FOR THALE OR WENT

The Sixth AFRC PMB *Arabidopsis* Newsletter July 1991

In This Issue:

Plus – attached:

Changes for the Next Newsletter

The deadline for the next *Arabidopsis* newsletter is **Monday, 7 October, 1991**. Owing to the increased time it is taking the ACM to compile the ever expanding newsletter, please note that the following measures apply from now on:

(i) Reminders will *only* be sent out by e-mail. So make a note in your Filofax, in your diary, on a post-it, on your lab-coat, wherever; but please remember the deadline.

(ii) Submissions will *only* be accepted if sent on disk or *via* e-mail. In particular, FAXes (except those coming in advance of a disk) will *not* be accepted.

These measures are designed to make the production of the newsletter quicker and hence expedite the time it takes to reach your bench-top (or litter bin). Your co-operation is much appreciated.

For those of you pondering over the crossword in someone else's newsletter, you can get your very own copy simply by sending in *any* form of usable submission, be it project report, protocol, or even cartoon.

(See also, How to Reach Us, *on Page 5.)*

For Thale or Went: the sixth AFRC PMB *Arabidopsis* Newsletter, July 1991.
Assistant Circulation Manager (ACM), David Flanders
The Cambridge Laboratory, The John Innes Centre, Colney Lane, Norwich, NR4 7UJ, U.K.
Tel: 0603-52571 (switchboard) or 0603-502200 (direct line). International +44-603-52571/502200.
FAX: 0603-56844 or 0603-502270. International +44-603-56844/502270. e-mail: ARABIDOPSIS@UK.AC.AFRC.JII

From Mary Anderson...

THE NOTTINGHAM *ARABIDOPSIS* STOCK CENTRE

THE NOTTINGHAM *Arabidopsis* Stock Centre officially opened its doors to the world on 2 April, 1991. It has been given a totally new look and is now a much more sophisticated operation, which has used the framework established by Jane Russell as a spring-board. It is funded by the AFRC Plant Molecular Biology Programme, the European Community BRIDGE Programme and the University of Nottingham. The Centre is managed by the Head of Centre, Bernard Mulligan. I am its newly appointed Director. At present, we are establishing the infrastructure of the Centre. We have one technician, Paul Anthony, already busy in the greenhouse. A second technician Patricia Fredericks has also been appointed and will be starting in the middle of June.

The Centre will function as a point of collection, maintenance, cataloguing and distribution of mutant strains and ecotype collections of *Arabidopsis*. The intention is that the Centre will work in close association with an American Resource Centre, which is under consideration, to provide an international resource network for the worldwide *Arabidopsis* Programme. To fulfill the changing demands of this fast developing area of research, we want to work in close association with *Arabidopsis* researchers. We see the Centre having two roles, that of a repository for a diverse collection of *Arabidopsis* lines, but also as a source of information exchange with the scientific community.

We intend to make the Centre and its resources as user friendly as possible. To this end, we have totally reformatted (and added to) the Nottingham Seed List. The new copy of the Centre's Seed List is included with this Newsletter. You will notice that the code assignments for the seed lines have been changed. This is to allow the holding Centre of material to be identified. Therefore, any seed that is now distributed through the Nottingham Centre will have an "N" prefix.

Bernie Mulligan

The size and scope of the Centre's holdings is going to increase dramatically. Over the next year we are going to incorporate the AIS collection of Professor Kranz from Frankfurt which contains over a thousand lines. As this collection is characterised and catalogued it will be made available for general release. As the Kranz collection is integrated, we shall create a user friendly database that will carry very detailed information about each line. Initially, the database will not have on line access, but we will gladly access any information required. We intend to produce a short-form Seed List as we characterise the lines. Eventually a detailed catalogue of all the accessions will be published. Now is your opportunity to request certain lines which we could prioritise in our bulking programme.

Mary Anderson

Watch this Newsletter for further developments.

We are also in the process of canvassing for the donation of novel lines. It is hoped that we can maximise the genetic diversity held at the Centre by encouraging the donation of lines from as many different sources as possible. I know there are people out there who have been generating new mutant lines. If you are prepared to let these lines be released (remember that the *Arabidopsis* Programme encourages free exchange of ideas and material), we will bulk the seed for you and offer a safe permanent repository for the line as part of this prestigious collection. We are also prepared to hold bulked seed until the details of a particular line have been published. The line will be named and assigned a code that would allow the line to be identified as yours, as well as being held at Nottingham. It is very important that we can organise a central holding Centre to which individuals can come. It will also save you a lot of time and effort in administrating requests.

We are also busy characterising the T-DNA tagged lines from Csaba Konz. If anyone else has produced T-DNA/transposon tagged lines we will gladly act as a repository for these valuable resources, but we are not prepared to characterise them yet. Therefore, it is best if they can be characterised before we receive them. Newsletter readers will be updated with any new developments.

We hope that the Centre will be used by many of the members of the *Arabidopsis* Programme in conjunction with the DNA Resource Centre of Jeff Dangl at Köln.

If you require any further information, please do not hesitate to contact me. I can be contacted at the address below, by direct telephone line, fax, or e-mail. I hope to hear from you soon.

Dr. Mary Anderson
Director of the Nottingham *Arabidopsis* Stock Centre
School of Biological Sciences
University of Nottingham
University Park
Nottingham, NG7 2RD
U.K.
Tel: +44 602 791216
Fax: +44 602 424270
e-mail: PBZMLH@UK.AC.NOTTINGHAM.CCC.VAX
or PBZMLH@VAX.CCC.NOTTINGHAM.AC.UK

From Jeff Dangl...

"Send us your poor, tired, huddled clones"

Dear *Arabidopsis* community,

As many of you already know, my lab is participating in the EEC BRIDGE *Arabidopsis* T-Project as a "DNA Resource Center". We have two goals: Firstly to collect, collate, and distribute "molecular tools" that serve the purpose of the T-Project, namely the identification of new plant genes with *Arabidopsis* as a focus. The second goal is to eventually collect as many cloned and characterised *Arabidopsis* sequences as possible. This is meant to serve the research community in much the way a strain collection does, namely, allowing people to avoid re-creating the wheel. Distribution of cloned sequences for use in experiments of biological interest, and for eventual sequencing programs is the goal. What we are not: We are not a sequence distribution/storage facility and we are not a substitute library of information regarding all DNA cloned and categorised from *Arabidopsis*, and vectors used.

The purpose of this column is to request that the research community please make "deposits" into the DNA resource center, as of now. I would like to standardise *how* we receive these materials, as all subsequent steps are then made easier. Enclosed in this mailing is a "Deposit Information Sheet". The data required on this page is basic, and absolutely necessary if further members of the community are to effectively use the resource center.

Jeff's Köln Deposit Information Sheet comes with this newsletter, as does Mary's Nottingham Seed List & Order Form.

I would like to assemble phage libraries in the first phase (starting now) and would like to make a request for cloned phage and plasmid sequences in the next issue of this newsletter in a few months.

Why phage libraries? Of critical interest to all *Arabidopsis* researchers is the ability to quickly come to genes of interest, via either chromosome walking or more standard methods. We felt that it would save a great deal of time and energy if various cDNA and genomic libraries could be centralised and distributed. The "Deposit Information Sheet" asks fairly detailed questions regarding how the libraries were constructed. Please fill it out *completely!*

We are especially interested in stage- and organ-specific cDNA libraries. As well, libraries made after various treatments/stresses would be helpful. I would ask that *either* unamplified or *once* amplified lysates be sent, and that as many coverages of the library as possible (in terms of titre) be sent. We will provide (hopefully) all libraries as *once* amplified lysate stocks. Thus, assuming we receive (a wildly high) 50 requests for a given library, we should be able to send an appropriate amount out. Please send phage in a *tightly* sealed tube (preferably with an O-ring cap). Leave little or no airspace in the tube, this minimises shaking during shipment which can break those poor little lambda tails. Another really critical point; please send a stab or plate stock of *all* required bacterial plating strains. As the number of specialised phage vectors increases, so does the number of crucial strains. If you feel that to ship the strains would break purchasing agreements with companies, then provide me with *exact* information of strain and company, and we'll buy the strain. If packaged correctly, lysates on chloroform travel nicely with express mail. Send me copies of shipping charges, and *we will reimburse you* for shipping costs!

If this letter sounds like one of those T.V. shopping ads ("Buy now and SAVE! SEND CASH!"), well that's life! We hope to activate this system quickly, meaning that we hope you all will feel compelled to send us your cDNA and genomic phage libraries *soon*, so that distribution can proceed. I will make another announcement, after our inventorisation is finished, that we are in "distribution mode". The speed at which "distribution mode" is achieved is, to an extent, dependent on the response to this call for deposits. At that time, I'll also tell how you can deposit your favourite cloned sequence.

Two more points: Deposition in the "DNA Resource Bank" implies that materials can be freely distributed to all EEC scientists requesting them. No exceptions. Second, we will, as far as possible, honour requests from non-EEC European countries. Having said that, we do, however, welcome deposits from the world over! Whether we can honour requests from outside Europe depends on how many we get, on shipping costs, and how fast a similar Resource Center can be set up in the States. For now, I am willing to send out phage libraries to *anyone* in the world who requests them. Hopefully, this will encourage a rash of deposits into what we hope will develop into an efficient distribution center.

