. It is very encouraging to see new mutants *etc.*, being donated as they are published, or in some cases, prior to publication. Thanks to Ian Bancroft, Caroline Dean, Ken Feldmann, Ian Furner, Maarten Koornneef, Clare Lister, Paddy Maher, Keith Mitchelson, Kevin Pyke, Jirina Relichova, William Sinclair, Alan Slusarenko, David Smyth, and Jan Zeevart.

Registering with NASC

If you have not already done so, may I suggest that you register with NASC. This will place you on our mailing list for information. **N.B. this is the LAST NEWSLETTER**, so you will not be getting information updates through this forum in the future.

If you require any further information about the Stock Centre, I can be contacted at the address below, by direct telephone line, fax, (**PLEASE NOTE THE NEW NUMBER**) or e-mail.

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Final Newsletter

THE DAY HAD TO COME, and it has. The ACM is extremely sorry to announce that this is the final Newsletter of the AFRC *Arabidopsis* Programme. This is because the three-year PMB I, of which the *Arabidopsis* Programme was a part, has come to an end. As announced in the previous newsletter, the momentum built up in PMB I will be continued in AFRC's recently-started PMB II. Owing to funding constraints, however, PMB II has fewer grants and, unlike its predecessor, is not divided into a "general" and an *Arabidopsis* half. As there is not a discrete *Arabidopsis* Programme, there will be no more newsletters.

The good news is that the unofficial U.K. *Arabidopsis* club, which the newsletter has helped to develop over the past few years, will not die with the demise of the newsletter. Caroline Dean intends to carry on co-ordinating those working on *Arabidopsis* in the U.K., who wish to maintain these links, in a slightly different fashion. Important details about this are given below.

The ACM thanks everyone who has contributed to the newsletter during the past three years -- particularly all those regulars from outside the *Arabidopsis* Programme. Without everyone's efforts, the newsletter would not have existed. He hopes you have found it useful and that in four months' time you might feel a slight loss as the familiar brown envelope no longer drops onto the doormat of your lab.

Hold on to your old copies. Who knows? They may become collectors' items.

U.K. *Arabidopsis* Co-ordination

AS ANNOUNCED ABOVE, this is the final A.F.R.C. *Arabidopsis* newsletter in hard-copy form. However, although there is no formal *Arabidopsis* programme within PMB II, Caroline Dean intends to carry on "co-ordinating" those *Arabidopsis* researchers that wish to continue as part of what has become in effect the U.K. *Arabidopsis* Club. It is important to do this in order to sustain the momentum built up by U.K. *Arabidopsis* researchers, both within and outside the A.F.R.C. Programme, over the last three years. This is particularly so with dissemination of technologies and materials from the John Innes site to *Arabidopsis* researchers elsewhere in the U.K. The co-ordination will, however, be somewhat differently slanted from that of the first *Arabidopsis* Programme.

The most noticeable change is that, in place of the newsletter, an U.K. *Arabidopsis* audited electronic bulletin-board will be set up (ARAB-UK). This will be managed by the A.F.R.C. computing section. It will be for use by U.K. researchers as a local newspaper ensuring good communication and interaction between U.K. labs and Norwich. Mapping experiments / cosmid screens / mutagenesis screens / *etc.* in progress at Norwich or elsewhere, will be advertised so that interested parties can visit and participate. ARAB-UK will also enable people to post questions about such things as: techniques, experiments, *etc.* which they may be reticent to put to global scrutiny on the international bulletin board; and will be used as a forum for announcements on U.K. meetings and mini-workshops.

It is hoped to have ARAB-UK set up sometime early in the New Year. If you are an U.K. *Arabidopsis* researcher, whether funded by an A.F.R.C. grant or not, and would like to be involved, please send an e-mail message with your name (or names), research interests, address, telephone and FAX numbers to the ACM at the usual address (ARABIDOPSIS@UK.AC.AFRC.JII). (If you have already replied to an earlier, similar request sent out by the ACM, you do not need to respond again.) Please note that all co-ordination information from Christmas on will only be sent out by e-mail.

ACM "Released" As Promotion Hopes Dashed

AT THE TIME OF WRITING, the ACM, whose funding terminates at Christmas, is desperately seeking gainful employment. And so if you know anyone who wants a second-hand scientific co-ordinator, with a previous existence as a cell-biologist, please let the ACM know at the usual address, as soon as possible. At one point it did look as though he would be given a position within PMB II, with promotion from Assistant to the coveted post of Associate Circulation Manager. In the end, however, this turned out not to be and the ACM, together with the newsletter office cat, are soon to be on the streets.

The ACM was last seen heading for an interview as science and wrestling correspondent of the Sunday Sport. Although rumour has it that the cat is the favourite candidate for the post.

The *Arabidopsis* Biological Resource Center at Ohio State

FOR READERS IN the U.S. and Canada, the following is a brief summary of recent announcements from Randy Scholl:

Orders for seeds and DNA are now being filled at the *Arabidopsis* Biological Resource Center at Ohio State University. Five general classes of stocks are available from the Center, as described in the April 1992 Seed and DNA Stock List. Included are: (i) mutants, (ii) marker lines for genetic mapping, (iii) ecotypes, (iv) RFLP bacteriophage clones, and (v) RFLP cosmid clones. All are available for immediate shipment. The Grill & Somerville and Ward YAC libraries are already being distributed and other clone libraries will be available in the near future.

At present, the above stocks are being provided free of charge. Orders are accepted by mail, FAX, or electronic mail to: The *Arabidopsis* Biological Resource Center at Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA. FAX: +1-614-292-0603.
e-mail (information): arabidopsis+@osu.edu
e-mail (seed orders): seeds@genesys.cps.msu.edu
(type "stockorder" on the subject line)
e-mail (DNA orders): dna@genesys.cps.msu.edu
(type "stockorder" on the subject line).

From Fred Lehle...

New EMS-mutated Seed

LEHLE SEEDS has just produced three new batches of EMS mutated M_2 seeds in the *Arabidopsis* backgrounds of Columbia, Landsberg *erecta*, and RLD. We are very pleased with the quality of these seeds and encourage you to give them a try.

All three batches have been scored for the frequency of albino embryo mutations in M_1 siliques. P-values, the frequencies of initial cells with albino embryo mutation were calculated according to Mednik (Mednik, I.G., (1988) *AIS* 26, 67-72) and are shown below. P-values are based on the scoring of about 250 M_1 siliques from about 50 M_1 parents for each of the three backgrounds.

These new M_2 seeds have been extensively cleaned and, as in the past, have been bulked by parental groups of about 6,250 M_1 parents per gram. Parental group samples are packaged using our new double-barrier pouch-system to provide the ultimate in seed quality protection and convenience in handling.

Cat No.	Description	P
M2E-1A-2	Columbia EMS M_2	0.41
M2E-4-2	Landsberg erecta EMS M_2	0.54
M2E-7-2	RLD EMS M_2	0.30

If you would like more information concerning these new M2 seeds kindly contact us by mail, FAX, or phone at our new address below. Thanks for your support.

Lehle Seeds, 3819 South Evans Suite 307, Tucson, AZ 85714 U.S.A. Tel: +602-889-3339 (new); FAX: +602-889-3403 (new).

From Hong Ma...

Mutant Information Request

THE FOLLOWING was placed on the *Arabidopsis* bulletin board:

Dear Arabidopsis biologists: Elliot Meyerowitz and I are putting together a list of Arabidopsis mutants and variants. If you have unpublished information or paper in press about new mutants, new alleles of previously defined genes, new information about existing mutants, including ,but not limited to map positions, phenotypes, growth requirements, and molecular data such as clones, nature of mutations, please send them to me by mail or by e-mail, if you don't mind the info to be included in our list. We are trying to find all available information and to assemble an as complete a list as possible. Other genetic variants such as different ecotypes, inbred lines and so on are also welcome.

Please be sure to include the method of mutagenesis if the mutants are new. Also list all available references. If you find a reference on existing mutants in recent journal which may not have been entered in the reference databases yet, I will also greatly appreciate your letting me know about it.

Thank you very much in advance for your cooperation.

Hong Ma; Cold Spring Harbor Lab. 1 Bungtown Rd., Cold Spring Harbor, NY 11724, U.S.A. Tel: +516-367-8309; FAX: +516-367-8369
MAH@CSHL.ORG

Arabidopsis at The Royal Society

THOSE OF YOU who are readers of *Tatler* will already be familiar with the contents of this article and its accompanying picture, but the rest of you will probably be shocked to read of the explosion onto the London high-society social scene of several notorious *Arabidopsis* parvenu(e)s.

The occasion was the Royal Society Soirée and Open Exhibition, held on 17 and 18 June this year. The object of this annual event is to: "Provide an opportunity, through exhibits and discussion, to promulgate science and its applications to a broad range of visitors from both the scientific community and other sectors, especially industry, commerce, Parliament, civil service, secondary education and the media." In keeping with these noble aims, a feature of this year's event was a display on the noble weed. This was entitled, "*Arabidopsis*: The Weed with a Purpose." It was prepared by the ACM & Caroline Dean from the John Innes Centre, Norwich; and Eric Holub & Ian Crute from Horticulture Research International, East Malling, together with Jim Beynon from Wye College. The specific aim of the display was to promote the work carried out on *Arabidopsis* as part of A.F.R.C.'s PMB Programme. It was one of 15 chosen out of 130 original applicants and was the only display on plants and the only one representing work funded by A.F.R.C. (Special thanks are due here to Chris Leaver for going into bat for *Arabidopsis* on numerous Royal Society committees.)

The *Arabidopsis* exhibit comprised four triangular "islands" of poster panels, which had been expertly made by HRI's Kay Webster, surrounding a central table on which were several hundred *Arabidopsis* plants and an orbital shaker. Kay's experienced eye also came into play with the positioning of the exhibit. Ian, through clever negotiation, had obtained a prime spot at the front of the main hall, but Kay ensured that it became a focus of attention by placing the display at an angle so as to face the entrance. As all the other exhibits, having been put up by scientists, were all orthogonal to the room, the one placed at an angle really stood out. Scientifically, the display was in two linked halves. The JIC portion concentrated on the advantages of *Arabidopsis* and the technologies in use and being developed at JIC to isolate known genes, while the HRI section focused on the identification of previously unknown fungal resistance genes.

The ACM, Eric, Ian and Jim, together with Penny Maplestone from A.F.R.C. Central Office, spent a busy and very productive two days championing the cause of *Arabidopsis* to people ranging from the great and the good to the person just off the Clapham omnibus.

The morning of day one was taken up with the media. Several famous BBC science journalists went trampling through the *Arabidopsis* display in order to get to those exhibits featuring work on humans and animals. The ACM did manage to grab a straggler as she went flying past and thus succeeded in getting a mention on BBC World Service for the *Arabidopsis* exhibit. The afternoon was given over to, it would appear, carefully selected school-students, who proved to ask many of the most taxing questions. A quick change (see below), and it was into the first formal evening for fellows and VIP guests, including the Duke of Kent, ...*continued on Page 25*

From Sue Albini...

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

This final report is a summary of the results of the experimental programme.

The technique of surface spreading plant meiocytes was used to produce two-dimensional synaptonemal complex preparations of *Arabidopsis thaliana*. All the stages of prophase I of meiosis have been examined and it appears that this species may have fewer sites of initiation of homologous synapsis than is observed in other higher plants. In addition to this, close presynaptic alignment seems to be prolonged. At pachytene there are five SCs. The SCs show a constant linear relationship to the bivalents and can be used for karyotype analysis. The three longest SCs can be identified from their relative lengths. The two shortest SCs both carry a nucleolus organising region at or near one end and are similar in length so they cannot be individually identified unequivocally. SC formation is often disrupted at the NOR. Matching the physical map to the genetical map could be done by examination of trisomics at prophase I and/or by *in situ* hybridisation of linkage group specific probes.

The technique of *in situ* hybridisation to plant SC spreads was developed during the course of this programme (Albini & Schwarzacher, (1992) *Genome* **35**, 551-559) and has been applied to meiocytes of *Arabidopsis thaliana*. Using ribosomal DNA as a probe, it was confirmed that there are two pairs of nucleolus organising chromosomes. The application of this technique, at the electron microscope level, to SC preparations of any species has great potential. It will enable the examination of genome organisation relative to the major events of meiosis, that is homologous chromosome pairing and recombination. In addition, very high-level resolution physical mapping will be possible.

Metaphase I of meiosis was examined in the ecotype, Columbia and as previously reported 60% of the chiasmata are located near the ends of the bivalents and 40% between the ends and the centromere.

In tetraploid Arabidopsis at metaphase I of meiosis, five quadrivalents or a mixture of quadrivalents and bivalents are present. In other higher plant species the smaller chromosomes do not form multivalents as frequently as the larger ones and this difference has been attributed to the size differences seen between the chromosomes within a species. However, since many of the small chromosomes of other species are often much larger than those of Arabidopsis this effect is not due to chromosome size *per se* but may be due to relative chromosome size within the karyotype.

> "This effect is not due to chromosome size *per se*."

At prophase I in tetraploid Arabidopsis, homologues pair two by two and show switches of pairing partner as has been seen in other autotetraploid species. In contrast to the Solanacea species, which also have small chromosomes, there is no multiple synapsis of homologues.

Having sweated away working on meiosis in Arabidopsis for three years I can safely say that it is not intractable and I hope that some of the potential that this research has shown can be realised. If anyone out there should ever feel the urge to study meiosis in Arabidopsis please feel free to contact us and we will endeavour to be of assistance.

S.M. Albini, G.H. Jones & J.S. Parker; School of Biological Sciences, University of Birmingham, P.O. Box 363, Edgebaston, Birmingham B15 2TT.
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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.

The intergenic spacer region of the ribosomal DNA repeat unit of Arabidopsis (containing a putative origin of replication) has been cloned intact, and as three EcoRI fragments, into our ORI vectors. These constructs have been transformed into Arabidopsis and several regenerating calli have been obtained from each. The transgenic calli are being used to (*i*) establish a suspension culture and (*ii*) produce transgenic plants from each construct.

We then plan to isolate plant cells in which intramolecular recombination has taken place by two methods. (*i*) Protoplasts will be isolated from suspension cultures and from leaves of transgenic plants and cultured on a medium containing antibiotic selection (hygromycin or kanamycin for the HYG and NEO vectors respectively). (*ii*) Seeds from the transgenic Arabidopsis plants will be plated on similar selective media. The protoplasts should only be able to form calli, and the seed be able to germinate, if intramolecular recombination has occurred and the circular DNA containing the antibiotic resistance gene and the replication origin is replicating.

We hope by the end of this year when the current project finishes, to have demonstated the presence or an origin of replication within the ribosomal repeat unit. Interestingly, the presence of an origin of replication was demonstrated recently in an animal ribosomal repeat using a 2-D gel method.

T.D. Jones & K.W. Buck; Dept. of Biology, Imperial College, London SW7 2BB.
UMBAG01@UK.AC.IC.CC.VAXA

From Neil Butt...

Cell cycle control genes in *Arabidopsis*.

We now have a complete sequence for our ubiquitin conjugating enzyme. Discussions with Richard Vierstra (University of Wisconsin -- who has cloned and characterised about 12 Arabidopsis UBC genes) indicate that the gene is the same as his UBC6 gene. Vierstra's lab have done very little work on UBC6, other than determine its sequence and place it in a small UBC sub-family comprising two other members. The function of the encoded proteins has not yet been identified, although our work has led some way to suggesting a rôle for UBC6. Northern analysis shows the transcript of this gene to be present only in flowers, seed and young »»

seedlings. However, promoter-GUS gene fusion experiments show GUS activity only in specific regions within flowers. We suspect that in normal plants the UBC6 transcript is stored in the seed until required, probably during germination. Analysis of the 5'and 3' untranslated regions and intron sequences of this gene show several elements which may have a rôle in RNA storage, *e.g.*, in the production of secondary structure within the RNA. These structures may protect the message from being spliced and explain why the northern produces a band several hundred base pairs larger than that expected for the mature RNA.

"Repeats direct cell-cycle dependent gene expression in plant cells?"

Specific repeat elements (MCBs) have been found in yeast that are thought to be essential in cell-cycle (G1/S) dependent gene expression. We have been investigating whether these repeats also direct cell-cycle dependent gene expression in plant cells. Band shift assays with nuclear extracts show that plant proteins do bind these repeats. Transgenic plant lines (both Arabidopsis and tobacco) have now been produced in which these elements have been cloned upstream of -60 and -90 35S CaMV promoters and the GUS gene. We have a large number of transgenic plants growing at present, which indicate that the elements do regulate GUS expression. Further studies are underway to determine whether this occurs in a cell-cycle dependent manner. To try and overcome any positional effects due to insertion of the constructs into promoter region, we are setting up transient expression assays. The constructs are being introduced by electroporation into the tobacco cell line BY2, which can be synchronised with aphidicolin.

Other genes are still being analysed, potential suc1 homologues have been identified which cross-react with anti-suc1 antibodies. These are currently being characterised. Our possible cdc25 clone is proving quite difficult to analyse because of the high level of introns that appear to exist. This has made DNA analysis of a relatively divergent gene awkward. Recently, a possible cdc10 gene has been isolated and this is now being sequenced.

Finally we would like to thank anyone who has supplied us with reagents, vectors, libraries, or information which have enabled us to generate these results.

N. Butt, A. Clarke, P. Layfield, J. Burke, A. Moore & F. Watts.
BAFP3@UK.AC.SUSSEX.CENTRAL

From Jeremy Carmichael & Jim Murray...

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

The aim of this project is to isolate plant homologues of genes that are known in yeast and other eukaryotes to have key rôles in controlling growth and progress through the cell cycle. The approach used is by complementation of yeast mutants with a plant cDNA library constructed in a yeast expression vector. Great strides have been made since our last newsletter report.

Most cell division in plants is confined to the early embryo and to limited meristematic regions in shoot and root tips. Other cells have largely ceased dividing, and are found to have exited the cell cycle in either G1 or G2, but may be stimulated to re-enter the division cycle in response to certain stimuli. The two main control points in the cell cycle are at the G1/S and the G2/M boundary. We are particularly interested in the G1/S transition, as several lines of evidence point to G1 arrest being more important in plants. We have therefore decided to concentrate on the transition from the arrested G1 state and the subsequent activation of specific genes required for DNA replication in S phase.

In yeast, it is known that passage through the point in G1 called START when cells become committed to a further round of division, requires the activation of the p34 (cdc2/cdc28) kinase by association with G1 cyclins. For transition through START, G1 cyclins must accumulate to a sufficient level that the cdc2 p34 kinase becomes activated, and G1 cyclins are probably the limiting component in this process. Moreover, G1 cyclins in yeast are regulated at the level of transcription, indicating that control of G1 cyclin transcription is likely to be the ultimate target of many signals stimulating cell proliferation. We obtained a yeast strain from which all three G1 cyclin (CLN) genes have been deleted, and which is absolutely dependent on galactose for growth, since it is kept alive by a copy of CLN3 cloned behind a galactose inducible promoter. We also have available an Arabidopsis cDNA library with cDNAs cloned behind a constitutive yeast promoter. We have used these tools to obtain putative clones for Arabidopsis G1 cyclins. Screens of more than 2×10^6 yeast transformants initially yielded about 70 putative colonies. The plasmids were rescued from yeast back into *E. coli* using a novel rapid technique (Soni & Murray, *NAR* in press). These clones have now been refined into 12 clones in six groups defined on the basis of restriction pattern and hybridisation. The clones have been tested for their ability to complement on re-transformation of the original yeast strain, using a novel micro-manipulation assay to check for the growth of individual cells. We have also shown that some of the clones also rescue a second yeast strain that is temperature sensitive for growth because it is defective in a transcription factor (swi4) required to activate G1 cyclin expression. This is strong evidence that the clones encode active cyclins. Sequencing of the clones, and northern and Southern analysis of Arabidopsis RNA and DNA is currently in progress. Although mitotic cyclins have been reported from plants, these would be the first plant G1 cyclins known.

"This is strong evidence that the clones encode active cyclins."

We also have some biochemical evidence for conserved mechanisms of cell cycle activation between yeasts and plants. After START, the expression of genes required for DNA synthesis in yeast is co-ordinately activated. In *S. cerevisiae*, the sequence required for this activation, and an activity which binds to it (called DSC1; DNA synthesis control) have been characterised.

Experiments were conducted in collaboration with N. Lowndes & L. Johnston (NIMR, Mill Hill) to observe if a DSC1-like binding activity could be found in Arabidopsis extracts. Preliminary results indicate that plant extracts contain a specific binding activity for these sequence motifs, which although 100% conserved between the divergent yeasts *S. cerevisiae* and *S. pombe*, has not to date been reported from a higher eukaryote.

The Plant Molecular Biology and Arabidopsis Programmes were initiated to stimulate interest and research in plant molecular biology. In that, it certainly seems to have been successful. It is, however, unfortunate that the pump-priming exercise could not be adequately backed up to allow the full strengths of the UK research now developing to be exploited. However, Arabidopsis as we know is a short lived plant, so as senescence sets in, we bid a fond farewell to Dave Flanders and our other colleagues and retire to the corner to dehisce quietly for a while. Then pack our bags for the brave new world of Some More New Initiatives and ... pig genome sequencing (?). We haven't seen the T-shirt yet.

Jeremy Carmichael & Jim Murray; Institute of Biotechnology, University of Cambridge. Tel 0223-334754.
JAH.M@UK.AC.CAMBRIDGE.PHOENIX

From George Coupland...

Isolation of the flowering-time gene, CO.

Since the map position of the co locus is known (16.4cM; chromosome 5), we are taking a map-based cloning approach to isolate the gene. Our strategy is to chromosome walk to CO and then use sub-clones from the region to complement the co mutant.

We built a 1.7 Mb YAC contig around four RFLP markers in the co region. At the Arabidopsis meeting in July, we reported the mapping of CO onto 300kb of DNA contained within three overlapping YACs. We decided that this region was too large for complementation experiments and resolved to fine map CO. To achieve this, we required more RFLP markers in the region and more chromosome crossover points that were closely linked to CO.

Firstly, we obtained DNA markers by hybridising nine YAC end probes in the vicinity of CO to the Olzewski cosmid library. Twelve independent cosmids were isolated and three are being used routinely as RFLP markers. Second, we have used phenotypic selection to enrich for recombinants between co and two closely linked markers on either side, lu and alb-2. This approach relies on the fact that alb-2 is lethal when homozygous. Therefore, since lu and alb-2 are on the same chromosome, lu homozygotes are only recovered in the F_2 generation if there has been a recombination event between them. We have screened about 10,000 F_2 plants from a cross of a lu/alb-2 heterozygous line with a co homozygous line and isolated about 190 plants containing chromosome cross-overs between lu and alb2. Of these, 30 recombination events fall into the 300kb region containing CO. Our recent mapping results suggest that CO is positioned on a 150kb sub-region cloned within two over-lapping YACs.

Once we have completed analysis of all the recombinants we will be ready to start complementation experiments with DNA from the region sub-cloned into transformation vectors.

J. Putterill, F. Robson, K. Ingle, S. Dash & G. Coupland.

Identification of *Ds*-induced mutations in *Arabidopsis*.

A two-component *Ac*-based system was used to isolate transposon-induced mutations in Arabidopsis. A stabilised fusion of the CaMV 35S promoter to the *Ac* transposase gene was used to drive excision of a *Ds* element from the streptomycin-resistance gene. The *Ds* was marked with a hygromycin-resistance gene inserted within it. In these experiments, seven *Ds* transformants were used. All of them contain a single copy of the T-DNA at one locus, except one which contained two copies of the T-DNA. To induce transposition, the *Ds* transformants were crossed to the same transformant carrying the transposase fusion. Approximately 10,000 hybrid seed were made. Around 2,500 of these were planted and the F_2 families harvested independently. These families were sown on medium containing streptomycin and hygromycin and seedlings resistant to both antibiotics were recovered and scored for β-glucuronidase (GUS) activity. A GUS gene is present on the same T-DNA as the transposase fusion. The strepRhygRgus- seedlings contain a transposed copy of *Ds* and have lost the transposase through meiotic segregation. The transposed copy of *Ds* should therefore be stable at its new location. At least one of these seedlings was recovered from 450 F_2 families, and in some families strepRhygRgus+ individuals were also collected. All of these were self-fertilised and the F_3 generation screened for mutants.

> "The albino mutation is almost certainly caused by *Ds* insertion."

So far, 200 F_3 families have been screened both on agar and soil. Four families contained individuals showing mutant phenotypes that were not observed in any other family. These four mutants were: a dwarf which segregated 3:1 in one family; an anthocyaninless which was present in all individuals in three F_3 families derived from the same F_2 family; an albino which segregated in 14 F_3 families derived from the same F_2 family; and a curly leaf which appeared in all individuals in one F_3 family.

The albino mutation is almost certainly caused by *Ds* insertion. Five of the 13 F_3 families were derived from GUS+ F_2 individuals and each of these contained variegated individuals. The other nine families segregated no variegateds. The variegated individuals were grown to maturity and produced green siliques which are likely to contain germinal revertants.

The dwarf mutation is very closely linked to the hygromycin-resistance gene inserted within the transposed *Ds*. This mutant phenotype is much more apparent in soil; on agar the mutants are difficult to distinguish from wild-type suggesting that the phenotype might be caused by a form of auxotrophy. In the original F_3 family sown on soil, 37 wild-type and 10 mutants were scored. Seeds harvested from the 10 mutants were ➤

sown on hygromycin-containing medium and all were resistant. Of the 37 wild-type seedlings, 26 were heterozygous for hygromycin resistance and 11 were hygromycin sensitive. The 26 resistants segregated dwarf mutants among their progeny while the sensitives did not.

Similar genetic analyses of the anthocyaninless and curly leaf mutants are in progress. In addition, DNA has been extracted from all four mutants and the molecular analysis of these lines has been initiated.

D. Long, M. Martin, J. Swinburne, J. Goodrich, E. Sundberg, K. Wilson, K. Lee & G. Coupland.

Induction of *Ds* excision by a heat-shock promoter fusion to the *Ac* transposase gene.

A fusion of the soybean hs6871 promoter to the *Ac* transposase gene was made and introduced into Arabidopsis. In total, six independent tranformants that contain the T-DNA at a single locus were analysed. Plants homozygous for the T-DNA were identified in the T3 generation and these were crossed to plants homozygous for *Ds* inserted within a streptomycin resistance gene. Both a simple *Ds* and one marked with a hygromycin resistance gene were used. The F1 plants were grown to maturity and heat shocked. Two heat shock regimes were used. The first of these, called regime B, was 4 hours of heat shock at 42°C (in a cycle of 1 hour on, 1 hour off) three days a week for two weeks from nine days after germination until bolting. The second regime, called C, was 4 hours of heat shock (again in a cycle of 1 hour on, 1 hour off) three days a week for 3-4 weeks from a stage when 4-5 inflorescences were visible and each of these had small siliques until senescence.

After F1 plants had been exposed to regime C, in the F2 generation 19.7% to 57% of seedlings were variegated, presumably as a consequence of excisions which occurred during embryo development, and 0-0.65% were full green, because an active copy of the streptomycin resistance gene had been transmitted in one of the gametes. Non-heat shocked F1 plants were grown as controls and only 0-0.6% of their progeny were variegated and none were full green. Moreover, those families derived from seedlings exposed to heat shock regime B, were very similar to those which had not been heat shocked. The vast majority of excisions identified phenotypically after heat shock regime C are therefore probably due to induction of transcription of the heat shock promoter.

The variegated F2 plants derived from heat shocked individuals varied in their patterns of variegation. They were scored as extremely highly variegated, highly variegated and lowly variegated. These plants were grown to maturity and again some of them were heat shocked under regime C. All of the progeny of two heat-shocked F2 plants (one highly variegated and one extremely highly variegated) were either full green or variegated, and it was therefore assumed that these plants were homozygous for both the *Ds* and transposase T-DNAs. Twenty-nine of these variegated F3 seedlings were grown to maturity and the F4 seeds harvested without heat shocking the F3 individuals. These F4 families were then sown on streptomycin-containing medium. Nineteen of the F4 families contained full greens, at a frequency of 0.9-92% of all seedlings, while the other 10 families showed no full greens. Very few variegateds were detected in these F4 families, presumably because the F3 plants were not heat shocked during embryo development.

The high proportion of full greens detected in the F4 cannot be due to late excisions in the F3 plant, since they were not heat shocked at that stage. We believe that the high proportion of full greens detected in the F4 generation is due to a *Ds* excision event occurring early in development and giving rise to a sector of streptomycin resistant cells in the embryonic shoot apex as it is formed in the developing F3 seed. This has implications for the time of development of the cotyledons and the embryonic shoot apex. For example, the individual which segregated 92% full greens in the F4, was nevertheless variegated in the cotyledons, so the reversion event must cover almost the whole shoot apex, but not the cotyledons.

At present, we and Eva Sundberg, a new member of the lab, are studying the frequency of *Ds* re-insertion after excision driven by the heat shock fusion and are exploring the possibility of using this for transposon tagging.

Lluis Balcells & George Coupland.

From Simon Covey...

CaMV infection of *Arabidopsis*.