We are also prepared to send upon request the Erwin Grill and Eric Ward YAC libraries, and the 96 phage lambda clones serving as the backbone RFLP probes of the Meyerowitz RFLP map. The YACs are not trivial to receive, so please "set up" for YAC work before you request them. Moreover, you must first sign an agreement with CIBA-GEIGY before I can release the Ward YAC bank. Iwill provide agreement forms upon request. The phage are sent as lysate stocks, with accompanying information provided by the Meyerowitz lab.

Further suggestions, questions, ideas can be faxed to me at: +49-221-5062-613.

I am also on the Arabidopsis e-mail network . Thank you, on behalf of the community, in advance for your contributions.

DANGL@VAX.MPIZ-KOELN.MPG.DBP.DE

Arabidopsis Publications

The ACM notes with pleasure that *Arabidopsis* papers in press are beginning to be mentioned in project reports (*e.g.*, see Kevin Pyke's). Could all AFRC grant holders please supply him with details of any publications arising, directly or indirectly, from their *Arabidopsis* grants? Reprints would also be much appreciated. Information of publications from other contributors to the newsletter are of course, also gratefully received.

Arabidopsis Bulletin Board

THE ELECTRONIC *Arabidopsis* bulletin board (bb) has changed hands. It was originally set-up by Chris Somerville as info-athal and was maintained courtesy of John Sticklen of MSU. It is now run by BIOSCI as **arab-gen**. All subscribers to the original bb will be aware of this and should be happily sending and receiving messages from the new board. For those wishing to *subscribe* to the new bb, send an e-mail message to **biosci** at the appropriate node for your geographical location (see below). To send a *message* to the bb, send it to **arab-gen** at your local node. The message will then automatically be sent to all arab-gen subscribers worldwide.

Your geographical location	Address to request subscription	BIOSCI node location
• Scandinavia & Cont. Europe	biosci@bmc.uu.se	Sweden (Internet)
• Ireland & Cont. Europe	(irlearn.ucd.ie see below!)	Ireland (EARN/BITNET)
• United Kingdom	biosci@uk.ac.daresbury	UK (JANET)
• North & South America	biosci@genbank.bio.net	USA (Internet/BITNET)

Plain English can be used in subscription requests at all sites *except* IRLEARN, which requires a special format for automatic processing. The procedure for automatic subscription is described further below.

Once you have subscribed by sending a mesage to BIOSCI at the appropriate node, you send messages to the *Arabidopsis* bb by replacing biosci with arab-gen in the address.

As an example, if you are at an AFRC institute using VMS on a VAX:

- To *subscribe* to the bb:
  $ mail <CR>
  mail> **send** <CR>
  to: **cbs%uk.ac.daresbury::biosci** <CR>
  subj: **subscription request to arab-gen** <CR>
  Then enter a message requesting subscription to arab-gen and your real (not user) name.
  CTRL/Z to exit>
- To send a *messsage* to the bb:
  $ mail <CR>
  mail> **send** <CR>
  to: **cbs%uk.ac.daresbury::arab-gen** <CR>
  subj: (whatever it is) <CR>
  Then enter your message.
  CTRL/Z to exit

To cancel a subscription, for example if you change your e-mail address, please send a message requesting this to the same address as to which you sent your subscription (*e.g.*, biosci@uk.ac.daresbury). Steve Marshall, the BIOSCI administrator at daresbury, asked the ACM to ensue that subscribe/unsubscribe requests are *not* sent to arab-gen.

The following section is for **irlearn** (European Academic Research Network) users only. U.K., U.S. and all other users please follow the directions above. To subscribe to arab-gen at irlearn, send a message to listserv@irlearn.bitnet or listserv@irlearn.ucd.ie containing the following line in the body of your mail message:

SUBSCRIBE ARAB+GEN your_personal_name
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If you wish to cancel your subscription, send a similar message to listserv@irlearn.bitnet replacing subscribe with the word signoff and omit your name, *i.e.*,
SIGNOFF ARAB+GEN

From J. Michael Cherry...

Codon-usage table

THIS CODON-USAGE table was placed on the *Arabidopsis* bulletin board:

Gly	GGG	80.00	7.37	0.10
Gly	GGA	292.00	26.89	0.37
Gly	GGT	310.00	28.55	0.39
Gly	GGC	108.00	9.95	0.14
Glu	GAG	344.00	31.68	0.56
Glu	GAA	275.00	25.32	0.44
Asp	GAT	278.00	25.60	0.55
Asp	GAC	227.00	20.90	0.45
Val	GTG	187.00	17.22	0.25
Val	GTA	60.00	5.53	0.08
Val	GTT	305.00	28.09	0.41
Val	GTC	187.00	17.22	0.25
Ala	GCG	84.00	7.74	0.10
Ala	GCA	151.00	13.91	0.19
Ala	GCT	382.00	35.18	0.48
Ala	GCC	184.00	16.94	0.23
Arg	AGG	143.00	13.17	0.23
Arg	AGA	209.00	19.25	0.34
Ser	AGT	103.00	9.49	0.13
Ser	AGC	118.00	0.87	0.14
Lys	AAG	426.00	39.23	0.67
Lys	AAA	210.00	9.34	0.33
Asn	AAT	127.00	11.70	0.33
Asn	AAC	263.00	24.22	0.67
Met	ATG	297.00	27.35	1.00
Ile	ATA	70.00	6.45	0.11
Ile	ATT	257.00	23.67	0.41
Ile	ATC	306.00	28.18	0.48
Thr	ACG	82.00	7.55	0.13
Thr	ACA	168.00	15.47	0.26
Thr	ACT	197.00	18.14	0.30
Thr	ACC	99.00	18.33	0.31
Trp	TGG	155.00	14.27	1.00
End	TGA	74.00	6.81	0.59
Cys	TGT	3.00	8.56	0.45
Cys	TGC	115.00	10.59	0.55
End	TAG	20.00	1.84	0.16
End	TAA	32.00	2.95	0.25
Tyr	TAT	99.00	9.12	0.34
Tyr	TAC	194.00	17.87	0.66
Leu	TTG	253.00	23.30	0.26
Leu	TTA	72.00	6.63	0.07
Phe	TTT	145.00	13.35	0.35
Phe	TTC	265.00	24.40	0.65
Ser	TCG	92.00	8.47	0.11
Ser	TCA	152.00	14.00	0.19
Ser	TCT	198.00	18.23	0.24
Ser	TCC	157.00	14.46	0.19
Arg	CGG	41.00	3.78	0.07
Arg	CGA	52.00	4.79	0.08
Arg	CGT	128.00	11.79	0.21
Arg	CGC	43.00	3.96	0.07
Gln	CAG	231.00	21.27	0.53
Gln	CAA	202.00	18.60	0.47
His	CAT	96.00	8.84	0.40
His	CAC	144.00	13.26	0.60
Leu	CTG	106.00	9.76	0.11
Leu	CTA	91.00	8.38	0.09
Leu	CTT	257.00	23.67	0.26
Leu	CTC	203.00	18.69	0.21
Pro	CCG	85.00	7.83	0.16
Pro	CCA	74.00	16.02	0.33
Pro	CCT	177.00	16.30	0.34
Pro	CCC	84.00	7.74	0.16

Produced by J. Michael Cherry with the GCG program

CodonFrequency from 31 genes found in GenBank 63. Duplicates, pseudogenes, mutant and synthetic genes were not included. Coding regions were specified using the Feature Table of each entry, then checked for accuracy. If more than one stop codon was found the sequence was not included.
CHERRY@FRODO.MGH.HARVARD.EDU

From Rob Dean...

Restriction Sites

The following was placed onto the *Arabidopsis* bulletin board:

This is a table for some common restriction enzyme recognition sites in the *Arabidopsis* genome. This table was composed using Jamie Cuticchia's computer program and data I obtained through searches. The table itself was made by John McDowell using the information from the computer printouts of dAta.

	Enzyme	Recognition Site	Frequency	Ave Size (bp)
1	Apa I	GGGCCC	0.0026	38,460
2	Xma I	CGGCCG	0.0053	18,870
3	Sma I	CCCGGG	0.0066	15,150
4	Sac II	CCGCGG	0.0092	10,860
5	Kpn I	GGTACC	0.0118	8,470
6	Xho I	CTCGAG	0.0144	6,940
7	Bam HI	GGATCC	0.0144	6,940
8	Xba I	TCTAGA	0.0158	6,330
9	SalI/HincII	GTCGAC	0.0158	6,320
10	Spe I	ACTAGT	0.0197	5,070
11	Sac I	GAGCTC	0.0249	4,016
12	Pst I	CTGCAG	0.0289	3,460
13	Eco RV	GATATC	0.0302	3,310
14	Eco RI	GAATTC	0.0368	2,590
15	Cla I	ATCGAT	0.0394	2,530
16	Hind III	AAGCTT	0.0617	1,620
17	AhaIII/DraI	TTTAAA	0.0703	1,422

The table was put together using the known sequences of *Arabidopsis* as found in Genbank and Uembl. The computer program (which fits a markov chain) takes these sequences and searches for trinucleotide, tetranucleotide, and hexanucleotide counts (compares random to expected). John took the hexanucleotide counts and looked for common restriction sites which he then put in table form.
DEANRE%GANDAL.DNET@ASMUS1.GENETICS.UGA.EDU

From Richard Williamson...