Much of our most recent data appeared in the abstracts for the Arabidopsis programme meeting during the Summer. We are still in the process of characterising one of our mutants with an altered response to CaMV infection (Dv1), but our scope for pursuing this beyond the end of the current grant is somewhat limited. Uninfected leaves of Dv1 are different from those of wt plants in that they are pale green with dark veins. We have made thin sections of these leaves and looked at them in the EM to see if they had any subcellular abnormalities. So far, we have found no obvious differences in the chloroplasts or other sub-cellular structures between Dv1 and wt plants. This is the case both for infected and uninfected plants. However, in both types of plants we have found particularly well developed virus inclusion bodies. These structures are characteristic of CaMV-infected plants. They are large electron-dense structures in which most of the CaMV particles in the cell are normally found. They are composed mostly of the gene VI polypeptide, the virus translational trans-activator, but their precise function is not clear. Although it has been variously suggested they function in replication, translation and feedback regulation of the CaMV replication cycle. In Arabidopsis, the inclusion bodies contain large quantities of virions many of which are clustered around the edge of the bodies or around the edge of internal vacuoles. These vacuoles which honeycomb the inclusion bodies, in some cases, have an internal core of unknown function particularly well developed in Arabidopsis.

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From Caroline Dean...

The *Arabidopsis* genome project.

Jo West & Renate Schmidt

The walking efforts on chromosome 4 have resulted in an 1.3 Mb YAC contig spanning RFLP markers 10086, 4564, 326, 580 and 226. This contig involves more than 60 YAC clones from five different libraries. A detailed analysis of these clones showed that at least 20% of the YACs are chimaeric.

Although we will continue chromosome walking in this region of chromosome 4, this approach is too labour intensive to link up one whole chromosome. A chromosome walking step often only adds 50-100 kb to an existing contig, in contrast a marker as a probe on the EG and EW YAC libraries identifies and maps on average 240 kb. Furthermore, in a lot of cases closely linked markers hybridise to the same subset of YAC clones. Using the YUP library (from Joe Ecker), with the bigger average insert size of 250 kb, these cases become even more prevalent. Therefore, we are concentrating our efforts on the use of new markers as probes on the YAC library rather than on global chromosome walking. As a first step, we have used 33 markers mapping to chromosome 4 and the top half of chromosome 5 which were made available to us by Bob Whittier (Japan). We could readily identify YAC clones corresponding to this set of markers in the three YAC libraries tested (EG, EW, YUP libraries). On chromosome 4 -- we included recently the RFLP markers from the bottom half of chromosome 4 in our analysis -- this has been especially fruitful. Using a total of 55 RFLP markers we identified and mapped more than 350 YAC clones.

To date, 11 YAC contigs could be established which span 2 or more RFLP markers and some of these contigs are in the megabase size range.

The genetic control of flowering time in Arabidopsis.

Jon Clarke

Three wild ecotypes and two derived lines of *Arabidopsis thaliana* have been extensively characterised at the physiological, genetic, and molecular genetic level for their flowering time phenotypes. A late flowering locus, fri, segregating between these, has been mapped to the top of chromosome 4. Fri had not been previously identified by mutagenesis. Rick Amasino (Madison), has recently mapped the fla locus to the same region. We hope to show that fla and fri are alleles of the same locus. This will confirm the suggestion that fri is the major locus conferring late flowering in Arabidopsis and is maintained in ecotypes from distant geographical locations. Fri is being fine mapped and a YAC contig within the region will provide the basis for map based cloning.

"fri has been mapped to the top of chromosome 4."

The late flowering phenotype of fri is modified depending on genetic background. The genetic basis for this is currently under investigation. We intend to determine the number of loci involved, the epistatic relationships between them, and their map positions.

In lines containing this dominant locus, the delay in flowering time can be corrected by vernalization, far-red light and GA. A mutagenesis screen of a line containing fri will be conducted by Garry Whitelam (Leicester) to identify mutants which have lost their response to far-red light and possibly GA. Combined with the vrn locus (see John Chandler's report below) we hope to identify common steps in the signal transduction pathway(s) to floral induction.

The mechanism of vernalization.

John Chandler

From a total of 36,000 EMS-mutagenised fca plants (having a late flowering phenotype and responsive to vernalisation), seven "vrn" mutants which have lost the ability to respond to vernalization to varying degrees, have now been isolated. These have been shown to give a 3:1 segregation of early:late flowering plants after back crossing to Ler, thereby showing the mutated locus does not itself cause late flowering, and therefore affects the cold response. All the vrn mutants are recessive, and are being further backcrossed to Ler.

Mapping the vrn locus showing the cleanest segregation during back-crossing has begun using RFLPs: a mapping population has been established by crossing the vrn mutant to a WS ecotype containing an allele of fca. RFLP polymorphisms between Landsberg and WS ecotypes are currently being checked for 30 markers distributed over the genome, before looking for linkage between the vrn locus and RFLP markers in the F_2 of the mapping cross. In case the vrn phenotype is altered by modifiers from the WS background, a second mapping approach of other vrn mutants is being performed, involving crossing them to mutants from each arm of each chromosome, in a double mutant combination with fca (to avoid segregation of fca in the F_2 progeny), and observing co-segregation of the vrn phenotype with a particular mutation.

To understand the pathways involved in the vernalization response of fca, analysis of the vernalization response of double mutants of fca with a series of hormone, starch and lipid mutations is underway. Preliminary data show the kinetics of the response is altered by the presence of gai and abi mutations, even if the degree of response to a full vernalization treatment may not be altered.

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From John Doonan...

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

No final report from John, but see his immunofluorescence protocol -- ACM.

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From Ian Furner...

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

As this is the last issue of the newsletter and the end of this initiative, I would like to express my gratitude to the people who have made the initiative both productive and enjoyable. My thanks to Bernard Mulligan, Caroline Dean & Don Boulter for organising the initiative and for ➳

three good meetings. I would particularly like to thank Dave Flanders (ACM) for producing the newsletter and for unflagging energy and enthusiasm (I still do not understand the bit about the pigeon-toed ballet dancer. Perhaps I had had too much port by then). Now the real report:

The fate map of the dry seed meristem of Arabidopsis is now out (*Development* (1992) **115**; 755-764). A paper describing the first three fasciated mutants by Ottoline Leyser and I will shortly be out in Development. A third paper by Nigel Kilby, Ottoline and I on promoter methylation in Arabidopsis is now out in *Plant Molecular Biology* (1992) **20**, 103-112). In the lab, Joanne Pumfrey and I have been continuing work in three areas: fate mapping, cell autonomy, and mutants.

Fate mapping

A fate map of the dry seed meristem of plants homozygous for the clv1-2 mutation has been made using X-rays and alb1. (Clavata is one of the fasciated mutants that Ottoline studied). The overall pattern of sectors is similar to wild type; short narrow sectors on the early leaves and longer wider sectors on the late ones. In clavata plants, sectors more frequently affect the late leaves and inflorescence, compared to wild type. Unlike comparable sectors affecting wild type plants, late sectors on clavata plants rarely come to dominate the L2 layer of the meristem. Sectors on clavata plants frequently (30%) have small patches of green tissue within albino sectors. These results can be explained by proposing that: (*i*) the enlarged dry-seed shoot apical-meristem of clavata plants contains more cells that will form the late leaves than wild type; (*ii*) During growth the enlarged clavata meristem is composed of more cells than wild type and chance (stochastic) events resulting in the fixation of marked lineages are comparatively rare; (*iii*) Divisions from L1 into L2 are comparatively frequent in the leaf primordia of clavata plants.

We have started to prepare a fate map of the late flowering fca mutation in order to examine how suppressing bolting affects cell fate. So far, 170 albino sectors have been scored.

The alb1 sectors show poor cell expansion and this makes it difficult to score them in the internal tissues of flowers (affected flowers do not open). We have recently been using the yellow T-DNA tagged CS mutant of the CH42 locus to generate marked sectors. X-irradiation of heterozygous seeds results in yellow sectors on the mature green plant. Such sectors are healthy and flowers within sectors are morphologically normal and fertile. We intend to use such sectors to generate a fate map relating the sepals, anthers and carpels. (The petals do not contain chlorophyll and cannot be scored.)

Cell autonomy

A trait is cell autonomous if genetically mutant tissue and genetically wild type tissue show their respective phenotypes in chimeras containing both. For example, alb1 is cell autonomous. Developmental traits often fail to show cell autonomy. We have recently been carrying out some experiments to look at the cell autonomy of the floral homeotic mutation leafy (lfy1). Plants homozygous for this mutation have flowers replaced by shoot-like structures

> "lfy1 is non-cell autonomous."

and have bract-like leaves in the inflorescence. Seeds heterozygous for lfy1 and the adjacent cell autonomous colour marker yi in coupling were X-rayed. So far three yi sectors affecting the inflorescence have been found and all three look morphologically wild type. This result suggests lfy1 is non-cell autonomous and that diffusable "factors" from wild type tissue result in the absence of the phenotype. Perhaps wild type expression of the leafy gene in L1 is sufficient to allow normal development of the lfy1-yi L2 beneath it. This experiment presumes that the wild type alleles of these genes are lost together. We intend to test this presumption by looking at seeds set in such sectors.

Mutants

We have collected a large number of mutants affecting the shoot apex. We have decided to concentrate our efforts on two groups of mutants. They are the short plastochron (shp) mutants and the early flowering (elf) mutants. The three short plastochron mutants found in our screen and a similar line obtained by Nick Harbard are allelic. (Nick's line was described as 'bushy' in an earlier edition of the Newsletter.) A mutant with a similar phenotype obtained by Igor Vizir is being crossed to one of the lines to look at complementation. Currently we are mapping the elf1 gene to morphological markers. The phenotype common to all four lines is the reduction in the time interval between the emergence of successive leaves. The stronger alleles show altered phyllotaxy, fasciation and very low fertility. The five early flowering mutants fall into at least two complementation groups (elf1 and elf2). The mutants produce four to six small leaves and flowers. Four of the five lines are infertile. We are attempting to map the mutation in the fertile line.

Work has been started on the Feldman T-DNA lines. 10,000 plants have been grown to produce families for the seedling screen. One sterile fasciated mutant has been obtained so far. We are currently looking at co-segregation of this trait in heterozygous families. The complete sterility of homozygotes makes the genetics more difficult, but the approach is feasible.

We have enjoyed the initiative and feel it has served its purpose in giving Arabidopsis a good start in the U.K. We hope the interest in and momentum of Arabidopsis research in this country will continue irrespective of the vagaries of fashion and funding.

Ian Furner & Jo Pumfrey; Dept. of Genetics, University of Cambridge.

From Nic Harberd...

Genetic map positions of gai & ga4.

The semi-dominant gibberellin-insensitive dwarfing-mutation, gai, maps to the top arm of chromosome 1 of Arabidopsis, within a region flanked by dis1 (a recessive mutation conferring distorted trichomes) and th1 (a recessive mutation conferring thiamine requirement). Interestingly, ga4, a recessive GA-sensitive dwarfing mutation, maps very close to gai. Previous experiments by Maarten Koornneef yielded no detectable recombinant chromosomes with cross-over points between gai and ga4. In further experiments we have pollinated plants homozygous for GAI and ga4 with gai GA4/GAI ga4 heterozygotes (the GAI ga4 chromosome also carried the flanking marker

mutations an1, dis1, th1 and tt1). The F1 seeds obtained from this cross were planted out. All plants are expected to display the dwarf phenotype (GAI ga4/gai GA4 and GAI ga4/GAI ga4), except for those carrying a wild-type recombinant chromosome (GAI ga4/GAI GA4), which will be tall. (Note that plants containing the reciprocal recombinant

> "...gai and ga4 are distinct loci."

chromosome, GAI ga4/gai ga4, will display dwarf phenotype and thus will not be detectable.) Three tall and 943 dwarf individuals were obtained, giving a map distance of approximately 0.6cM between gai and ga4. The three tall individuals were allowed to self-pollinate and their (F_2) progeny were examined for segregation of the markers (an1, dis1 and th1) flanking gai and ga4. All three F_2 families segregated for dis1. This marker is relatively close to gai-ga4 (approximately 4cM distal of this region). The presence of dis1 in all three families confirms that the current published gene order for the top arm of chromosome 1 (dis1-gai-ga4-th1) is correct. These observations conclusively establish that gai and ga4 are recombinationally separable and are indeed distinct loci. Furthermore, the confirmation of gene order marks significant progress in the genetic and molecular description of the gai-ga4 region.

We are making good progress with the additional projects we have described in previous editions of this newsletter.

Nicholas Harberd, Marie Bradley, Pierre Carol, John Cowl, Jingrong Peng, Marion Rawlins & Paul Sinacola.

From Nick Harris & Phil Gates...

Development of the silique of *Arabidopsis*.

Not very much new to report since all of the results presented at the July meeting in Norwich. Our funding ceased in July and the last week or so were spent 'tidying up', storing tissue blocks, glycerols *etc.* Jacqui Spence completed her M.Sc. thesis during the summer and by the time you are reading this will have started on an AFRC studentship to Ph.D. Lesley Edwards completed some more cloning from the subtractive libraries and since leaving has added Benjamin to their family. Jacqui will be working on 'pod' shatter in Arabidopsis so some of our programme will continue.

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From Pat Heslop-Harrison...

Localisation and characterisation of tandemly repeated DNA sequences in Arabidopsis.

Jola Maluszynska, the 'post-doc' working on the Arabidopsis project, has now returned to Poland to take up a Professorship in the University of Katowice. We plan to continue our collaboration on the molecular cytogenetics of Arabidopsis, and have applied for EEC Look East/Look West grants, particularly to examine modulation of rDNA expression in Arabidopsis (see reports in previous newsletters). Meanwhile, we are filling her position here.

We reported some work with the *A. thaliana* centromeric repeat in previous newsletters. The high copy number sequence pAL1 is located at the centromeres of all ten chromosomes. However, Jola showed that it is not present in any of the other species of Arabidopsis that we have investigated, including *A. griffithiana* which has been alleged to include the *A. thaliana* genome in its ancestry. Now, Gill Harrison has isolated analogous subcentromeric repetitive sequences from *Brassica campestris* and *B. oleracea*. When probed onto the tetraploid derived from these two species, *B. napus* (rape), preliminary results show that one or other of the clones hybridises to the centromere of each chromosome. However, two pairs of chromosomes include both sequences at the centromere, implying either mobility of the sequence or recombination between chromosomes from the two genomes. The important evolutionary implications of this are now being examined. (The technical aspects and pictures of the probing are in press in *Trends in Genetics*).

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From Eric Holub...

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

The project began at a time when the existence of naturally occurring pathogens of Arabidopsis was virtually unknown, so it's not unusual that we set ourselves a realistic objective of identifying any gene for disease resistance to either of two fungal pathogens, and if time permitted, to determine on which of the five chromosomes the gene resided. At the end of three years, we hoped to be proposing the first steps of fine-scale mapping. With hindsight, our initial objective was somewhat myopic as the figure overleaf illustrates. Needless to say, more difficult challenges lie ahead.

The first allele for resistance to *Peronospora (RPp1)* was mapped to chromosome 3 within eighteen months of starting the programme using evidence of linkage to the recessive morphological marker *glabrous-1* (*gl1*). This marker was chosen as an easy means of monitoring a cross between accessions Col0 *gl1* and Nd0, so the linkage with *RPp1* was an encouraging bonus. We have since confirmed its loation relative to RFLP markers. RAPD probes are being selected to add new markers within this region. In addition, at least fifty recombinants between *gl1* and *RPp1* loci have been selected and will be used to reduce the interval between markers flanking *RPp1*.

RPp1 has several interesting characteristics. It is the only allele found thus far which causes a "pitting" »

phenotype which is clearly visible to the naked eye three days after inoculation. The pits typically expand until the entire inoculated cotyledon has become necrotic. However, the reaction stops there so the plant is capable of growing apparently without debilitation. *RPp1* has been found in the East Malling population of Arabidopsis, the same location from which the diagnostic isolate of *Peronospora* EMOY2 was collected. The corresponding allele for avirulence of the isolate to *RPp1* is shared by a second isolate from near Southampton (HIKS1). Such evidence may allow us to speculate about the importance in the wild of at least one of the recognition alleles which we hope to eventually isolate and clone from Arabidopsis.

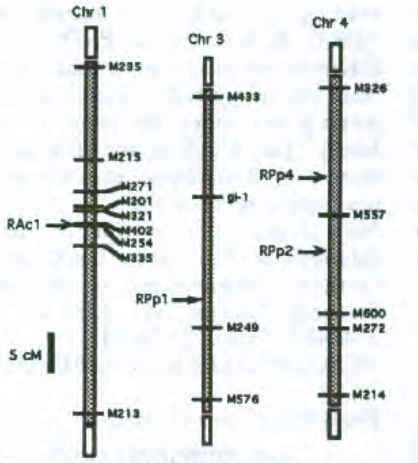

Figure. Probable location of three loci for genotype specific recognition of *Peronospora* (*RPp1, 2, & 4*) and one locus for recognition of *Albugo* (*RAc1*).

"RPp2 and RPp4, were observed to be at linked loci."

Using the same F2 Col0 gl1 x Nd0 population, two additional alleles, *RPp 2 & 4*, were observed to be at linked loci and were subsequently mapped to chromosome 4. These alleles provide an important contrast to the pitting allele because *RPp2* causes a more discrete, non-expanding flecking reaction, and *RPp4* causes a delay and reduction in asexual sporulation. By pursuing alleles which condition different phenotypes, we hope to reveal clues to differences that may exist in mechanisms of resistance.

A single allele for resistance to *Albugo* (*RAc1*) has also been mapped. A cross between accessions Wein (susceptible) and Kes37 (resistant) was used. The allele lies very close and probably within a cluster of currently available RFLP markers. The correct order of molecular markers needs to be confirmed for this cross; and key F4 families and 100 recombinant inbred lines (now at F7) will be analysed to determine the precise location of *RAc1* relative to the molecular markers.

Publications

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From Gareth Jenkins...

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

We are now at the stage of consolidating our work in PMB I and preparing for PMB II. As described in previous Newsletters, we have isolated several mutants with a long hypocotyl in blue light and have started to characterise these. In addition we have continued to study the existing blue light response mutant hy4, particularly with regard to gene expression. One of our main objectives was to isolate new photoregulatory mutants by screening mutagenised seed of various transgenic populations. Although arduous, this approach has proved successful. We have isolated several putative mutants by screening T3/M2 seed of transgenic plants and these are now being characterised. Everyone who works with Arabidopsis will know that obtaining mutants is often not that difficult; the problem is in identifying the most interesting mutants for detailed study. We are therefore trying to obtain sufficient information about our various mutants to decide which to focus on in PMBII.

Some of the recent work on the long hypocotyl mutants has been carried out by a very capable summer student, Shona Robertson. We would like to thank the Gatsby Charitable Foundation for supporting Shona.

For anyone who is interested, some of our work is discussed in a recent conference paper: Jenkins, G.I. *et al.* In: Plant Photoreceptors and Photoperception (Ed. M.G. Holmes & C.B. Johnson), the proceedings of the British Photobiological Society, Cambridge, September 1992; in press.

Gareth Jenkins, Bobby Brown, Jennie Jackson, Morgan Shaw & Shona Robertson; Departments of Biochemistry and Botany, University of Glasgow.

From Kerrie Jones...

Ammonium toxicity in *Arabidopsis*.

At the programme meeting in Norwich we reported on our difficulties in cloning Arabidopsis glutamate dehydrogenase (GDH) sequences. Using a PCR-based approach with highly degenerate primers we were able to isolate GDH sequences from a range of bacterial and fungal species, but were unsuccessful in doing so by amplification of DNA from higher plants.

Since the meeting, we have been screening Christine Raines's Arabidopsis cDNA library with antibodies directed against a mitochondrial GDH extracted from vine-leaves (kindly supplied by Prof. Roubelakis-Angelakis from the University of Crete). This antibody detects a 42kDa polypeptide in western blots of

Arabidopsis whole plant extracts. Our screen yielded 10 positive clones containing inserts varying between 1.4 and 2kb in length. Sequence analysis of the shortest and longest cloned inserts revealed their sequence identity and thus far we have obtained approximately 1.1kb of DNA sequence. Comparisons of the derived polypeptide sequences have not revealed significant homology with any other sequences in the database, so we cannot yet be certain of the identity of these clones. However, the majority of GDH sequences presently in the database are of bacterial or fungal enzymes which utilise NADP, whereas GDH activities reported in higher plants utilise NAD preferentially. Most other NAD-requiring hexameric GDHs show limited homology with the NADP-specific GDHs and, apart from the vertebrate enzymes, show little homology with each other outside strategic regions.

Additionally, we have transcribed and translated the longest cDNA clone *in vitro*. Analysis of the translation product revealed a 55KDa polypeptide. We are currently determining whether this might comprise a GDH precursor by immunoprecipitation with the vineleaf antibody.

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From Peter Jordan...

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

Progress has continued with the purification of 5-aminolaevulinic acid dehydratase and porphobilinogen deaminase from leaves of *Arabidopsis thaliana*.

Both enzymes are essentially pure and proteolytic fragments are being prepared for protein sequencing and characterisation. The sequencing of the hemL cDNA encoding the glutamate 1-semialdehyde aminotransferase is also nearing completion.

Work has commenced on the pea 5-aminolaevulinic acid dehydratase in preparation for detailed structural analysis.

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From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

The aim of this work has been to develop a gene tagging system based on the activation of the gus reporter gene by native gene regulatory sequences. We have used our modified version of the Valvekens root explant system (now published; Clarke, Wei & Lindsey (1992) *Plant Molecular Biology Reporter* 10, 178-189) to generate about 2,100 Arabidopsis transformants containing a promoterless gus gene. We have screened a proportion of these lines (about 400-500) both for GUS activation in diverse organs and tissues, and for aberrant phenotypes. Data on the GUS activation frequencies and range of expression patterns in transgenic Arabidopsis and other species are in press (Lindsey, Wei, Clarke, McArdle, Rooke & Topping: Transgenic Research).

Since we last reported, we have developed primers and reaction conditions to use inverse PCR to clone out T-DNA flanking sequences. This has been successful for several lines that exhibit GUS activation and/or aberrant phenotypes, and we have begun screening a wild-type genomic library for the tagged genes. Arabidopsis has proved to be much more amenable to the IPCR approach than, for example, tobacco and we have generated PCR products of up to approx. 2.5kb using T-DNA sequences as primers. The following lines, which contained single T-DNA copies, are being studied:

A. Vascular differentiation.

1. AtVT-1. This line expresses GUS in the vascular tissue. A developmental study has found that expression begins within 48 hours post-germination in the hypocotyl, and then in the root elongation and maturation zones and cotyledons of the developing seedling. In the older plant, the roots and true leaves only exhibit GUS activity in the vascular tissue, and the stem is GUS-negative. The level of GUS expression declines with age (between day 10 and day 26 post-germination), and is seen in the tapetum of the anther, but in no other floral organ. The 1.2kb IPCR product has been sequenced and has been found to contain the expected T-DNA sequences. This line has a GUS fusion transcript of approx. 3.3kb, and the T-DNA flanking sequence hybridises to a wild-type transcript of approx. 1.5kb (the GUS transcript itself is 1.8kb). We are currently characterising homologous λ clones that have been identified by probing a wild-type genomic library with the IPCR product.

2. AtVS-1. This line also expresses GUS in the vascular tissue, but the timing and pattern during development are different from those of AtVT-1. The 1.2kb IPCR product has been used to identify homologous λ clones.

3. AtVR-1. This line expresses GUS in a more restricted pattern than the other two lines, predominantly in the root vascular tissue. The 1.5kb IPCR product has been used to identify homologous λ clones.

4. AtVT-2. This line expresses in the vascular tissue throughout the plant and, like AtVT-1, also in the tapetum. This, and the following line, are being studied by a new Ph.D. student in the lab, Paul Muskett, who worked on them as undergraduate project student last year.

5. AtVF-1. This line expresses GUS in vascular tissue and also has an interesting phenotype, in which the flowers exhibit homeotic-like aberrations. Not all the flowers on a given individual are aberrant (typically about 50%), but the trait does segregate in selfed progeny. We are carrying out further genetic analyses and are using the 1.0kb IPCR product to screen a genomic library.

Paul will also be screening our transgenic population for mutants in the patterning and function of vascular tissues. ➤

B. Anther development
These lines will be studied as part of our PMBII project, in collaboration with David Twell.
1. AtTAP-1. This line expresses GUS only in the tapetum, and in the homozygous condition is infertile, apparently due to a lack of pollen production, under certain growth conditions (*i.e.*, in our growth rooms). The phenotype therefore appears to be conditional, and our hypothesis is that we have mutagenised by insertion a gene, expressed in the tapetum, that plays a rôle in pollen development. Since no restriction sites were available in the transformant that allowed IPCR of the T-DNA flanking sequences, a sub-genomic library has been prepared in λ ZAPII. Work is in progress to characterise further the mutant phenotype and to isolate recombinant clones, using GUS as a probe.
2. AtPS-1, AtPS-2. Both these lines express GUS in mature pollen (post-microspore mitosis). From one, AtPS-1, a 1.0kb IPCR product has been used to probe a genomic library, and homologous recombinant clones are being investigated.

We hope to characterise further putative mutants in anther/pollen development in the course of the work.

C. Zygotic embryogenesis and seed development
This work is funded by the CEC BRIDGE programme. Two lines show GUS activity in embryos and one in the endosperm. We have also identified putative mutants in embryonic pattern formation, and a *transparent testa* line.
1. AtEM-1. This line expresses GUS in the basal part of the embryo, and expression is initiated prior to the heart stage. In the seedling, expression is seen in the root tip and cotyledons, but becomes more restricted in the mature plant to the root tip and, less strongly, in the silique wall. A 1.1kb IPCR has been used to identify homologous λ clones.
2. AtEM-2. This line expresses GUS throughout the entire embryo at the heart stage, but only in the root region at the cotyledonary stage. In the seedling, expression is seen in the cotyledons, hypocotyl and root tip, but only strongly in the root tip, and faintly in the ovary and stigma, of themature plant. A 2.1kb IPCR product is being used to screen a genomic library.
3. AtEN-1. This line expresses GUS at a low level in the heart-stage embryo and faintly in the margins of the cotyledons of older embryos, but most strongly in the endosperm. In the seedling, expression is found in the cotyledons and root elongation / maturation zones, but in the mature plant expression is restricted to the tapetum. A 2.2kb IPCR product has identified homologous clones in a genomic library.
4. For the *transparent testa* line, a 1.3kb IPCR product has been generated. Before proceeding too far with this line we will carry out crosses with known *tt* mutants to look for allelism.

"This tagging system is valuable for the identification of genes, without the requirement for the generation of an obvious aberrant phenotype."

The demonstration that our 'promoter trap' vector can generate transcriptional fusions, and that cloned T-DNA flanking sequences are homologous to native transcripts in untransformed plants, is consistent with the view that this tagging system is valuable for the identification of genes, without the requirement for the generation of an obvious aberrant phenotype in the transformants. The approach is also useful for identifying genes which may be embryonic lethal or seedling lethal in the homozygous mutant condition, since GUS activity is dominant and detectable in heterozygotes throughout the entire life cycle of the transgenic. Thirdly, the approach can be used to screen for genes that are inducible by environmental, pathogenic or other factors, detectable by activation of GUS activity following the challenging of the plants with that factor.
Keith Lindsey, Mike Clarke, Wenbin Wei & Jennifer Topping; Department of Botany/Leicester Biocentre, University of Leicester.
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From Helen Logan...

Cell cycle control genes in *Arabidopsis*.

We have carried out western blots on cytoplasmic extracts from Arabidopsis, cauliflower and *Chlamydomonas* with various antibodies against yeast cell-cycle proteins. A newly raised polyclonal antibody to fission yeast $p63^{cdc13}$ (cyclin B) recognises bands of c. 60K in all three extracts. The reaction is abolished by presorption of the antibody with bacterially expressed p63. We will use this antibody to screen our λ ZAP cauliflower meristem library.
Helen Logan & Jeremy Hyams; Dept. of Biology, UCL.
(Congratulations to Helen on changing her name from North. And getting married too – ACM.)

From Andy Maule...

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

The work has moved along nicely since the last report and we are now beginning to dissect the complex functions of the CaMV movement protein (P1) using mutagenesis. The functions of P1 that we now have a handle on are: (*i*) the ability to mediate a systemic infection (assayed as local and systemic infection of plants after inoculation with mutants); (*ii*) the binding of P1 to RNA *in vitro* (assayed after expression of the mutated ORF in *E. coli*); and (*iii*) the formation of P1-specific tubular extensions from the surface of infected protoplasts, in a manner reminiscent of the modifications to plasmodesmata seen *in vivo*. This last phenomenon is intriguing. It indicates that P1 has the capacity to polymerise alone or with host proteins (?) into a tubular structure with a potential vectorial or delivery function for the transfer of virus particles to neighbouring cells. Similar structures have been seen by others for cowpea mosaic virus infection of host protoplasts and, in this case, the structures are also formed after transient expression of the respective movement protein in protoplasts.

This strategy will allow us to analyse our P1 mutants in protoplasts without the problems of efficient infections (discussed in our previous report).

Scanning deletion mutagenesis is a strategy based on the view that small (2-3 aa) deletions can affect local domains within a protein without affecting gross protein structure. We have employed this approach to make 20 separate deletions spread throughout P1 to identify domains determining the functions descibed above. Already we can see that the N- and C-terminal domains are not critical for overall systemic infection and that certain specific regions are essential for RNA-binding *in vitro*. These analyses will keep us occupied for some months. We are now, however, identifying a major weakness in our capacity to understand protein structure and function, that is our inability to predict 3-dimensional conformation.