Arabidopsis in Oz Conference

The programme for the Robertson Symposium at the Australian National University is almost settled with some 18 overseas speakers and a similar number of locals. It will be held on September 30 and October 1 and further details are available from Richard Williamson, Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, PO Box 475, Canberra, ACT 2601, Australia. FAX (6) 248 9995, phone (6) 2495087.

A number of people who had previously decided that they could not afford the trip have now changed their minds as some very low air fares are available. Remember that you can comfortably fit this in *en route* to the plant molecular biology meeting in Tucson!
WILLIAMSON@RSBS0.ANU.EDU.AU

AFRC PMB *Arabidopsis* Conference

It has been decided that the next meeting of the *Arabidopsis* part of the PMB Programme will *not* be this Christmas, as it has been with the previous two, but will be in July 1992. This end-of-programme (or perhaps end of the first series?) finale will be held at the University of East Anglia, Norwich, from Monday, 13 July until Wednesday, 15 July, 1992 (inclusive). More details in subsequent newsletters.

How to Reach Us

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

The deadline for the next newsletter is **Monday, 7 October.**

The AFRC is Sixty

As part of the AFRC's 60th aniversary celebration, a function was held at the Royal Society on 25 June. One of the posters on display expounded some of the merits of *Arabidopsis* research and of the AFRC PMB *Arabidopsis* Programme. The ACM thanks Emily Lawson and Jo Putterill from The Cambridge Lab., J.I. for tending the *Arabidopsis* exhibit and spreading the good word.

From Sue Albini...

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

The objectives of this project fall into three main areas: (i) a thorough ultrastuctural study of prophase I of meiosis in *Arabidopsis*, (ii) the development of DNA-DNA *in situ* hybridisation (ISH) to plant synaptonemal complex (SC) preparations and (iii) the application of the latter to the former.

Good progress has been made in the production of SC preparations of *Arabidopsis*, all stages of prophase I of meiosis have been identified and the refinements made to the technique have made it repeatable and reliable. SC prepartions can be obtained consistently. Yield, however, is still low. Low yield is probably a limitation that cannot easily be overcome because only low numbers of pollen mother cells (PMCs) at prophase I of meiosis can be collected at any one point in time. Steps have been taken to maximise the numbers of PMCs available at prophase I of meiosis. Plants were grown under a short-day low-temperature regime until flowering. This produces plants with large leafy rosettes and inflorescences with short thick stems and densely-packed numerous flowers. Working with plants like this makes it much easier to locate buds at the appropriate stage. Floral mutants which produce many more anthers than the wild type are also proving useful. One mutant, Flo 10, can have as many as 20 anthers per bud, all reasonably synchronous. This has proved a big advantage when trying to produce *Arabidopsis* SCs. Despite the problem of low yield, enough SC spreads of reasonable quality can be obtained and a detailed analysis of chromosome pairing, the pachytene karyotpe and SC lengths of *Arabidopsis* is underway.

The second objective, of developing DNA-DNA ISH to plant SCs, has progressed very sucessfully over the last three months. This year, the set up for ISH has been established in our lab. It is a completely new technique in the lab so a lot of effort has gone into getting it up and running. The first success (on the third run) was the detection of biotinylated rDNA hybridised to plant mitotic chromosomes. The sites of hybridisation were detected using the horseradish peroxidase-DAB colourimetric reaction. In order to learn more about ISH and different detection methods I travelled to Norwich to work with Trude Schwarzacher and Pat Heslop-Harrison (courtesy of the AFRC PMB travel fund). The aim of the visit was to attempt ISH on rye SC preparations, using rDNA labelled with digoxygenin, and then to detect the sites of hybidisation using fluorescein. The results obtained were beyond our expectations. It actually worked first time. We showed that digoxygenin labelled rDNA hybridises to the site of the nuclous organising region in plant SC complements. The method developed by the Karyobiology group to perform ISH to somatic nuclei of plants needed no modification when applied to meiotic nuclei. Since returning from Norwich, I have repeated the experiment using biotinylated rDNA and a

"Digoxygenin-labelled rDNA hybridises to the site of the nucleolus organising region in plant SC complements."

fluorescent detection technique. This too was successful on both rye SCs and mitotic metaphases. As both biotin and digoxygenin labelled probes have been applied singly, double-labelling experiments are a realistic proposition. The aims for the next half year are: (i) to establish ISH as a routine technique in our lab (ii) to repeat the ISH experiments using SCs of other plant species (iii) to use a range of DNA sequences as probes, from highly repeated to low copy number (iv). to develop ISH to plant SCs for the electron microscope (v) to apply the DNA-DNA ISH technique to *Arabidopsis* SC preparations.

SH to plant SC preparations will enable very fine resolution mapping of DNA sequences, because chromosomes at prophase I of meiosis are much more extended than at *e.g.*, mitotic metaphase. Futhermore, the way in which the genome, or selected parts of the genome, interacts with the SC during meiotic chromosome pairing and recombination can be investigated in detail using this technique.

S.M. Albini, G.H. Jones & J.S. Parker; School of Biological Sciences, University of Birmingham, P.O. Box 363, Edgebaston, Birmingham B15 2TT.
ALBINISM@UK.AC.BHAM.IBM3090

From Ken Buck...

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

We have now confirmed the presence of all regions of our hygromycin ORI vector in transgenic plants via PCR and Southern blotting. Work is presently continuing on the isolation and growth of protoplasts which will be subjected to hygromycin selection to identify intramolecular recomination events. We have also grown on some plants to produce seed. It may be possible to select seedlings that contain replicating circles in most or all of their cells, if we can plate out enough to detect intramolecular recombination.

We have also now repaired the NEO gene in our NEO ORI vector by site-directed mutagenesis. This vector will be used to transform leaf discs within the next few days.

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

From Neil Butt...

Cell cycle control genes in *Arabidopsis.*

Mutants in the gene CDC34 of *Sacchromyces cerevisae* are defective in the transition from G1 to S phase of the cell cycle. The gene is relatively short encoding a 295 residue protein that is extensively homologous to the yeast RAD6 gene. The protein itself is a distinct member of the family of ubiquitin carrier proteins and has been associated with the degradation of cyclins during the cell cycle. A homologous gene has been isolated from other organisms including wheat. We have successfully isolated a member of this family from *Arabidopsis*. The gene contains three introns which vary in size, the largest being approximately 200bp in length, and the other two approxmately 70bp.

The gene was found to be highly homologous to the wheat ubiquitin carrier protein in the coding regions.

This gene has been used to screen the λZAP cDNA library kindly donated by Christine Raines and Philip Horsnell (Essex), and six putative positives have been isolated. These cDNAs have been purified and are currently being sub-cloned into vectors suitable for DNA sequencing. Southern blot analysis of *Arabidopsis* DNA cut with a variety of restriction endonucleases indicates the presence of many homologous sequences. Investigation of the cDNAs will hopefully provide other members of this family, corresponding to the multiple bands observed on Southern blots.

The putative cdc25 PCR product reported in the last issue has proved not to be as encouraging as initially thought. However, this could be due to the presence of a large intron in the gene adjacent to our 5' primer, so not all hope has been lost in this sequence. The worrying nature of this product is that the 3' primer can not be found after sequencing, and this primer was constructed from a relatively conserved region in the yeast/human/*Drosophila* sequences.

"We have successfully isolated a member of this gene family from *Arabidopsis*."

Since the last, issue we have set up our cell suspension techniques using the method supplied by Nigel Blackhall at Nottingham. As a high proportion of these cells are probably in the mitotic cell cycle, we are attempting to produce sufficient suspension cultures to enable the preparation of RNA. This is required for the generation of a cDNA library from actively dividing cells to facilitate the isolation of cell division cycle genes.

We have recently been investigating the presence of certain cell division proteins within *Arabidopsis in situ*. We have generated several samples of anti-sera to cell cycle proteins which we are using on various sections of the plant to try and observe the specific expression of these proteins.

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

From Jeremy Carmichael & Jim Murray...

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

One of our strategies is to use complementation of yeast mutants to clone plant homologues of cell cycle genes. To this end, we have modified two existing yeast expression vectors, one for budding yeast and one for fission yeast, for directional cloning of cDNA inserts. The vector for *S. cerevisiae* is based on galactose inducible expression whereas the vector for the *S. pombe* library has constitutive expression from an alcohol dehydrogenase promoter.

Our *Arabidopsis* callus is growing particularly well now that the dust has finally settled in the new labs. We have been preparing the poly(A+)RNA, for our cDNA library, directly from tissue homogenates using oligo(dT) cellulose in a batch process modified from a protocol of C.A. Raines. This method is relatively simple and fast. We now have poly(A+)RNA from cultures derived separately from leaf, root and whole plant. We are checking for degradation of the RNA using northern blots, but at this stage the quality looks good. If so, it should be possible to prepare the cDNA in the next few weeks.