It has been suggested that virus movement proteins could control virus host range and to this end we have made hybrid viruses between CaMV and other related and unrelated viruses, exchanging movement proteins. In some cases these viruses share common hosts in others not. Comparison of the parental and hybrid viruses on the various host plants should show the importance of movement proteins in host range determination.

Well, here we are in full flow on this project and 'chop', no further funding. Richard is now looking for alternative positions, although he will be with us into early 1993. This is Christine's last year for a Ph.D. -- she hopes to find a postdoc position in Germany (a good catch for somebody!). This leaves Carole (the real expert!) and myself (the author!) to pursue all these exciting avenues. More pairs of hands, please!

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From Keith Mitchelson...

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

It's hard to believe that this will be our last report to the Newsletter. I hope there will there be another issue: A Bumper Christmas Issue full of rare gifts? If not, a Merry Christmas to all our readers!

YAC library screening

Elaine Durwood has maintained the YAC library for the last 15 months and has (uncomplainingly) carried out PFGE of YAC chromosomes and transfers to membranes. Ordered pools of 6 or 8 YAC clones are screened systematically (see attached protocol). This fractionation away from host chromosomes is necessary for detection of Arabidopsis fragments carrying VNTR elements as similar sequences are present in the yeast genome. Andy and I have hybridised polymorphic sequences to the YACs. Clones separated by PFGE, display conspicuous (putative) positive clones. The close similarity in sizes of different YAC molecules precludes the identification of individual clones in a pooled clone-PFGE screen. When *individual* YAC clones are similarly PFGE fractionated, positive clones are confirmed. The period has been devoted to finalising the screening of the Grill library. YACs from the last plates have been pooled & subjected to PFGE. The transferred DNA presently awaits probing. The Eric Ward YAC library was obtained from Jeff Dangl, MPIvZ, Köln and the first poolings/membranes are being prepared.

M13 Probe

Andy has identified additional YAC clones carrying DNA sequences that hybridise strongly to M13 repeat. The YAC clones identified by these VNTR markers need to be located to particular chromosomes/chromosomal loci for the utility in mapping experiments. The recovery of the repeat sequence and flanking DNA from YAC clones will restarted soon when he returns from Australia. Positive YAC clones from plates 2 & 5 screened negative for Arabidopsis sub-centromeric pAL1 sequence. The YAC clones identified by M13 or (potentially) 33.15 do not contain chloroplast sequence (Renate Schmidt).

33.15 Probe

I have identified (putative) positives to minisatellite probe 33.15 in half of the EG YAC plates. Confirmation with individual clones has not yet been carried out.

Phage λ library

Andy and I have screened an EMBL4 genomic library using M13 and minisatellite 33.15 probes, respectively. Presently, two M13 positive phage and one 33.15 are being sequenced for the repeat sequence elements. As reported previously, simple repeat probes failed to identify unique phage plaques. At that time, the library was plated using *E. coli* LE392 hosts. The failure may be partially due to instability of repeat sequences in *E. coli*. The library will also be rescreened using different *E. coli* hosts NM621 and NM612 (rec- & mcr-). I will also be looking into improving hybridisation conditions with simple sequence oligomeric DNA's. I have also isolated a number of λ clones carrying what appears to be repeat sequence DNA (not pAL1 or rDNA). I am subcloning these phage and will be looking at their location on rare-cutter fragments of Arabidopsis chromosomes. Polymorphic signals are also detected with Arabidopsis DNA elements produced by PCR amplification of selected oligonucleotide primers. We have screened the λ library for clones carrying these plant sequences. Some putative positive clones have been identified, but await further purification. Also since our last report (AFRC Final Meeting), the Recombinant Inbred Lines (WS x W100F) from Du Pont has been received. Elaine is producing seed to allow DNA production from plants. The generation of new minisatellite alleles in different RI lines may show if minisatellites have a recombinatory rôle in plants.

Andrew Porter, Elaine Durwood & Keith Mitchelson; Department of Molecular & Cell Biology, University of Aberdeen, Aberdeen AB1 9AS.

From Bernie Mulligan...

Genetic male sterility in *Arabidopsis*.

Further γ irradiation experiments are underway. Kriton Kalantidis has irradiated dry ecta seeds with 130 KRad and screened 1,000 M1 pods so far. Of the M2 families, 5.9% →

segregated pronounced chlorophyll mutants including inviable albinos, another 1.8% showed seedlings with light chlorosis, 0.8% of families produced plants with general growth problems and sterility, while, the number you've all been waiting for (Oh really?), 1.2% segregated male sterile plants, which showed no obvious reduction in female fertility or vegetative oddities. Higher and lower radiation doses will be tested over the coming months.

"..started transforming ms1 with wild type DNA clones to test for complementation."

Microscopy progresses: in our msZ mutant, the stamens don't elongate properly and while pollen is formed, it is not viable (see earlier newletters). Greg Briarty predicted problems with tissue organisation within the stamen filament. Preliminary, yet typically elegant, light micrographs produced by Janet Dawson, suggest that he is correct. Mapping continues; msK, msW and maybe msY are probably on chromosome 1, while msH is on chromosome 3.

Keith Lindsey's lab have kindly coached us in their methods for Arabidopsis transformation. Leonid Shlumukov and Rob Ribeiro have started transforming ms1 with wild type DNA clones to test for complementation.

Bern Mulligan, Greg Briarty, Zoe Wilson *et al*; Dept. of Life Science, Nottingham University. Tel: 0602-513236.
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From Jane Parker...

Analysis of two Arabidopsis loci which control resistance to Peronospora parasitica isolate NoCO2.

In January, 1991, Arabidopsis plants of the land race Columbia (Col-0) growing in our glasshouse became infected with the downy mildew pathogen, *Peronospora parasitica*. The fungal isolate is denoted NoCO2 for its origin in Norwich and maintenance on Col-0 plants.

Inoculation of Col-0 seedlings with NoCO2 conidia resulted in rapid mycelial growth in the cotyledons and leaves and abundant asexual sporulation after four to seven days. Pathogenicity tests revealed that Landsberg *erecta* (La-er) plants did not become infected with NoCO2, but exhibited a diffuse necrosis in response to inoculation with the fungal conidia.

Col-0 and La-er are two lines which are well characterised in respect of genetic and molecular markers and were therefore considered to be very suitable in a mapping programme. First of all we studied the inheritance of the La-er resistance by inoculating F_2 plants derived from a La-er x Col-0 cross with NoCO2. There was a 3:1 segregation of resistance to sensitivity which is consistent with the presence of a single locus in La-er controlling resistance to NoCO2. The resistance locus has been denoted RPp5, in accordance with the interactive scheme constructed by Eric Holub & Ian Crute at HRI, East Malling (see previous issues of this splendid organ! Thanks ACM). Interestingly, detailed analysis of F_1 and F_2 plants showed that the resistance locus is semi-dominant in its action.

We were fortunate in being able to use a La-er x Col-0 F_9 recombinant inbred (RI) population generated by Clare Lister & Caroline Dean (Cambridge Laboratory, Norwich) to map RPp5 relative to existing RFLP markers and newly generated RAPD markers. The RIs should allow greater mapping resolution than F_2 populations because more recombination events have occurred to attain homozygosity. Also, an RI mapping population is genetically fixed and therefore represents an inexhaustible supply of material for genetic analysis and cumulative mapping information. Clare's RFLP data on 100 RIs allowed us to place RPp5 on a central region of chromosome 4 and Veronique Szabo and I have been analysing further RIs in order to map its position more closely. So far, RPp5 lies 1cM from the Goodman RFLP probe 3845 and Veronique has identified a RAPD marker which also maps very close (0.5cM) to RPp5. We are now placing further markers on the map relative to RPp5 and we hope soon to be in the position to chromosome walk to the locus using data obtained from the RIs and selected F_2 recombinant plants. We will use aral and cs as flanking genetic markers.

One of the fascinating aspects of the Arabidopsis-*Peronospora* interaction which was first demonstrated by Holub & Crute is the diverse range of reaction phenotypes in different plant-pathogen combinations. Several distinct resistance phenotypes are now known to be due to the presence of different "Resistance" loci presumably interacting with the products of a correspondingly large array of fungal "avirulence" genes.

"This stronger reaction phenotype is the action of a single "R" locus unlinked to RPp5."

We have shown, using various diagnostic tests, that RPp5 in La-er prevents extensive fungal ingress and abolishes asexual sporulation. Fungal haustorial connections are made with the mesophyll cells, but these react adversely and eventually die. Commonly, a trail of dead cells is observed in the path of attempted fungal penetration. The incompatible phenotype of RPp5 is, however, relatively weak compared to some other "R" gene activities. In particular, we have examined the response of plants of the land race Ws-0 to NoCO2. In this interaction fungal ingress is contained more effectively than in La-er and the visible reaction phenotype is stronger. Cell death appears to occur earlier leading to discrete, but intense patches of dead cells surrounding points of attempted fungal penetration. We now know that this stronger reaction phenotype is the action of a single "R" locus which is unlinked to RPp5.

So we have the opportunity to clone two distinct resistance loci as a first step towards understanding their mode of action at the molecular level.

Jane Parker, Veronique Szabo, Brian Staskawicz (on sabbatical from U.C. Berkeley), Mike Daniels & Jonathan Jones, Sainsbury Lab.

From Kevin Pyke...

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

Since the Norwich meeting, we have concentrated on screening the Feldmann T-DNA insertional mutant population for changes in chloroplast number per cell and chloroplast size similar to the cellular phenotypes of arc mutants which we already possess. To date, we have screened 4,000 lines by microscopic examination of leaf tissue from individual seedlings and retained 10 lines for further analysis. We have just started doing the backcrossing and allelic crosses to establish whether any of these lines represent tagged alleles of existing arc mutants. Initial examination of two of these putative tagged mutants indicate their cellular phenotypes appear similar to arc1 and arc2 respectively.

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From Christine Raines...

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

The overall objective of this proposal was to investigate the molecular factors determining the control of development of the photosynthetic apparatus. One aspect of this programme was to study the transcriptional regulation of genes encoding enzymes of the photosynthetic carbon reduction (Calvin) cycle.

One of our first aims was to isolate and characterise the genes from Arabidopsis that encode enzymes of the Calvin cycle namely, fructose-1,6-bisphosphatase (FBPase), phosphoribulokinase (PRKase) and sedoheptulose-1,7-bisphosphatase (SBPase). To this end we constructed an Arabidopsis genomic library and a genomic clone encoding SBPase was successfully isolated using a wheat cDNA clone. Unfortunately we were unable to obtain genomic clones encoding FBPase or PRKase and were concerned that using heterologous cDNA probes may be contributing to our lack of success. We constructed an Arabidopsis cDNA library in the vector λZapII and screening of this using our wheat probes resulted in the isolation of cDNA clones encoding FBPase (Horsnell & Raines, 1991a), PRKase (Horsnell & Raines, 1991b) and SBPase. The corresponding genomic clones for all three enzymes have now been isolated and the upstream regions sequenced. This was all achieved within the first 18 months of this grant whilst Philip Horsnell was in my laboratory, Philip's health deteriorated in June 1991 and sadly Philip died on 30 November, 1991. On the 1 March, 1992 I appointed a new postdoctoral worker, Stuart Hodgetts, and currently he is preparing constructs using the FBPase and SBPase gene upstream sequences for insertion into Arabidopsis. These constructs will allow us to begin to identify regions important in the regulation of transcription of these genes and will also provide useful tools for our new project in which we will investigate the effect of metabolites on Calvin cycle gene expression.

Natalie Gray, my departmental technician has succeeded in producing transgenic Arabidopsis plants using a control construct with the CaMV promoter driving the GUS gene. Having established this technique in our laboratory we are now in a position to produce transgenic plants rapidly when the constructs are ready.

Another goal of this project was to study the expression patterns of the Calvin cycle genes in Arabidopsis plants grown under different environmental conditions. Using analysis of steady-state mRNA levels Nicola Willingham (SERC Ph.D. student) has been studying the effects of light and elevated CO_2 on the expression of the Calvin cycle genes.

The main thrust in the remaining few months of this project will be to define the *cis*-acting elements involved in regulating expression of the FBPase and SBPase genes using functional analysis of promoter deletions in transgenic plants. In parallel, we will also begin to study the *trans*-regulatory protein factors involved by using gel-shift assays and DNA-footprinting techniques.

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From Colin Robinson...

Isolation and analysis of Arabidopsis chloroplast biogenesis mutants.

It's nice to know that some things have not changed during the last three years. The ACM was steeling himself for his usual onslaught of telephone calls to Warwick, when he found, much to his surprise, his first call being answered. A pleasant voice told him that Colin was on sabbatical in the U.S.A. When the ACM enquired as to where the elusive Dr. Robinson was for his sojourn, he was told that no-one knew. A message informing those interested as to his location had been promised, but none had arrived.

Colin Robinson; Dept. of Biological Sciences, University of Warwick.

From Steve Slocombe...

Investigation of temporal and tissue-specific regulation of the stearoyl-acyl carrier protein (ACP) desaturase in *Brassica napus*.

Stearoyl-ACP desaturase carries out the first step in C_{18}-fatty acid desaturation and is an important component of the pathways of membrane lipid and storage oil synthesis. It was established that in the developing embryo the transcript peaks at an early stage of development. A seed-specific gene for the desaturase was derived from *B. napus* and a preliminary promoter analysis was undertaken, including transformation of tobacco with a promoter-GUS fusion construct. Microspore derived embryo cultures from *B. napus* were exploited to distinguish maternal factors from endogenous factors involved in embryo development. For example, the desaturase transcript appears earlier than that for oleosin in this system as observed in zygotic embryos growing on the plant. This project has contributed to the basis for two new projects. The desaturase gene promoter will be used to express antisense RNA against the Acetyl CoA carboxylase message in the rape embryo. This enzyme is an important controlling point in oil synthesis and manipulation of the enzyme levels ➳

in this way is expected to cast light on carbon partitioning in the embryo. A second project will involve the use of desaturase promoter-GUS constructs to examine oil synthesis in Arabidopsis mutants with reduced synthesis and perception of abscisic acid.
Steve Slocombe & Denis Murphy; John Innes Centre, Norwich.

From Alison Smith...