Jeremy Carmichael & Jim Murray;
Institute of Biotechnology, University of Cambridge. Tel 0223 334754.
NC104@UK.AC.CAMBRIDGE.PHOENIX

From George Coupland...

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

June Swinburne

Recently, I have introduced a new *Ds* construct into *Arabidopsis* and will use it in the two component *Ac/Ds* transposon-tagging system. This *Ds* element differs from the one we have used until now in that it is marked with a hygromycin resistance gene. This modification should aid our tagging procedure in that it will allow identification of lines in which the element has re-inserted after excision. This *Ds* also has a CaMV35S promoter inserted 245bp from one end directing transcription out of the element. Insertion of this *Ds* could therefore cause dominant, over-expression mutations as well as recessive, loss of expression mutations. The *Ds* is inserted within a streptomycin resistance gene so that excision can be selected for with the antibiotic. A kanamycin resistance gene is the transformation marker.

So far, 22 independent transformants have been isolated. A number of lines are segregating approximately 3:1 for both hygromycin and kanamycin resistance and are all streptomycin sensitive. These are likely to be single locus transformants.

A control construct which has the element in the other orientation such that the CaMV35S promoter within the transposon directs transcription towards the streptomycin resistance gene has also been tested. Many transformants with this construct segregate 3:1 for streptomycin resistance as well as resistance to kanamycin and hygromycin. The promoter within the transposon is therfore capable of driving transcription of adjacent genes.

When homozygous lines containing one copy of the *Ds* T-DNA have been identified, this element will be incorporated into our tagging strategy.

June Swinburne & George Coupland.

Isolation of the flowering-time gene *fg*.

fg is located on the upper arm of chromosome 5, 2cM proximal to *transparent testa*-4, which is believed to be a mutation in the gene encoding chalcone synthase. We are chromosome walking using YAC libraries from RFLP markers in the vicinity of chalcone synthase towards the *fg* gene. We have one contig which includes RFLP markers 6833 and chalcone synthase as well as several cosmid clones provided by Brian Hauge (Boston). Our contig in this region is covered by at least seven overlapping YACs spanning a distance of around 600kb. At one point this contig is not covered by overlapping YACs, but is linked with a cosmid clone. Marker 6833 is positioned asymmetrically on this contig approximately 60kb from one end. Our second contig is around »

the marker 5962 which is 2cM proximal to 6833 on the Goodman RFLP map. We now have four YACs from two libraries that hybridise to this marker. These YACs span around 200kb. We have isolated end probes from the ends of two YACs in this contig, but these contain repetitive DNA which hybridises to a large number of YACs and therefore they have not helped to extend the contig. We have subsequently isolated two more end probes and are now using these to screen the library. Our third contig is around the Meyerowitz marker pCIT1243. This is 2cM proximal to chalcone synthase on the Meyerowitz unpublished map of June 1990 map. We have identified four YACs that hybridise to this marker, and using end probes from one of these YACs have identified YACs extending in both directions. Our strategy so far has been to walk in both directions from our three contigs in an attempt to overlap them. The overlap would then allow us to orientate the contigs on the chromosome.

Since the last newsletter we have analysed recombinants that were selected phenotypically to contain cross-overs between *tt*-4 and *fg*. We crossed a doubly marked *tt*-4 *fg* Landsberg *erecta* strain to Niedersenz and in the F2 selected three recombinants from around 650 plants which contained cross-overs between *tt*-4 and *fg*. The first of these was a *tt*-4 homozygote which is heterozygous for *fg*. As expected, the chalcone synthase probe on DNA from this strain only detects Landsberg *erecta* RFLPs. However, the probe derived from one end of our chalcone synthase contig detects Niedersenz RFLPs. From this analysis the order of the markers appears to be 6833-CHS-5962. We are confirming this with more markers and recombinants. Now that we believe we have orientated our CHS contig we can concentrate on walking in the direction which is towards 5962 and *fg*. To determine whether our contig already contains *fg*, and to position 5962 relative to the gene, we are analysing recombinants proximal to *fg*. This will take a bit longer.

Since 5962 and 6833 were reported to be 2cM apart, we would expect this, on average, to represent 300kb. If our prediction of the orientation of our CHS contig is right, these markers must be separated by at least 550kb of DNA. This discrepancy might be due to the markers having been originally mapped inaccurately or might indicate that the frequency of recombination in this region is less than expected on average.

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

Mapping T-DNAs which contain SPT::*Ds*.

In maize, tobacco and tomato, *Ac* transposes preferentially to genetically linked sites. If this is also true in *Arabidopsis*, it would be advantageous to know the map position of the T-DNA containing *Ds* prior to its activation. This would give us a means of increasing the frequency with which genes in that region are inactivated by insertion of the transposon. To do this, we have used the Inverse Polymerase Chain Reaction (IPCR) to isolate the DNA adjacent to the T-DNA in four lines transformed with *Ds*. The four IPCR fragments are 350bp, 500bp, 550bp and 1.4kb in length. Each has been sequenced and contains the expected sequences derived from the right border of the T-DNA. The exact point at which transfer occurred varies from 65bp to 22bp from the end of the right border direct repeat.

"This T-DNA is therefore on the upper arm of chromosome 1."

All four fragments have been used as probes against the Erwin Grill YAC library. Two hybridise to YACs which were previously shown to hybridise to RFLP markers. One hybridises to three YACs which Jing-Rong Peng (Cambridge Lab., Norwich) had already shown hybridise to RFLP markers PhyA and 322. This T-DNA is therefore on the upper arm of chromosome 1. A second IPCR fragment hybridises to YACs which Renate Schmidt (Cambridge Lab., Norwich) had earlier shown to hybridise to marker 4564. We believe this T-DNA is on the lower arm of chromosome 3. We are currently trying to map our other T-DNAs by RFLP analysis.

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

From Simon Covey...

CaMV infection of *Arabidopsis*.

We continue our screening of mutant (M_2) *Arabidopsis* with a severe strain of CaMV. The primary objective here is to isolate mutants which show attenuated or altered symptom characteristics. So far, we have screened about 3000 plants. We have found a small number of these that have shown less severe symptoms than the normal response, but we failed to obtain seed from them. A further six have remained asymptomatic after two rounds of inoculations and we will be testing the progeny in due course. At least one other mutant with unusual symptom characteristics has been found and from which we have been able to get viable seed which we can re-challenge with virus. It is arduous at times, but we keep reminding ourselves that just one genuine mutant affecting susceptibility to CaMV will open the flood gates! We are also looking at genetic variants of CaMV with altered symptom characters, including a very mild virus strain, to identify other interesting host/virus combinations including changes from tolerant to susceptible. More on this later.

Nothing more to report yet on the 2,4-D sensitivity phenomenon (see last Newsletter). We have also been exploiting *Arabidopsis* tissue cultures to manipulate the CaMV replication cycle. Although expression of the CaMV minichromosome in the nucleus of infected plants is regulated by the so-called constitutive 35S and 19S RNA promoters, we have known for some time that they are not well expressed in some organs/tissues (roots, callus, old leaves etc) of host plants in the context of the infection cycle. We have found a similar effect in *Arabidopsis* in that callus derived from infected leaves contains elevated levels of an inactive minichromosome. We have started to regenerate plants from this callus with the aim of re-activating the minichromosome to study how developmental factors influence its expression.

Simon Covey, Andrew Bannister & Andy Maule; John Innes Institute, Norwich.
COVEY@UK.AC.AFRC.JII

From Caroline Dean...

Transposon tagging.

Emily Lawson

While developing our transposon-tagging system, we have shown that a deletion, which removes 537 bp of the 650 bp long leader of the *Ac* transposase transcript, increases *Ac* activity in the *Arabidopsis* genome. Plant DNA has been shown to be methylated at CpG and CpNpG motifs, and this leader sequence is particularly rich in these sequences. The deleted derivative therefore has less methylateable sequence and so I have been trying to ascertain if the cause of the difference in activity of the two elements could be a lower level of methylation in the deleted version. Methylation of plant DNA can be studied using enzymes which recognise sequences containing CpGs and CpNpGs, and which are sensitive to methylation. So far, blots using the isoschizomers *Eco*RII and *Mva*I (recognising CCA/TGG) have shown no methylation of several lines carrying inactive or very low activity wild-type or deleted elements. As I write the blots using the more frequent cutters, *Hpa*II and *Msp*I (recognising CCGG) are being exposed, but I feel safe in saying that it looks like methylation is not the answer. This is hardly surprising when one considers that the *Arabdopsis* genome has a very low level of methylation when compared to most plant species (About 5% according to Priutt & Meyerowitz (1986), compared to an average of 30-40% for a range of plants examined by Gruenbaum (1981)).

Leutwiler, L.S., B.R. Hough-Evans, & E.M. Meyerowitz (1984) *Mol. Gen. Genet.* **194** 15-23.

Gruenbaum, Y., T. Naveh-Many, H. Cedar, & A. Razin (1981) *Nature* **297** 860-862.

The *Arabidopsis* genome project.