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

Those of you present at Tom Blundell's talk to the Arabidopsis conference in July will have seen him present a 3-D structure of an enzyme protein. This was *E. coli* porphobilinogen deaminase -- or hydroxymethylbilane synthase (HMBS)! Our project has been to isolate and characterise the gene for HMBS from Arabidopsis. As reported in the last Newsletter, Saw Hoon Lim isolated a clone from a genomic library. Since then, the gene has been localised on the insert and 1.7 kbp upstream of the translation start site has been sequenced. By comparison with other light- and tissue- specific promoters, it contains numerous potential *cis*-acting motifs which may be involved in transcriptional regulation. To test their rôle, Saw Hoon has introduced constructs containing various lengths of the promoter (-1705, -1275 and -304 relative to the initiation codon) fused to the GUS reporter gene into both Arabidopsis and tobacco. Despite the fact that the Arabidopsis transformation was carried out one month earlier than that with tobacco, we do not yet have any Arabidopsis material for analysis. Fortunately, rooted explants of tobacco have already been produced, and these have been assayed for GUS. The "full-length" (-1705) HMBS promoter directed GUS expression to a similar extent as the triply-enhanced 35S CaMV promoter, whereas the -1275 fragment was about 10 times less active. Further deletion (to -304) increased GUS activity to about a third of that with the 35S promoter, suggesting the presence of a silencer element between -1275 and -304. The results were confirmed by histochemical analysis, since the intensity of the blue precipitate in tissue sections stained with X-gluc correlated with those obtained with the fluorimetric GUS assay. Furthermore, light- and tissue- specific expression was observed, with high levels in the leaves and only slight expression in roots, principally at the tip.

The other aspect of the HMBS work was to introduce sense and antisense constructs for the enzyme into plants, to increase and decrease activity levels. The transformation has been carried out, and we are now just waiting for enough plant material to regenerate for biochemical analysis. In the meantime, Mike Witty has overexpressed the protein in *E. coli*, and we hope to be able to purify it in order to raise antibodies. Ashley Wallace-Cook has completed a comprehensive study of the expression of HMBS in different organs of the plant and during greening, and correlated the results with enzyme activity levels.

As far as the work on ferrochelatase is concerned, the cDNA which I isolated by functional complementation of a yeast mutant, has been confirmed to encode the chloroplast-localised enzyme since the polypeptide transcribed and translated *in vitro* has been shown to be imported into isolated intact pea chloroplasts, and processed to the mature size. But it also is imported into yeast mitochondria, although not as efficiently as a *bona fide* mitochondrial protein. This explains how the protein could complement the yeast mutant since the ferrochelatase enzyme is required in the mitochondrion to function. The next experiment we are about to do is to see if the precursor is targeted to plant mitochondria. This work is being done by Maria Angelica Santana, a graduate student from Venezuela.

Lastly, but not least, Helen McPartlan has joined the group as the new post-doc and will carry on the transgenic plant analysis started by Saw Hoon, who will leave in February to start as a lecturer at the National University of Singapore.
Alison Smith, Saw Hoon Lim, Michael Witty, Helen McPartlan & Ashley Wallace-Cook; Dept of Plant Sciences, University of Cambridge.
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From Nelly Tsvetkova...

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

Since our last report we have made progress in examination of ultrastructural changes in fatty acid defective mutant strains of Arabidopsis using thin section and freeze-fracture electron microscopy. The FAD mutation affects the chloroplast pathway of the lipid biosynthesis. A result of this mutation is a 49% decrease in the level of unsaturation of the major thylakoid lipids. Although the fad b and fad c mutants differ in their acyl chain specificity, they both exhibit similar changes in the chloroplast ultrastructure when compared to the wild type. There is, for example, reduction in the number of the thylakoid membranes per granum from 6.3 for the wild type to 4.3 and 4.2 for fad b and fad c mutants, respectively. This results in a decrease of the appressed-to-non-appressed membrane ratio, which is 1.5:1 and 1.4:1 for fad b and fad c, and 2.8:1 for the wild type. This change in the chloroplast ultrastructure is not compensated by an increase in the amount of the stromal membranes.

"...change in chloroplast ultrastructure is not compensated by an increase in the amount of the stromal membranes."

Chloroplasts isolated from the wild type and the fad b and fad c mutants have shown major differences in the size and distribution of particles found in the exo- and protoplasmic fracture faces. These particles are believed to correspond to photosystem II, photosystem I and light-harvesting protein complex. The most striking difference between the patterns observed in the wild type and fad b and fad c mutant thylakoids is the formation of continuous crystalline-like arrays of particles on the fracture faces of the mutants. One possible explanation of the observed patterns is that the reduced level of lipid fatty acid unsaturation affects the lipid/protein ratio and protein/protein interactions, which could bring the complexes of photosynthetic components together

in crystalline-like structures. Similar arrays have been observed in barley chloroplasts treated with detergents. In order to investigate this further, we plan to study the chloroplast ultrastructure of Arabidopsis wild type in presence of galactolipase and detergents and to examine the particle size and distribution compared with those of the fad b and fad c mutant strains. An optical diffraction analysis of the observed crystalline-like patterns will be performed as well. Having completed our initial studies on the phase behaviour of saturated galactolipids using dynamic X-ray diffraction methods, we plan to isolate molecular species of unsaturated galactolipids from the fad mutants of Arabidopsis in the final phase of the project.

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GUEST SUMMARIES

From Ivo Cetl.

Towards exploration and conservation of natural genetic variation in Arabidopsis.

Each programme of sampling, exploration and conservation of natural genetic variation of ecotypes of *A. thaliana* should reflect the genetic structure of particular populations. The programme should be based on the gene groups showing the most complicated pattern of natural genetic variation. Considering the two well-known gene groups in *A. thaliana*: flowering-time genes; and those controlling electrophoretic allozyme differences, the variation pattern of the former seems to be more complex than of the latter. Thus, all procedures in establishing gene banks of natural genotypes should issue from our knowledge of the genetic structure of natural populations as to the genetic differences in flowering time. Simultaneously, the procedures should be able to be extended to include other groups of genes and chromosomal aberrations.

Three kinds of genetic variation in flowering time were suggested in the previous newsletter: (*i*) Genetic variation only between populations, genetic variation within populations absent. (*ii*) Genetic variation not only between populations, but also within them -- in the latter case present only between individuals, but absent within them. (*iii*) Genetic variation not only between populations and within populations -- in the latter case not only between individuals, but also within them.

It appears advantageous to conserve the gene pool of particular populations as different highly homozygous lines rather than as original samples. Accordingly, three kinds of conserved material should be distinguished: (*i*) When the natural population consists of individuals of one and the same genotype homozygous for the alleles of all genes the sample may be represented by the descendants of whichever member of the population: one population -- one homozygous line. (*ii*) When the natural population consists of individuals of two or more genotypes homozygous for the alleles of all genes, the sample may be represented by the descendants of two or more different members of the population: one population -- two or more different homozygous lines. (*iii*) When the natural population consists of individuals of two or more different genotypes partly homozygous and partly heterozygous for the alleles of all genes the sample may be represented not only by the descendants of two or more different homozygous members of the population as in (*ii*), but also by the descendants of two or more different heterozygous members whose descendants show segregation and recombination in one, two or more genes: one population -- two or more different homozygous lines from natural homozygotes plus two or more different homozygous lines from each of the natural heterozygotes.

In order to distinguish between (*i*), (*ii*), and (*iii*) as well as to ensure and verify the phenotypic uniformity and stability of final homozygous lines, pedigree breeding experiments are necessary. They should start with families derived from individual members of the original population sample and continue with consecutive subfamilies obtained by means of repeated natural or controlled self-fertilization. Such a procedure makes it easy to obtain quantitative data on the populations studied, the identification of particular genes and alleles by means of segregation and recombination of natural heterozygotes and, finally, the expression of gene frequencies in the original population sample.

The procedure is shorter with allozyme variants or DNA segments where heterozygosity of particular members of natural populations may be detected immediately while in other cases, as a rule, an additional cycle of reproduction appears necessary.

Valuable information, of course, may also be obtained by means of studying original samples. Nevertheless, the best way enabling to conserve at least a majority of genetic variation present in various groups of genes and to prepare the final product, *i.e.*, various homozygous lines ready to use in detailed investigation of natural genetic variation, is safeguarded by means of the breeding procedures mentioned.

Valuable natural heterozygosity of some individuals in some natural populations or the possibility to obtain recombinants between different conserved lines may lead to the construction of recombinant inbreds for molecular mapping (Burr & Burr, 1991). At the same time, a parallel study of the two gene groups, namely, of the flowering-time genes and of those controlling the electrophoretic variants, might help in searching for closely linked couples of loci of the two groups and so facilitate cloning and sequencing at least of some flowering-time genes.

The most serious problem connected with the standardisation of the programme considered is the size of experimental samples. The numbers of individuals necessary in each population sample and in each cycle of reproduction as well as in each gene group studied as yet unknown.

Some examples of natural populations subdivided into different ➳

homozygous lines are now available in our collection. Unfortunately, we succeeded in saving only a part of the material collected during the 60's and 70's, *i.e.*, a series of homozygous lines different not only in flowering time, but also in the morphology of vegetative and generative organs obtained from the population Je (Jehnice) collected as early as in 1963, from populations Da (Drahonin), Jm (Jamolice) and others. Large numbers of different homozygous lines originating from different homozygotes and also from segregating heterozygotes were obtained in the population samples Bh (Babí hora) and Sn (Senorady). Since the late 80s, I am trying, above all, to determine the numbers of individuals necessary in particular stages of the programme in each population collected.

Burr, B. & F.A. Burr (1991) *Trends in Genetics* 7, 55-60.

Ivo Cetl; Dept. of Genetics and Molecular Biology,Faculty of Science, Masaryk University, Kotlarska 2, 611 37 Brno, Czechoslovakia.

From Abed Chaudury...

A mutant that couples space and time.

We would like to describe a mutant of Arabidopsis, ampl (altered meristem programme) that indicates that spatial and temporal aspects of development can be coupled.

The ampl mutant was originally isolated as one in which a high fraction of the seedlings have multiple cotyledons. While less than 1/1,000 seedlings from a congenic wild-type Columbia had seedlings with higher or lower than two cotyledons, 10-30% of the seedlings from a homozygous ampl have multiple cotyledons. They include tricot, tetracot, and even monocot seedlings. The rate of leaf formation, leaf phyllotaxy, and the rate of increase in biomass is higher in ampl than in wild-type. The mutant plant also flowers (12-15 days at 16h day) earlier than wildtype (16-20 days at 16 h day).

Recently, we found that ampl also lacks photoperiodic response. The mutant flowers as early in short days (8h light) as it does in long day.

The alterations of a spatial (cotyledon number and leaf phyllotaxy) and temporal (flowering time and photoperiodic response) control of development by the same mutation suggest that these two processes (*i.e.*, spatial and temporal) can be coupled. This demonstration has implications for the evolution of form in higher plants. For instance, a trait that is under under high selection pressure (*e.g.*, photoperiodic changes) may sometimes bring with it novel changes in phyllotaxy which may not have any adaptive value.

> "...spatial and temporal processes can be coupled."

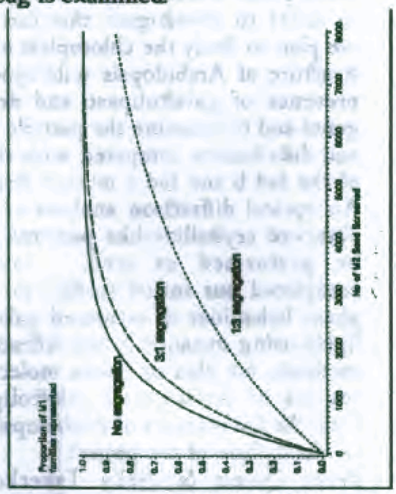
By making double mutants with a number of other mutants that affect leaf development and flowering time, we are trying to understand the mechanism of action of Ampl function We have already shown that the ampl phenotype is epistatic over the high apical dominance of hy2 mutants which lack phytochrome, suggesting that the ampl phenotype is not controlled by a functional phytochrome. Double mutant studies with fca and fb, two different flowering time mutants are also under way.

Abed Chaudhury; CSIRO Division of Plant Industry, Canberra, Australia.

From Ray Ellis...

Screening for herbicide resistant mutants.

To isolate mutants of Arabidopsis resistant to a test herbicide (an inhibitor of the photosynthetic apparatus), we purchased from Lehle Seeds 512,000 M2 seed derived from 64 parental groups (each bag of 8,000 M2 seed derives from an average of 1,000 M1 parents). To optimise our search strategy we computed the number of M1 families sampled as a function of the number of M2 seed screened per bag. Since M1 plants are heterozygous for any nuclear mutation, our calculations assumed segregation of the conferred trait in the M2; *e.g.*, to sample 80% of M1 families in any one bag 2,200 or 6,800 M2 seed need be screened for 3:1 or 1:3 segregation ratios, respectively. The calibration curve demonstrates that a doubling in number of M2 seeds sampled less than doubles the number of M1 families sampled. Thus for a specified maximum number of M2 seed to be screened, as many parental groups as possible should be sampled, even though only a fraction of each bag is examined.



From a screen of 180,000 M2 seeds sampled across all parental groups available we found only one resistant seedling. On screening the remaining seed from this parental group we discovered a further three resistant individuals. Analysis of selfed M3 progeny from the strongest of the candidate plants confirmed heritability of herbicide resistance; absence of segregation indicated that the M2 parent was homozygous for the trait.

Ray Ellis, Karen Sturgess, Belinda Neil & Derek Pike; ICI Seeds, Jealott's Hill Research Stn. FAX 0344-414818.

From Lieve Gheysen...

Nematode resistant Arabidopsis mutants.

Almost 5,000 EMS mutagenised Arabidopsis plants have now been screened to search for an altered response to infection with the root-knot nematode, *Meloidogyne incognita*. Five mutants have been isolated with lower to much lower infection rates than that of control plants and the resistant phenotype was shown to be transmitted to the next generation. Two types of mutations could be distinguished, depending whether or not the mutation had a visible effect on the root morphology. In the three mutants with abnormal root organisation, it is likely that there is a direct or indirect relation between the morphology of the root and the resistance to *M. incognita*. Detailed characterisation of these

mutants should give us some clues about the interaction between root anatomy and susceptibility to nematode infection. The two other mutants could be even more interesting in enabling us to identify genes which are essential for the development of a compatible plant/nematode interaction. Although more research is needed to characterise the isolated mutants and to possibly identify additional ones, our results indicate the feasibility of isolating nematode resistant Arabidopsis plants. This type of mutational analysis should allow to genetically dissect the different steps in this plant/pathogen interaction.

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From Eric van der Graaff..

About auxins and gene targeting.

Arabidopsis plants harbouring a tobacco auxin inducible promoter fused to GUS were crossed with different auxin mutants. So far no apparent changes in expression patterns were observed. Dianne has isolated two Arabidopsis homologues of the 103 tobacco gene, that are both single copy genes and do not show cross-reaction. RNA analysis revealed that both genes are expressed constitutively in leaves. One of the genes can be induced in roots by auxins and kinetin. The Arabidopsis homologues are currently being sequenced.

To obtain a large number of transformants in the gene targeting projects, a highly efficient transformation protocol is a must. Annette's efforts to establish an efficient co-cultivation system of Arabidopsis protoplasts with Agrobacterium have not led to sufficient progress to be usable in targeting experiments. Neither the liquid culture experiments varying the hormone regime nor the 2-6DB experiments yielded proper division rates. Furthermore, much variability was encountered. The focus was, therefore, put on the root transformation system. Different parameters are being checked to optimise the root transformation system.

Targeting experiments with a target line that carries a defective hygromycin gene in its genome (U. Halfter *et al.* (1992) *MGG* **231**, 186-193) are being performed. The first hygromycin resistant clones have been obtained and are being analysed using PCR analysis. The amanitin selection in Arabidopsis does work, but at least a 100-fold higher amanitin concentration is needed for selection when compared to selection in mouse ES cells. Stephan has tried to improve the amanitin selection by using membrane destabilising chemicals and by coupling amanitin to biotin, but neither of these approaches resulted in a better selection. The AtrpII gene construct will still be used because it offers more homology than previous systems in plants and the introduced mutation provides only a very limited non-homology. Stephan will try to identify targeted recombinants by direct PCR screening on pools of transformed clones. Such an approach, if successful, will not be limited to selectable genes. Eric has used the leaf transformation method described by van Lijsebettens *et al.* (1991 *TAG* 81, 277-284), which was improved on several points, to transform T-DNA constructs harbouring the *Agrobacterium tumefaciens* genes 1 & 2, gene 4, gene 6b and the *A. rhizogenes* gene rolC to Arabidopsis to study the effect of altered phytohormone content on the morphology of Arabidopsis. Also constructs harbouring the promoters of gene 1, 2 and 6b fused to GUS were used to transform Arabidopsis to study the expression patterns of these genes. The T_2 seeds of the primary transformants were shown to be kanamycin resistant and the transgenic seedlings are now grown for bulk seed formation. The T_3 seeds will be analysed for altered morphology and GUS expression patterns using the earlier described Arabidopsis root assay.

Dianne van der Kop, Annette Vergunst, Stephan Ohl & Eric van der Graaff.
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From Roger Innes..

Mapping and characterisation of the disease resistance gene, Rps3 in Arabidopsis.

We have been busy mapping and characterising the disease resistance gene *Rps3*, which confers resistance to *Pseudomonas syringae* strains that carry the avirulence gene *avrB*. We now believe that this gene may be the very same gene that Jeff Dangl's group has been mapping, *Rpm1*. The data supporting this view are threefold. (i), the ecotype specificity of *avrB* and *avrRpm1* are identical. This means that all the ecotypes that fail to recognise avrB, also fail to recognise avrRpm1. (ii), both *Rpm1* and *Rps3* map to the top of chromosome III by RFLP analysis, and the resistance phenotype co-segregates 100% (50 F_2's tested to date). (iii), four independently isolated mutants (two in our lab and two in the Staskawicz lab) that no longer respond to *avrB*, also fail to respond to *avrRpm1*, but retain their ability to respond to a third avirulence gene, *avrRpt2*. It is still formally possible that there are two very closely linked genes at the *Rpm1/Rps3* locus and that the mutations all knocked out two genes simultaneously, but this scenario seems unlikely.

"*Rpm1* and *Rpm3* are the same gene?"

We are now collaborating with Jeff Dangl's group and Brian Staskawicz's group to analyse our mutants at a molecular level. Jeff reports that they are very close to localising *Rpm1* to within 2 or 3 cosmids of DNA. He has agreed to send us these cosmids as soon as they are defined. We will use these to search for alterations of either mRNA's or genomic DNA in our mutants. Hopefully, this will allow us to assist Jeff in localizing *Rpm1* more precisely, and to determine if *Rpm1* and *Rps3* are indeed the same gene.

If *Rpm1* and *Rps3* are the same gene, several very interesting questions come to mind. Is such "promiscuity" in an R-gene going to be common? The sequences of *avrB* and *avrRpm1* are very different, although the first five amino acids are identical. Is this limited sequence conservation important? Both *avrB* and *avrRpm1* are also recognised by soybean, but the cultivar specificities are not identical. Some cultivars that respond to *avrRpm1*, do not respond to *avrB*. Do these differing specificities in soybean simply represent alternate alleles of the same R-gene? Look for answers to these questions, and others, in future issues of this esteemed publication.

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From Javed Mirza...

Plant hormone mutants in *Arabidopsis*.

Plant hormones are crucial factors involved in almost every aspect of plant development from seed germination to seed formation. However, there are major uncertainties about the biosynthetic pathways of plant hormones and their subcellular action is not clear. Biochemical analysis of plant hormone mutants and cloning of the genes related to these hormones could be of basic importance to plant developmental physiology. With this in mind, we have screened several mutant lines of *Arabidopsis thaliana* that are affected in their response to either auxins, cytokinins, or polyamines.

For the last four months, we have been busy in characterising the lines screened for NAA-resistance. Out of 25 lines originally isolated for NAA-resistance, 15 lines have now been confirmed as resistant to NAA. All of these mutants have distinct phenotype such as dwarf or semi-dwarf stature, reduced apical dominance, more frequent and prominent cauline leaves, trichome variations, light green stem, light green inflorescence, long hypocotyl, short root hair, and agravitropism. The other 10 lines that have turned out to be not resistant to NAA, also have distinct phenotypes including yellow seed colour, male sterility, long hypocotyl, absence of root hair, short root hair, twisted stem, more frequent branching with numerous cauline leaves and various flower abnormalities. Two of the root hair mutants have smaller / less frequent root hairs that are resistant to the promotory effect of NAA. Several mutant phenotypes are due to single recessive mutations, others are being studied. The mutants have been crossed with various gene markers for allelism, linkage and mapping. The marker lines were obtained from the Nottingham Arabidopsis Stock Centre. **Here I must appreciate the quick response of the Arabidopsis Stock Centre for efficient supply of seeds.** We are now also going to start characterisation of cytokinin and polyamine mutants. These mutant lines have been screened for resistance/susceptibility of seedling roots to BAP or putrescine, and for resistance to germination inhibition by spermine. Hopefully, a mini report of these mutants will be ready for the next issue of this newsletter.

We are going to develop a research collaboration with Prof. Dr. Willy Hilgenberg of J.W. Goethe-University, Frankfurt, for "biochemical studies of Arabidopsis auxin mutants." As a part of this programme, our research student, Saiqa Rasheed has been awarded a DAAD long-term scholarship. She will start research work at Frankfurt from December 1992.

Javed I. Mirza; Institute of Biology, B.Z., University, Multan, Pakistan.

From Brian Osborne...

The Cre-lox recombination system and *Ds* in Arabidopsis.

The work I'm describing is taking place in the lab of Barbara Baker at the Plant Gene Expression Center. I started by constructing the vectors I'll describe, and was joined at that point by fellow post-doc Uwe Wirtz, who will also continue to study *Ds* transposition in *O. sativa*. I have constructed *Ds*'s and T-DNAs containing lox sites that are substrates for Cre-mediated site-specific recombination. We expect that *Ds*-lox will transpose to linked sites, as well as to unlinked sites and sites on other chromosomes. These insertions will be stable as the source of *Ac* transposase on another T-DNA will segregate away from *Ds*-lox in selected progeny of *Ds*-lox x *Ac* transposase cross. When *Ds*-lox is linked to a T-DNA-lox, Cre-mediated recombination acting on the lox sites in those elements may generate a deletion between those elements. We can select for the deletion as lox-lox recombination between the sites fuses a promoterless acetytransferase (AAC-III) ORF within *Ds*-lox to the 35S promoter within T-DNA-lox, conferring gentamicin resistance (a control, *in vitro* generated construction introduced into Arabidopsis demonstrates that Cre will correctly generate the transcriptional fusion). Cre-mediated recombination can also generate translocations and inversions in the same selectable manner, depending on the location of the transposed *Ds*-lox.

We've collected about 120 independent Arabidopsis transformants containing the *Ds*-lox inserted into a 35S::surA,B gene (chlorsulforon resistance) within T-DNA-lox. The *Ds*-lox contains markers for selection in yeast (HIS3), *E. coli* (supF), a plant marker (35S::bar), and a number of "rare-cutter" sites. The T-DNA contains a similar set of selectable and screenable markers. We're identifying plants with single T-DNAs, and these T-DNAs will be mapped in an RI population kindly supplied by C. Lister & C. Dean.

Our *Ac* transposase fusions are placed into T-DNAs that also contain the maize R-Lc cDNA fused to a "double" 35S. Alan Lloyd in R. Davis's lab has shown that such a fusion confers a hirsute and more darkly pigmented phenotype to Arabidopsis. Thus, plants containing *Ac* transposase fusions are hairy, and desired progeny without transposase, with transposed *Ds*-lox, are relatively glabrous and chlorsulfuron resistant. We're experimenting with a variety of promoters fused to *Ac* transposase: 35S::Ac (from M. Honma & C. Waddell), a "double" 35S with "promoter up" mutations, a LAT promoter ("Late Anther Tomato", from S. McCormick), and a promoter active in vegetative and floral meristems.

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From Valentin Tolstikov...

Arabidopsis pollen.

Besides the groups working with Arabidopsis which were mentioned by Natalya Klueva in her report in the seventh issue of the Arabidopsis Newsletter, there are a number of other groups, or rather microgroups similar to those mentioned on the territory of the former U.S.S.R. I represent one of these.

My scientific interests can be summarised as follows: study of artificially induced abnormal ontogeny of pollen and chemical mutagenesis of pollen in terms of their utilisation for the develoment of microgametophyte selection techniques.

In particular, I was able, with the aid of colchicine solution and by means of *in situ* inhibition of the first mitosis of microspores, to obtain a high yield of mature viable uninucleate Arabidopsis "pollen" (Figure). In fact, such "pollen" is a population of overmature microspores, though not haploid, but dihaploid. Clearly, this "pollen" (pseudopollen) has, to a great extent, if not wholly, lost its gametophytic features, since it forms neither generative cells, nor sperms. It would be very interesting to use it as a material for *in vitro* cultivation. Mitosis of such "pollen" could apparently be induced by means of hormones.

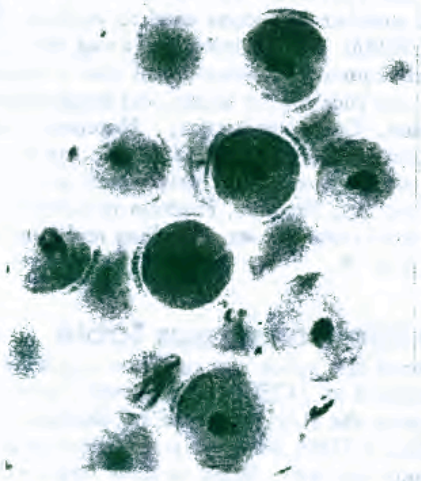

Figure. Mature uninucleate "pollen" of Arabidopsis obtained from a diploid line by means of blocking the first mitosis of microspores.

If this should be possible, then, by preliminarily creating genetic variety in the "pollen", *e.g.*, by means of chemical mutagenesis in the premeiotic period, and by cultivating it in the presence of various selecting agents, we shall obtain an attractive selection scheme.

Of particular interest is the fact that the mature uninucleate "pollen" can be obtained not only by means of inhibiting the microspore first division, but also by blocking the chromosome separation in meiosis. In both cases the mature uninucleate "pollen" has a similar morphology, but a different genetic nature and, probably, different share of gametophytic features.

I am planning to proceed with work in this direction using *Arabidopsis thaliana* in the immediate future, and I hope that I shall be able to report on my progress in the next issues of the Arabidopsis Newsletter.

V.F.Tolstikov; Don Institute of Agricultural Science, Department of Genetics of Plant Mineral Nutrition, 346714 Rostov-on-Don Region, Aksay District, Rassvet Settlement, Russian Federation, Russia.

From Richard Wiliamson...

Arabidopsis root mutants.

We have completed an extensive screen for mutants causing radial swelling in seedling roots and from this have focused on 25 lines. These are all temperature-sensitive mutants that more than double their diameter within 48h of being moved from 21 to 31°C. We consider a temperature-sensitive phenotype important to maximise our chances of recovering mutations in genes with essential functions. In particular we want to identify genes that cause cytoskeletal defects and those that cause major defects in wall biosynthesis. We know from the use of inhibitors acting on these processes that radial swelling can result from interfering with the cytoskeleton or wall biosynthesis.

By feeding 14C-glucose to seedlings and preparing a crude wall fraction, we have investigated incorporation into major categories of wall polysaccharides. We have evidence that some of the lines have sharply reduced incorporation into specific fraction after 24h at 31°C when they are compared to themselves maintained at 21°C or to the wild type incubated at 31°C. Sorting them into complementation groups is in progress.

Richard Williamson, Tony Arioli, Jacek Plazinski, Ann Cork, & Rosemary Birch; Plant Cell Biology Group & Plant Science Centre, RSBS, Australian National University, Canberra.
WILLIAMSON@RSBS0.ANU.EDU.AU

Methods in *Arabidopsis* Research

THE ACM RECEIVED A, somewhat long, advertisement for the above book, which is edited by C Koncz, N-H Chua & J Schell. From a quick peak it seems generally very good, but, notwithstanding its title, some of the chapters seem a little thin on actual methods. Nevertheless, it is probably a compulsorary purchase for all Arabidopsis labs. Indeed, the blurb states, "The book offers a wealth of methodical and theoretical information as well as valuable references. It should be of use to students, teachers, as well as advanced researchers and those breeders who want to use molecular techniques in breeding." Rather than bore you with the rest of the copy in full, here is a list of authors and contents to whet your appetite.

A Heuristic Glance on the Past of *Arabidopsis* Genetics (G P Rédei); Classical Mutagenesis (G P Rédei & C Koncz); Genetic Analysis (M Koornneef & P Stam); Introduction to the Arabidopsis Genome (E M Meyerowitz); Chromosome Walking in *Arabidopsis thaliana* using Yeast Artificial Chromosomes (S I Gibson & C Somerville); Genetic and Physical Linkage of the *Arabidopsis* Genome: Methods for Anchoring Yeast Artificial Chromosomes. (E Matallana, C J Bell, P M Dunn, M Lu & J R Ecker); Genetic Linkage of the *Arabidopsis* Genome: Methods for Mapping with Recombinant Inbreds and Random Amplified Polymorphic DNAs (RAPDs) (R S Reiter, R M Young & P A Scolnik); Genome Mapping in *Arabidopsis* (B M Hauge & H M Goodman); T-DNA Transformation and Insertion Mutagenesis (C Koncz, J Schell & G P Rédei); T-DNA Insertion Mutagenesis in *Arabidopsis*: Seed Infection/Transformation (K A Feldmann); Transposon Tagging in *Arabidopsis* (G Coupland); Protoplast Transformation and Methods to Create Specific Mutants in *Arabidopsis thaliana* (T Altmann, B Damm, U Halfter, L Willmitzer & P C Morris); Cloning *Arabidopsis* Genes by Genomic Subtraction (T P Sun, D Straus & F M Ausubel); Gene Isolation with the Polymerase Chain Reaction (A Gasch, T Aoyama, R Foster & N H Chua); Microsequence Analysis of *Arabidopsis* Proteins Separated by Two-Dimensional Polyacrylamide Gel Electrophoresis: A Direct Linkage of Proteins and Genes (G Bauw, M Van Montagu & D Inzé); Analysis of Protein/DNA Interactions (R Foster, A Gasch, S Kay & N H Chua); Genetic Definition of Loci Involved in *Arabidopsis*-Pathogen Interactions (J L Dangl, E B Holub, T Debener, H Lehnackers, C Ritter, & I R Crute); *Arabidopsis* Mutant Collection (M Anderson & B *Mulligan*).