Alma Balestrazzi, Gerda Cnops & Renate Schmidt

Alma has joined us for a couple of months from Pavia to share the glories of "chromosome crawling".

After having isolated corresponding YAC clones for the 33 RFLP markers on the top halves of chromosomes 4 and 5 from the EG library (see March Newsletter); we have, in the last few weeks, isolated another 70 YAC clones corresponding to these markers from the EW library (Columbia ecotype). All these clones have been sized on CHEF gels and have been confirmed with Southern blots. With the identified EW clones we have now identified more than one YAC clone for nearly all of the RFLP markers, this duplication will help us to identify potential chimaeric clones.

"The glories of chromosome crawling"

We recently had a lot of problems with unequal yeast colony growth, which badly affected the screening. After using YEPD-medium which is complemented with 100 mgl^{-1} adenine all colonies now grow equally well. The modified version of the protocol also allows us to use even more densely plated master filters (1536 colonies per 7.5 x 11 cm nylon membrane).

We are concentrating at the moment on walking in the region around RFLP markers 226, 326, 580, 210, 4108, 6837, 10086 and 4564. Plasmid rescue of the left ends of YAC clones in *E. coli* as an alternative method to IPCR works very efficiently. On the one hand we are using end probes to verify the contig we identified with the RFLP marker hybridizations (see March Newsletter), whilst on the other we are currently expanding the contig by walking.

The mechanism of vernalization.

John Chandler

An EMS-mutagenised population of the fca locus, confers a late flowering phenotype that is responsive to vernalization, continues to be screened to isolate individuals no longer responding to vernalization. These mutants, if not themselves late flowering, have mutations in the perception and/or transduction of the cold signal. Of 240 families screened so far, at least 10 putative mutants have been isolated which are significantly later-flowering than fca after a vernalization treatment. Of these, at least two are not later flowering than fca without vernalization. The phenotype of further putative mutants is currently being quantitatively checked, before starting a crossing program to Landsberg *erecta*, to analyse the genetic nature of the mutation. The same EMS-mutagenised population also continues to be screened for suppressor mutants of fca which flower at the same time as wild type Landsberg *erecta*. A lot of variation towards early flowering is released following mutagenesis, but only those individuals with the earliest flowering phenotype are being checked for phenotype in the next generation, before a crossing programme to fca.

In order to see how the response of fca to vernalization and flowering time might be altered by the presence of a second mutation, the fca mutant has been crossed to a range of starch, lipid and hormone mutants, . So far, seed has been obtained for mutants of fca with gai, Abi 1, 2, 3, and two different starchless mutants. An F_2 population is currently being screened for fca/starch overproducer double mutants. So far, the F_2 segregation data for the fca x gai cross show that the gai mutation does not affect the flowering time of fca.

Still mapping *fca*!

Rob Ewing & Lore Westphal

We are still mapping the *fca* locus, which confers late-flowering in *Arabidopsis thaliana* ecotype Landsberg *erecta*,using Meyerowitz's Lambda RFLP markers and, more recently, Goodman's cosmid RFLP markers. Combining these data should also enable us to integrate both RFLP maps in our region of interest on chromosome 4. We have also started utilising end fragments of YAC clones as probes. These YAC clones are part of contigs identified by Gerda Cnops and Renate Schmidt. In order to extend our collection of individuals showing recombination between *fca* and tightly linked RFLP markers, a cross between the triple mutant *hy4 fca cer2* in Landsberg *erecta* and ecotype Columbia will be pre-screened for recombinants employing the phenotypical markers available. These recombinants will be checked subsequently for recombination between *fca* and adjacent RFLP markers.

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

Jonathan Clarke

I am currently using the tech- ➤

nique of interval mapping to locate the locus/loci influencing the late flowering and vernalization response of line H51 (containing the *FRI* Locus from the winter annual Stockholm [St] in the background of a summer annual Limburg 5 [Li5]) in a cross with Landsberg *erecta* (L.*er*). Segregation data from 50, randomly selected, F_3 families gave a ratio of 20 early flowering : 27 segregating : 3 late flowering families. This suggests the presence of two loci with a number of possible epistatic interactions. A genetic analysis of the flowering character of Li5 in a cross with L.*er* conducted in the same environment had revealed no segregation for flowering time in the F_2 population. However, during an analysis of the effects of photoperiod on flowering time I observed that under 10 hour short days the developmental rate of Li5, as indicated by the number of rosette leaves at the time of flowering, was more rapid than that of L.*er* while no difference in flowering time was detected. From the evidence so far, we conclude that a single locus from L.*er* modifies the expression of *FRI* and accounts for the segregation ratios observed in the H51 L.*er* F_3 families.

I am just harvesting the F_3 seed from 100 F_2 individuals from a St/L*er* cross. This material will be the basis of a Quantitative Trait Loci Analysis (QTL) of Stockholm's late flowering character. From the segregation seen in these F_2s, which had been fully vernalized, it is clear that a likely of vernalization insensitive/less sensitive loci are present. Segregation for photoperiodic response is also being assessed.

C. Dean *et al*; Norwich.
ARABIDOPSIS@UK.AC.AFRC.JII

From John Doonan...

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

Antibodies against nuclear structural proteins have identified several clones from the Clontech *Arabidopsis* expression library. Molecular analysis of some of the JIM63 clones is underway. Screening for nuclear pore gene is about to commence using NSP-antibodies raised against mammalian pores, but which cross-react well with plant pores.

John Doonan; Dept. Cell Biology, John Innes Institute, Colney Lane, Norwich, NR4 7UH.
DOONAN@UK.AC.AFRC.JII

From David Evans..

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

Since our last entry, we have made a number of significant advances in our work, but also have some continuing problems. Work to reconstitute the purified calmodulin-stimulated ATPase has been going extremely well and Per Askerlund has achieved the first functional reconstitution of calmodulin-stimulated calcium transport and ATPase activity from plants. The specific activities are very good when compared with mammalian systems. However, the coupling efficiency (ATP:Ca) still leaves a little to be desired. Having a reconstituted system opens lots of possibilities for studies on regulation at the molecular level and rapid progress will now be possible on this front. In a parallel project, an SERC student in my lab has just produced a family of monoclonal antibodies that recognise the pump. As well as being useful for further cloning/sequencing approaches, these antibodies will be very useful for structure funtion relationship studies and studies on post-translational regulation. Work on cloning and sequencing continues to be like running uphill through treacle. Joy Boyce continues to put in long hours probing libraries by a variety of means and while we have a few 'positives' to follow up, nothing is yet looking definite. Hopefully, we should learn something as we follow up positives detected by the new antibodies from an expression library and should get some fragmentary sequence. PCR work in the Coates' lab so far has also failed to produce any ATPase like sequence. Finally, the Coates' lab has successfully completed production of a new genomic library. People interested should note that it is interestingly packaged, human (male) and rather demanding on media. Enquiries for samples from the library to the proud parents, David & Janice Coates. Janice has now retired from the AFRC grant.

D.E. Evans, D. Coates, J.M. Boyce, J. Coates & P. Askerlund; Plant Sciences Oxford and Pure & Applied Biology, Leeds.
DEEVANS@UK.AC.OXFORD.VAX

From Gary Foster...

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

The expression of genes during the development of the male gametophyte, the microspore, is of interest both academically and commercially. The cytological aspects of pollen development have been studied at length, but little is known about the genes expressed specifically during microspore development. We have cloned and sequenced six microspore-specific cDNAs (F2sL, F2S, E2, I3, CEX1 and CEX2) from *Brassica napus*. The predicted polypeptides of the I3 and CEX1&2 cDNAs are proline-rich and contain putative N-terminal membrane-spanning domains. Transcripts are detected in buds and anthers of male fertile plants, but not in green tissues, roots or in cytoplasmic male sterile buds. The I3 and CEX genes are expressed following microspore release and expression continues until the onset of pollen grain formation in *B. napus*. The corresponding *Arabidopsis thaliana* gene (APG) for one of these cDNAs (CEX1) was isolated (using the related I3 cDNA as a probe) and the coding and upstream regions identified. The putative microspore-specific promoter within this upstream region was linked to GUS and transformed into tobacco (0.5kb and 1.5kb upstream region) and *Arabidopsis* (1.5kb upstream region). Expression of GUS in tobacco was not detected in green tissues, stems or roots, but was present in anthers. GUS activity appeared in the microspores shortly after their release from the meiotic tetrads, and was maintained until the interphase after the first pollen mitosis. Strong GUS

activity was also detected in the tapetum for a short time prior to this mitosis in tobacco. Strong GUS activity was also detected in the microspores of *Arabidopsis* when a 1.5kb upstrean region was used with GUS fusion constructs, though this is currently being further analysed. Northern analysis of the E2 transcript also showed high expression in developing microspores as well as in the tapetum, as detected by *in situ* hybridisation. The upstream region from E2 has now been obtained by inverse PCR from *B. napus* genomic DNA and is currently being sequenced. Current work includes functional analysis of these cDNAs by sense and antisense constructs with the *A. thaliana* promoter in transgenic plants.

So after isolating microspore specific cDNAs from *Brassica napus*, the equivalent gene from *Arabidopsis*, the promoter of which was tested in tobacco, we have now returned to our beloved hero, and tested the promoter in *Arabidopsis*!

All of this work is leading up to our original aims, which are to increase the frequency of germinal transpositions of *Ds* and to rescue the flanking DNA sequences via YAC vectors.

"GUS activity appeared in the microspores shortly after their release from the meiotic tetrads."

To date the isolation of a plant gene of interest for which the product is unknown, but a mutant phenotype has been observed, is classically carried out by either chromosome walking or by transposon tagging. An improvement on both of these approaches might be achieved by a combination of both processes carried out *in vivo*. We have placed the essential components of a Yeast Artificial Chromosome (YAC) vector into the maize transposable element *Ds*, thus a gene of interest may be tagged by close linkage to the transposing YAC vector and large flanking regions rescued as a YAC vector into yeast. In subsequent generations the preference for *Ds* to transposase to linked sites could be utilised to capture limited contiguous YAC clones to a specific region of the chromosome, and thereby allow for a limited chromosome walk *in planta*. We are currently demonstrating the rescue of these molecules from plant DNA by transformation into yeast. These constructs will be stabilised within the plant by expressing the transposase from the *Ac* element *in trans* via the microspore-specific promoter which we have isolated from *Arabidopsis*, thus allowing one transposition per generation to occur. This promoter, isolated from *A. thaliana* via microspore-specific cDNAs from *B. napus*, gives no expression in green tissues, stems or roots of tobacco, *A. thaliana* and *B. napus*, but activity was detected in the microspores shortly after their release from the meiotic tetrads, and was maintained until the interphase after first pollen mitosis. It is now being tested whether this promoter when used to express transposase from *Ac*, will increase the frequency of germinal transpositions of the trans-activated element, *Ds*. Excision reconstitutes a streptomycin gene, thus allowing screening for transposition in the F_1 generation, with reinsertion being monitored by a hygromycin gene within the *Ds*. This is a feature highly desirable in transposon tagging, where the generation of large numbers of plants with unique stable inserted elements is required.

Dr. Gary Foster, Dr. Rod Scott, Dr. John Draper, Mr. Mike Roberts, Mr. Rob Blundell & Ms Sue Robinson; Botany Department, University of Leicester, LE1 7RH.
Tel: 0533 523393; FAX: 0533 471001.

From Ian Furner...

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

The fate mapping work using X-ray induced sectors on plants heterozygous for the alb 1 mutation is going well. I have now looked at 212 sectors on the leaves, stems and flowers of treated plants. The plants were treated as dry seeds and a reasonable presumption is that each sector is the product of a single cell in the seed apex. Marked sectors affecting all the aerial structures have been observed. However, the frequencies are very different. For example, half the sectors appear on the first leaf apex and the great majority of these are confined to these structures. In contrast only one seventh of the sectors appear on the flowering spike and these usually include tissue in the cauline leaves. This result suggests that the first leaf apex is a large target and the flowers a small one.

"...the first leaf apex is a large target and the flowers a small one."

The pattern of sectors suggests that *Arabidopsis* has the L1, L2, L3 layers in the meristem characteristic of most dicots. However, L3 does not usually contribute to the cauline leaves and flowers. This might have something to do with the rosette habit of the plant. The phenotype of shoots from the axils of sectored plants corresponds to that of the centre of the leaf immediately beneath them suggesting that the axillary meristem is initiated next to the centre of the leaf, but is much narrower. Sectors contribute to a variable number of nodes before they become fixed (contributing all subsequent L2 tissue) or lost from the meristem. In general, clones on the latter leaves are larger and contribute to several leaves and frequently to the flowers. In small subset of plants the sectors are very large, contributing to most, if not all leaves. In such plants the meristem must consist of very few cells (I estimate two to five) and it is unclear whether this implies either the existence of some seeds with very undeveloped meristems or a sub-population of peculiarly radioactive seeds with the large marked contribution due to "repopulation" of the damaged meristem. I have been looking at sectors affecting the flowers and I have succeeded in producing a rough fate-map based on the frequency with which any two structures are of the same colour (green or white). The result is a dome with the phylotaxy superimposed on the top. I am currently trying to increase the sample size to make this map accurate.

Joanne Pumfrey has joined the laboratory as a Research Assistant. She is currently working on the meristem mutants. Most of the work at this point is still screening EMS derived M3 and M4 populations for interesting phenotypes. We intend to ➤➤

start a more detailed morphological characterisation of the lines obtained so far in a few weeks.

I have been working on a new fasciated mutant (Ac1-37). It was found in the progeny of a plant containing the 35s-Ro1C-*A c* construct. Unfortunately, it does not appear to be a tagged mutant, since blots of this line show no evidence of transposition events. It is presumably a somaclonal event of some kind. Somaclonal variants are quite common in transformants produced by Valvekens's protocol. I am currently testing allelism with previously described mutants and mapping it to morphological markers.

Paul Davison (the postdoc on the project) has been trying to obtain apex specific cDNA clones. His initial experiments with a library derived from *in vitro* produced apicies were discouraging and no useful clones were obtained. He has now hand dissected 11 grams of seedling shoot tips and produced a second library. This will be screened with shoot tip cDNA and leaf cDNA to look for specific clones. Carol Merideth has recently published an article describing five apical meristem clones obtained by using cDNA derived from cauliflower apicies. I believe this project is still worthwhile and that is is likely that at least some of the clones we obtain will be unique and distinct from those obtained using a heterologous probe.

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

From Nic Harberd...

An attempt to clone the gai locus by phenol-enhanced DNA re-association.

We are making progress on several fronts. A comprehensive update will be provided in the next issue.

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

(Nic has promised an epic for next time to follow on from the above haiku ~ ACM.)

From Carole Harker...

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

Seven independent transgenic *Arabidopsis* plants homomzygous for CaMV gene I, the gene encoding the protein (P1) implicated in cell-to-cell spread of the virus, have been obtained. These plants are at a stage where they can be tested for their ability to express the P1 protein, its subcellular location and the phenotypic consequences of gene I expression. Segregation analysis indicate that all the selected plants have gene I insertions at single loci, this is being confirmed by genomic Southern analysis. Two methods are being used to determine the ability of these plants to express P1; firstly, western analysis of samples obtained from the subcellular fractionation of these plants, and secondly, a more direct test, the ability to complement P1 mutants of CaMV.

"This is the first direct evidence that P1 is the cell-to-cell spread factor of CaMV."

Only those transgenics that express P1 at the correct level and intracellular site will allow complementation and hence spread of the P1 mutant. The P1 mutant that is being used for this has had the central third of the P1 protein removed and has been tested on turnips for its ability to spread and replicate. Agroinoculation of leaf discs and whole plants have demonstrated that this mutant is replicationally competent, but unable to spread. This is the first direct evidence that P1 is the cell-to-cell spread factor of CaMV. Several other P1 mutants are being constructed and analysised to identify functional domains of the P1 protein.

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

From Nick Harris & Phil Gates...

Development of the silique of *Arabidopsis*.

Jacquie Spence has been using *in situ* hybridisation, immuno- and enzyme- histochemistry to look at various changes associated with the cellular differentiation that occurs during silique development. We have been particularly interested in the patterns established in the fruit prior to fertilisation and how these are related to the changes which occur after fertilisation, and during seed development and fruit ripening. Lesley Edwards has been labelling and using *in situ* probes, assiduously collecting tiny, early gynoecia, and has started with RNA extractions prior to subtractions and the construction of libraries. We continue to screen EMS-induced material and have found some silique variants (in M_2 and M_3) which look to be of interest, with changes in form, rate of development, etc.. Green fly, root fly and red spider mite made a combined assault in early May, but (we hope) have been repulsed: 'imported' ladybirds were quite successful for a while but they all 'flew away home'.

N Harris & P Gates; University of Durham, Department of Biological Sciences, Durham DH1 3LE
Tel: 091-3742417 Fax: 091-3743741
NICHOLAS.HARRIS@UK.AC.DURHAM

From Pat Heslop-Harrison & Jola Maluszynska....

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

Arabidopsis thaliana has five pairs of chromosomes (2n-10), of which two pairs have rDNA genes (see Araba-Daba-Dopsis; The Plant Journal, in press, issue 2). The rDNA sequences occupy about 8% of the total genome, so we decided to look at other species of *Arabidopsis*, because it is interesting to study the evolution and variation in this type of tandemly repeated sequence, and other species might be more amenable for *in situ* mapping of single copy clones. Although DNA amounts have never been measured in these other species (How accurately has it been measured in *Arabidopsis thaliana*? - see bulletin board recently), visual microdensitometry indicates they have similar contents of DNA to *Arabidopsis thaliana*. However, in some cases the DNA is distributed over

many more chromosomes. *A. griffithiana* and *A. pumela* both have 16 chromosome pairs (2n-32), and major sites of rDNA on three pairs, with minor sites on another pair. *A. wallichii* has eight pairs of chromosomes (2n-16), and only one major pair with rDNA.

"Does anybody have seed of any other *Arabidopsis* species which they could let us have?"

What are the longest fragments which can be separated by PFGE? Two of the above species apparently have chromosomes which are half to a third of the average size of *Arabidopsis thaliana* chromosomes. Could these be separated by PFGE? If so, the resulting blot could then be used to screen YAC libraries to map the YACs to smaller linkage groups. There may be translocations, fissions and fusions between the chromosomes in these species with respect to *Arabidopsis thaliana*, but it is unlikely that the genome would be scrambled! Indeed, if the translocations vary between the species, they might be used to help link clones, since characterisation of the PFGE spots would be easy by hybridisation with the RFLP markers (as in wheat, rye and barley - Mike Gale and colleagues).

Does anybody have seed of any other *Arabidopsis* species which they could let us have?

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

From Eric Holub...

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

At the March general meeting of the AFRC-PMB programme in Reading, we described a hypothetical model based on three matching pairs of genes to explain specific resistance of *A. thaliana* to different isolates of *Peronospora parasitica*. This working model was proposed as the simplest explanation of differential interactions observed between six ecotypes of the host (Tsu0, Nd0, Oy0, Col0, La-er, and RLD) and four isolates of the fungus. Thus far, Tsu0 and RLD have been susceptible and resistant, respectively, to all isolates tested, using lack of asexual sporulation as the criterion for resistance. The other four ecotypes are differentially resistant to at least one isolate. Plants selected from each of the six ecotypes were included in the half-diallele cross that we described in the March 1991 newsletter. We have concentrated effort on producing F_2 and S_2 generations of seed from crosses made between these parents. Ten of 21 possible F_2 and S_2 stocks have been produced, but the remaining stocks have been slow to advance because of seed dormancy.

As a prelude to fine-scale mapping, we will proceed by confirming the existence of these putative genes for resistance to *P. parasitica*, and determining their chromosome location. The first step entails assessing the validity of or modifying the proposed model by examining segregation among F_2 progeny from the half-diallele cross of the six ecotypes. Jim Beynon (Wye College) is helping us with this work, and we have invited Alan Slusarenko (Zurich, Switzerland) to assist as well by testing F_2 progeny with his isolate of *P. parasitica*. Chromosome locations will be determined by examining the co-segregation of resistance genes with available RFLP markers (obtained from Elliot Meyerowitz). F_3 families from two crosses, Nd0 X Oy0 and Col0 x La-er, have been given to us to test for segregation of disease resistance by Jeff Dangl (Cologne, Germany) and Mike Daniels (Sainsbury Laboratory), respectively. Jeff is utilizing the Nd0 X Oy0 cross to map a gene for resistance to an isolate of *Pseudomonas syringae* pv. *maculicola*. He has already begun to map this gene and has formally agreed to share with us the relevant co-segregation data. Jim has also been active with us in preparations for future work with RFLP markers by establishing polymorphisms between other parents that we have been used in crosses. There is certainly a long way to go, but the combined information of formal genetic analysis and chromosome location will be an invaluable foundation for deciding the future direction of co-ordinated research of several groups likely to be involved in fine-scale mapping of genes for resistance to *P. parasitica*.

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

From Gareth Jenkins...

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

Since the last Newsletter, the number of *Arabidopsis* plants in our growth rooms has increased exponentially and the shelves are now bulging with M_1, M_2, M_3, T_1, T_2, T_3, T_3M_1, T_3M_2 *etc* plants. Soon we will need an administrative assistant to keep track of all the plants! The project is also expanding in numbers of humans. We will have at least one Ph.D student joining the project in October and there will be two temporary assistants working with us over the summer on screening and preliminary characterisation of mutants.

As a reminder, the main objective of our project is to isolate novel photoregulatory mutants. Our principal strategy is produce transgenic *Arabidopsis* expressing chimeric genes consisting of a photoregulatory promoter linked to an appropriate reporter gene and to screen mutagenised transgenic seedlings for aberrant light-regulated or organ-specific expression of the reporter. Such plants are putative photoregulatory mutants. We are using several different promoters and are investigating the suitability of various reporter genes and have therefore made a number of different constructs. It took some time to obtain all the relevant promoters and reporters, to make the constructs, transform, collect T_2 seed, mutagenise homozygous populations of T_3 seed and collect the T_3M_2 seed, but we have finally got there! Large scale screening of a chalcone synthase promoter-GUS T_3M_2 seed population starts next week and other T_3M_2 populations will soon be ready. We have decided to try to screen using GUS having talked to other labs ➤

who are doing this and having undertaken our own feasibility study. We have been using luciferase and have obtained expression, as monitored using the Aberdeen University CCD camera, but the levels of expression are lower than we had hoped and further work will be needed before we can use luciferase for large scale screening. Hopefully, if the GUS screening goes well, it won't be necessary.

In addition, while we were carrying out the above work, we initiated a programme of conventional screening. M2 seed were collected from groups of 20 M1 plants and over 10,000 seedlings were grown in a blue-enriched light environment. We screened for mutants that showed abnormal responses to blue light, such as a long hypocotyl, long petioles, low anthocyanin content or poor leaf expansion. A further 5,000 seedlings were screened in a red light environment (which contained no blue light) for plants that showed a blue light phenotype, *i.e.*, short hypocotyls and petioles, fully expanded leaves and high anthocyanin. These screens yielded several potentially interesting mutants of the kind we wanted and we are now re-screening them under blue and red light regimes. Hopefully some of these plants will be specifically deficient in blue light responses. The screen also gave us other classes of putative mutants, such as those with altered leaf number or leaf shape, some of which we will look more closely at.

In the next Newsletter we will hopefully be able to report on the preliminary characterisation of some new mutants and the screening of transgenic populations.

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

From Kerrie Jones...

Ammonium toxicity in *Arabidopsis*.

We have screened both an *Arabidopsis* cDNA library (from Christine Raines) and an *Arabidopsis* genomic library (from David Coates) for the gdh gene(s), using our *Arabidopsis* PCR product as a probe. So far, none of the positives isolated have proved to be gdh, but we are analysing further clones. The cDNA library is also being screened with a heterologous probe and is being sreened for complementation of an *E. coli* auxotrophic mutant. A new series of experiments involves exploring the concept of expressing the *E. coli* gdhA gene in *Arabidopsis*. Previous work here at Leeds has demonstrated increased ammonium tolerance in a cyanobacterium carrying a foreign gdh gene. A construct with the gdh gene under the influence of the CaMV35S promoter will be transformed into *Arabidopsis* and examined for GDH expression and for ammonium- tolerant phenotype.

Kerrie Jones (1,2), Mike McPherson (1) & Andy Cuming (2); 1 Dept. of Biochemistry & Molecular Biology; 2 Dept. of Genetics, Leeds University, Leeds U.K.
KERRIE@UK.AC.LEEDS.BIO.VAX

From Peter Jordan...

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

Work is progressing satisfactorily on the isolation and cloning of the cDNAs of the hemL, hemB, hemC and hemE genes from *Arabidopsis thaliana*. In addition, refinements in the purification of the related enzymes are in progress and it is hoped that N-terminal sequencing will be possible shortly.

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

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

We have been generating more plants containing native gene-gus fusions, and now have about 500 independent transformed lines. A major effort is in screening floral organs for gus activation, and we have identified a number of interesting lines. These include one in which expression has been detectable only in anthers, one only in mature pollen and one, in the stigmatic surface. These will be characterised in more detail, while the general screen continues. T2 seed is being bulked up and the plants will be screened for expression in embryos (as part of our BRIDGE project) and in roots. We are also looking for stable phenotypic mutants in this population.

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

From Keith Mitchelson...

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

I would like to introduce Beata Luczak and Andy Porter to our band of happy readers (Hi there!). Beata is a post-graduate Tempus student from Szczecin in Poland and Andy is a post-doc newly arrived from Rothamsted, where he worked as part of the zero pesticides group on glucosinolates in Brassicas and *Arabidopsis*.

After much hard work testing different hybridisation conditions and different restriction enzymes Beata has developed the conditions for detection of hypervariable RFLP's using a number of different sequence probes. Thus far, results are very promising. Difference signals are detectable between four *Arabidopsis* ecotypes with three of the five probes so far tested. Several untested probes are in the pipe-line to be screened and thus we hope to have results with a variety of different probes in the near future.

Andy has commenced the screening of YAC clones (Colombia library) with several of the positive probes. Putative positive clones appear to be present in YAC chromosomes from an initial screening of pooled cultures. These clones produce an intense autoradiographic signal readily distinguishable from a weak signal seen with all yeast host chromosomes. *Arabidopsis* hypervariable elements which hybridise weakly with these probes will therefore not be easily detectable using pooled YAC clones. Screening of individual clones will commence shortly.

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

From Steven Neill...

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

In vitro translations of mRNA isolated from turgid, ABA-treated and wilted leaves have already demonstrated the production of at least one polypeptide in response to ABA and one in response to stress *per se*. We have extended these studies to investigate the kinetics of these responses and to look at gene expression in the wilty *aba*^{1} mutant.

We have prepared a cDNA library from water-stressed leaves and are currently screening this library. To pick out stress-inducible clones, we had originally intended to use cDNA probes representing turgid *aba*^{1} (*i.e.*, ABA-deficient) tissue. However, whilst this mutant is indeed unable to synthesise ABA in response to water stress, it is not, strictly speaking, an ABA-deficiency mutant. Rather, the genetic lesion has resulted in an inability to synthesise violaxanthin, the putative carotenoid precursor of ABA (Zeevaart; Taylor, both in press). As the mutant is very wilty and there is no reason to suppose that it is impaired in its ability to perceive the signal of water stress, there is a risk that mRNA isolated from mutant tissue would contain stress-induced transcripts. Consequently, our screens are using cDNA probes from turgid and wilted wild type tissue. The mutant will be used to differentiate between clones induced by stress and ABA.

Alternatively, it may be possible to use PCR to generate sufficient cDNA from very small amounts of tissue, *e.g.*, from a single tissue-cultured, non-stressed *aba*^{1} plant). In conjunction with Mike Bulman, a graduate RA supported by the SERC, we are currently attempting such PCR amplification of cDNA, both to produce mutant cDNA and to generate sufficient "driver cDNA" for use in subtractive library construction.

Steve Neill & Jackie Williams; Bristol Polytechnic.

From Helen North...

Cell cycle control genes in *Arabidopsis*.

We have been trying to construct cDNA libraries from carrot suspension cells and cauliflower meristematic tissue in an *S.pombe* expression vector in the hope that we may be able to complement yeast cell division-cycle mutant functions with plant proteins. Unfortunately, despite several attempts, we are unable to obtain significant numbers of clones by directly constructing the library in the expression vector. It has been decided to change strategy and construct a λ library and then subclone into the expression vector. This will additionally enable us to screen the λ library with yeast *cdc* gene probes which we also have available.

Meanwhile, our test transformation with a *S.pombe* genomic library on the *cdc 7* mutant have been promising. In order to gauge the numbers that are needed to yield various classes of complementing plasmids, all positive rescuing plasmids are being followed through. Four thousand five hundred transformants were obtained which were able to grow on minimal media and by complementation of an auxotrophic marker on the plasmid. When tested for their ability to grow at the restrictive temperature, 120 of the transformants complemented the *cdc 7* mutation. These were examined by light microscopy for their phenotype and only those that looked morphologically normal were pursued. The plasmids from the 71 remaining were isolated from *S. pombe* and transformed into *E. coli* and plasmid prepared. As these plasmids were to be retransformed back into *S.pombe*, to confirm their ability to compliment the *cdc 7* function, they had to be purified on caesium chloride. It was possible to isolate plasmids from only 66 of the *S. pombe* transformants and these are now being retransformed back, restriction mapped and classified into groups.

From Jane Parker...

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

Our extensive screening of *A. thaliana* ecotypes for variation in the reaction phenotype to *X.c. campestris* strain 8004 and strain 8004 containing the avirulence gene (*avr*Xca) has uncovered three ecotypes which are susceptible to 8004(*avr*Xca) by the criterion of disease symptom development. These are Aa-0, Pr-0 and Kas-1. Analysis of bacterial growth in a cloned population of Kas-1 plants shows that 8004(*avr*Xca) grows to a higher titre than in Col-0 (our resistant line) and disease symptoms are evident after 4 days. However, its growth in Kas-1 is not as great as 8004 minus the *avr*-containing cosmid. We are checking that there is no great loss of cosmid in Kas-1 which could lead to symptom development, although previous tests have shown that the the cosmid is stable in Col-0 and *Brassica* plants with only 5-10% loss after 6 days. We have yet to complete growth curves in Pr-0 and Aa-0. Interestingly, there is one ecotype (Ll-6) which is resistant to *X.c. campestris* strain 8004. The plants are symptomless after 6 days and growth is about 50-fold less than in Col-0 plants. Ll-6 is therefore a source of resistance genes directed against strain 8004.

Chris has made good progress in sequencing the *avr*-functional region of the 25 kb cosmid insert identified by Tn-5 mutagenesis (described in the last report). We are just about to search for homologies and 'interesting' features.

"Ll-6 is therefore a source of resistance genes."

We have also marker-exchanged some of the Tn-5 insertional mutants of the *avr* gene into the *X. campestris* parental strain 1067 to test if these become pathogenic on ➤

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

normally resistant Col-0 plants. They did not! This may be because strain 1067 has additional *avr* genes directed against Col-0. Alternatively, the *avr* gene product has a vital function in 1067 pathogenic fitness. Or is it because 1067 *is* pathogenically defunct? Answers hopefully in the next installment.

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

From Kevin Pyke...

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

The screening of an M2 population for chloroplast accumulation mutants has led to the isolation of 18 different lines, all stable to the M5 generation. We have now analysed the changes in chloroplast number during mesophyll cell expansion in four mutants representing the most extreme variants within our mutant collection. All show trends in chloroplast accumulation which are consistently different from those observed in wild type. For all four mutants the total chloroplast cover in a cell of a given size is very similar, but partitioned in different ways in different lines. At the extreme, mutant YKA80 shows a rapid increase in chloroplast number with cell expansion, but the chloroplasts remain small. In contrast, YKL75 shows a minimal increase in chloroplast number during cell development, but extensive chloroplast expansion. The relationship between chloroplast number and cell size for the mutants and for the wild type suggests that the number of proplastids present in the youngest dividing mesophyll cells is very similar in all lines, *i.e.*,. 14-16 proplastids per cell.

> "The number of proplastids present in the youngest dividing mesophyll cells is very similar in all lines."

We have begun the genetic analysis of the mutants by reciprocal backcrossing to wild type and analysing the two subsequent generations. Preliminary tests for allelism have also been carried out.

A paper is in press in *Plant Physiology* describing the screening procedure of stained leaf cell preparations using image analysis. Another paper, entitled, "Temporal and spatial development of the cells of the expanding leaf of *Arabidopsis thaliana* (L.) Heynh." by Pyke, K.A., Marrison, J.L. and Leech, R.M., is in press in the Journal of Experimental Botany.

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

From Peter Quinn...

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

Following the departure of Srinivas Volety from the project earlier this year a search has been underway to locate a replacement post-doctoral assistant. In the meantime, the project has been moving forward with the assistance of Nelly Tsvetkova who is currently visiting the lab. under the auspises of a FEBS Postdoctoral Fellowship from the Central Laboratory of Biophysics of the Bulgarian Academy of Sciences in Sofia. She is experienced in biophysical studies of membrane lipid-water systems using thermal and diffraction methods and freeze-fracture electron microscopy. Her initial task will be to prepare suitable quantities of lipids from chloroplast membranes for analysis.

Peter Quinn; Biomolecular Sciences Division, King's College London W8 7AH.
UDBC600@UK.AC.KCL.CC.OAK

From Chris Raines...

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

At the time of the last newsletter, Phillip Horsnell had just isolated a number of genomic clones encoding fructose-1,6-bisphosphate (FBPase) and two for phosphoribulosekinase. We now have around three kb of sequence for the FBPase which includes two kb of upstream sequence. By the end of this week work will start on preparing deletions and constructs for analysis in transgenic plants. Analysis of the phosphoribulosekinase clone is continuing and a number of subgenomic fragments have been subcloned. End sequencing of these clones indicates that they contain some 5' upstream sequences.

C.A. Raines, N.R. Baker & M. O'Farrell; Dept. of Biology, University of Essex.
RAINC@UK.AC.ESSEX *or*
RAINESC@UK.AC.AFRC.ARCB

From Colin Robinson...

Isolation and analysis of *Arabidopsis* chloroplast biogenesis mutants.

The main focus of the screening programme is on the isolation of thylakoid import mutants, which should be able to germinate, but not carry out photosynthesis. Such mutations should be lethal, and we have therefore built up a library of EMS-mutated M1 seeds (in groups from five plants) in order to be able to retrieve the mutation. The screening protocol involves growing M2 seedlings from a number of parental groups in the dark for five days, and then transferring to light for two days. Seedlings that do not green are analysed by harvesting the etiolated "leaves" and immunoblotting using antisera against thylakoid lumen proteins to detect accumulated precusor forms. A proportion of wild-type seedlings (ca. 5%) fail to green when grown under this regime, and so the screening protocol is not perfect. Nevertheless, this method should markedly reduce the number of plants that need to be screened. We have been using this approach for two months and one possible mutant has been identified using antisera raised against the 33 kDa oxygen-evolving complex protein. We are now growing seeds from the parental stocks to determine whether the mutation can be identified and maintained as the heterozygote form.

Colin Robinson & Alison Creighton; University of Warwick.

From Steve Slocombe...

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

Two stearoyl-ACP desaturase cDNA clones recently received from John Shanklin (MSU-DOE Plant Research Laboratory, Michigan State University) have been sequenced. These clones had been derived from our developing rape embryo library using antisera raised against the desaturase from avocado mesocarp tissue. The two predicted peptide sequences are 94% identical and 80% identical with published data for castor and cucumber embryo desaturase cDNAs. Screening for the genomic clones is currently in progress with a view to studying the regulation of oil synthesis genes during embryogenesis in collaboration with Michigan and with Antoine R. Stuitje (Department of Genetics, Vrije Universiteit, Amsterdam).

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

From Alison Smith...

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

We have all but finished the sequence of our pea cDNA clone for HMBS and unfortunately, while its length is close to the size expected to