From Mike Cherry...

Update to Codon-usage Table

This update to the *Arabidopsis* codon-usage table was placed on the *Arabidopsis* bulletin board by Mike Cherry.

The table is in GCG format. It should be fairly easy to convert this table to formats used by other software products. The ACeDB software used by the AAtDB database will produce a codon usage table from any set of sequences known to the database. This is an option of the DNA analysis window.

AmAcid	Codon	Number	/1000	Fraction
Gly	GGG	964.00	9.49	0.12
Gly	GGA	3241.0	31.91	0.39
Gly	GGT	3001.00	29.55	0.36
Gly	GGC	1078.00	10.61	0.13
Glu	GAG	3487.00	34.33	0.54
Glu	GAA	3002.00	29.56	0.46
Asp	GAT	3275.00	32.24	0.61
As	GAC	2117.00	20.84	0.39
Val	GTG	1811.00	17.83	0.26
Val	GTA	736.00	7.25	0.11
Val	GTT	2799.00	27.56	0.40
Val	GTC	1586.00	15.61	0.23
Ala	GCG	888.00	8.74	0.11
Ala	GCA	1708.00	16.82	0.22
Ala	GCT	3711.00	36.54	0.47
Ala	GCC	1510.00	14.87	0.19
Arg	AGG	1253.00	12.34	0.24
Arg	AGA	1617.00	15.92	0.31
Ser	AGT	1181.00	11.63	0.15
Ser	AGC	1109.00	10.92	0.14
Lys	AAG	3933.00	38.72	0.60
Ly	AAA	2592.00	25.52	0.40
Asn	AAT	1691.00	16.65	0.40
Asn	AAC	2501.00	24.62	0.60
Met	ATG	2725.00	26.83	1.00
Ile	ATA	902.00	8.88	0.16
Ile	ATT	2224.00	21.90	0.41
Ile	ATC	2362.00	23.25	0.43
Thr	ACG	675.00	6.65	0.12
Thr	ACA	1444.00	14.22	0.26
Thr	ACT	2001.00	19.70	0.36
Thr	ACC	1468.00	14.45	0.26
Trp	TGG	1119.00	11.02	1.00
End	TGA	106.00	1.04	0.40
Cys	TGT	868.00	8.55	0.54
Cys	TGC	745.00	7.33	0.46
End	TAG	51.00	0.50	0.19
End	TAA	108.00	1.06	0.41
Tyr	TAT	1072.00	10.55	0.37
Tyr	TAC	1805.00	17.77	0.63
Leu	TTG	2021.00	19.90	0.23
Leu	TTA	889.00	8.75	0.10
Phe	TTT	1737.00	17.10	0.42
Phe	TTC	2448.00	24.10	0.58
Ser	TCG	752.00	7.40	0.10
Ser	TCA	1406.00	13.84	0.18
Ser	TCT	2108.00	20.75	0.27
Ser	TCC	1145.00	11.27	0.15
Arg	CGG	345.00	3.40	0.07
Arg	CGA	478.00	4.71	0.09
Arg	CGT	1115.00	10.98	0.22
Arg	CGC	362.00	3.56	0.07
Gln	CAG	1782.00	17.54	0.50
Gln	CAA	1783.00	17.55	0.50
His	CAT	978.00	9.63	0.50
Hi	CAC	970.00	9.55	0.50
Leu	CTG	851.00	8.38	0.10
Leu	CTA	838.00	8.25	0.10
Leu	CTT	2399.00	23.62	0.27
Leu	CTC	1806.00	17.78	0.21
Pro	CCG	788.00	7.76	0.16
Pro	CCA	1688.00	16.62	0.35
Pro	CCT	1759.00	17.32	0.36
Pro	CCC	657.00	6.47	0.13

Of the 410 Arabidopsis sequences in AAtDB 1-2 from GenBank 73 and EMBL 32 including updates through October 15, 1992. A total of 315 sequences had a coding region defined and contained zero or one termination codon. The total number of codons used to produce this table was over 100,000. Table constructed using the GCG program CodonFrequency. Meaning of the columns: AmAcid, three letter code for the amino acid designated by the codon; Codon, Codon sequence; Number, Total number of occurrences of this codon in the input set; /1000, Number of occurrences of this codon per 1000 codons in the input set; Fraction, Fraction of occurrence of this codon is used from the set of codons representing the same amino acid.

Splice Site Consensus Table

Mike also placed this splice site consensus table on a-mail. It was produced by ACEDB 1.8 software (Durbin & Thierry-Mieg) using the AAtDB release 1-2 database. Of the 410 Arabidopsis DNA sequences contained within AAtDB, 289 sequences were found to have intron-exon boundaries stated. A total of 383 introns were defined.

The "|" denotes the exon-intron boundary. The columns represent the nucleotide position relative to the exon-intron boundary. The rows present the total number of the different nucleotides observed in the dataset.

5' consensus:				--- intron --->						
A	105	143	235	45	2	4	256	221	86	104	124
C	98	122	56	11	3	1	17	58	28	58	68
G	71	61	30	290	371	7	40	18	196	37	46
T	109	57	6	37	7	371	70	86	73	184	145
X	0	0	0	0	0	0	0	0	0	0	0

3' consensus:					--- intron --- >						
A	71	79	64	127	22	368	7	97	82	104	99
C	53	49	41	24	225	3	2	48	56	56	64
G	77	65	33	136	8	8	370	183	73	101	123
T	182	190	245	96	128	4	4	55	172	122	97
X	0	0	0	0	0	0	0	0	0	0	0

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This Issue's Quote

"His style has the desperate jauntiness of an orchestra on a sinking ship." Edmund Wilson (on Evelyn Waugh)

continued from Page 4...

Arabidopsis at The Royal Society

who looked a little startled when the ACM asked him if he would like to try some weed.

Day two brought your intrepid *Arabidopsis* pioneers face-to-face with that most horrific of beasts: the general public. This brought in many types, from kids to teachers to ambassadors' wives. Curiously enough, the ACM met more journalists on this open day than during the press preview. One of the highlights was a shell-suited Essex man and similarly-bedecked small son, the latter barely the height of the display table, both watching an orbital shaker, labelled "Crute/HRI" (probably to prevent it ending up in the John Innes black hole), seemingly transfixed on the *Arabidopsis* root cultures swirling hypnotically around in their flasks. After several minutes, the small boy finally looked up at Ian and asked; "What's a Crute?"

By the end of the afternoon, those on the stand must have spoken to several hundred people in total and all were glad of a quick respite while changing into formal attire for the second, and perhaps even more grand, formal evening. This brought a reprise of one of the main highlights for many of the first evening: the ACM -- normally a paragon of the Australian sartorial-school of shorts/tracksuit bottoms and T-shirts, and owner of one tie (bought in 1980) -- wearing (hired) formal evening attire. Although his appearance as a hypertrophic penguin was nearly upstaged by Penny, whose velvet number was somewhat reminiscent of a boudoir *chaise longue*.

Caroline Dean joined the *Arabidopsis* crew for the afternoon and evening of the second day. Upon arrival, with the general public swarming around, she somewhat foolishly asked the ACM's advice on opening lines with which to ensnare the punters. After a day and a half's experience he had settled on firstly, "Are you a biologist?" and then, if the answer was in the negative, "Are you a gardener?" The latter being a way of introducing the "weed with a purpose." These lines she used to great effect with the public in the afternoon and with the vast majority of the FRS's in the evening, who were non-biologists . She came a little unstuck with this patter at one point with an aged gent, festooned with medals and regalia, who was eyeing the wonder weed. On the blind-side of his name badge, she asked of him the hitherto successful ice-breaking question, "Are you a biologist?" He turned out to be Sir Ralph Reilly, the former head of PBI.

Such minor gaffes aside, the whole event was a great success in raising the profile of *Arabidopsis* research, particularly amongst those with "influence."

Thanks are due to Jon Clarke for growing the plants used and to Mary Anderson for supplying seeds with a give-away leaflet, "Grow you own *Arabidopsis* mutants," which went like hot cakes.

Shown right are Penny, Caroline, the ACM, Ian, & Jim tending the *Arabidopsis* display at The Royal Society (Eric took the photograph and so escapes humiliation.)

Thanks to...

- Barrie Allen for offset-litho printing this newsletter.
- Mary Anderson for the Nottingham update.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying some of the graphics.
- Joan Green for the Current Awareness List.
- Sharon Green for help with photocopying & collating.
- Black Rot for the crossword.
- Renate Schmidt for suggestions & proof-reading.
- Morton Suglevand for help with photocopying.

Stanzas for Scientists

RATHER LIKE MOST WARS, the poetry is only recognised afterwards. As the Arabidopsis Armistice approaches, here, at last, is a poem sent in by one of the readership.

ARABIDOPSIS BLUES
(dedicated to the Arabidopsis post-doc)

This era cannot pass away
without some tribute given
to ACM, Black Rot and co
and the weed with which we're smitten

This hallowed rag was really grand
a must for far flung parts
the demented ramblings of ACM
we took him to our hearts

And looking back in years to come
when unemployment bites
we were there at the beginning
our cv's will recite

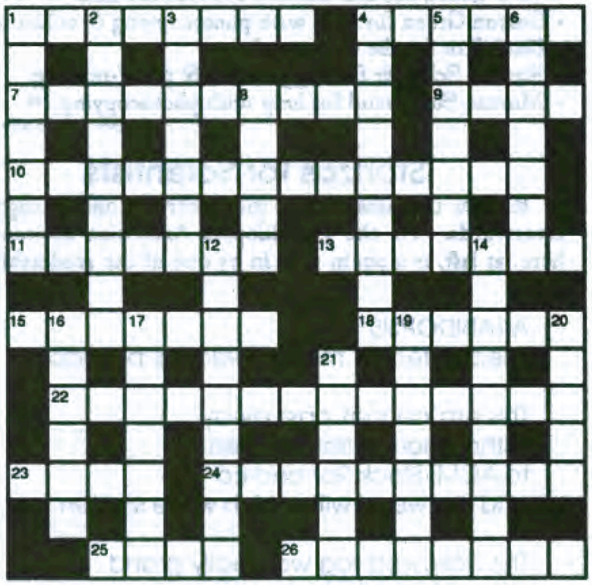
From the heady heights of Nottingham
to the drunks in Aberdeen
on the long road to Vienna
some will know what I mean

And dear Arabidopsis
we've never asked her fair
that with the help of science
her secrets we might share

So farewell Arabidian nights
the times they were stupendous
it's such a drag the system's made
the career structure horrendous

Anon

Arabidopsis Prize Crossword



Swan Song

by Black Rot

FIVE OF YOU managed to complete the previous crossword, *Albertville*, despite a couple of very minor errors missed by the ACM on his trial run through. Perhaps surprisingly, only one of the entrants mentioned the mistakes. Not surprising, however, was that all were former competitors and all, except one, were previous winners. All deserve medals for persistence, but the lucky ones, drawn out of a recycled envelope by Sharon Green, who is currently doing an admirable job filling in for new mother Rosi Joy here in Mol. Gen., were **Brian Forde** from Rothamsted who wins the Newsletter £5 book-token, and **Jo Ross** of the Cambridge Lab. at the John Innes Centre, who, for the second newsletter in succession, wins the site prize of a bottle of Bulgarian Beaujolais. The highly-skilled, but unrewarded, runners-up were Mary Anderson (Nottingham), Alison Smith (Cambridge Univ.), and Jim (no relation) Smith (J.I.C.). This means that Mary is the only regular entrant not to have won a prize during the duration of the newsletter. To compensate for this, the ACM hereby promises to buy her lunch on her next visit to Norwich. (She will have to hurry of course, because he will not be there much longer.)

Black Rot assures the ACM that this final, all-cryptic, crossword is error free, but as with all of them the ACM takes full responsibility for any mistakes. Unlike certain government-appointed officials, the buck (or pound) firmly stops here. As usual, a lot of the answers are on the same subject, which is strongly suggested by the title.

On his last day in the newsletter offices before the bailiffs move in, the ACM will draw out the winner and send them the last £5 book-token remaining in the petty-cash drawer. Answers will then be sent to all entrants.

For the sadists amongst you, the minor glitches in the previous crossword were, to quote the ever-observant Alison, "The verb to remove nerves is enervate and not ennervate...Also betroth is to engage or affiance, not to marry." Such dedication reminds the ACM to take this opportunity to thank Alison and other regular entrants for their support of this back-page diversion. He also particularly thanks Black Rot for the hours spent in preparing the crosswords. From the feedback the ACM has received, many of you have greatly enjoyed this aspect of the newsletter and Black Rot's efforts have been much appreciated.

Clues Across

1. Northern weather changeable for gallinule (5,3)
4. Plover and eagle both in Colorado town (6)
7. Tests after cunning learner trapped auks (10)
9. Stable home for seagulls (4)
10. Veteran who shot three under par (7,7)
11. The spanish lasso thrown in loose soil (7)
13. Placate orang-uotang with meal of legumes (7)
15. Break a path for *Otis*? (7)
18. Lifeboatmen dressed in brown see common shorebird (6)
22. Eden's starling ? ... (4,2,8)
23. ... who mimics youth we're told (4)
24. Crane rocks with manufacture of rails (10)
25. Fledgling hawk adopts detailed grey aspect (4)
26. Peregrine, heard to be, earlier than the early bird ? (8)

Clues Down

1. Rover's happy response to grey, yellow or black and white variety (7)
2. Spasmatic observers of 21 ? (9)
3. Birds as breakers of locks - surely not (7)
4. Another member of Shakespeare's schoolboys movement class ? (9)
5. Plates make animal rear up on hind quarter (7)
6. Lear and Elgar unite to foster Gareth (7)
8. Form of fatigue induced by rock ? (5)
12. Perhaps RAF is core unit of these (3,6)
14. Type of game demanded by BR ? (3,6)
16. Surgeon cuts Heep short in Italy (6)
17. Noddy's home, we hear, in three parts (7)
19. ... and German river in unoxygenated state (7)
20. Manipulates, they say, our necessities (5)
21. Falcon starts nest, beset by renal problem (6)

Here are the answers to *Albertville*, the crossword in *Thale of the Century*: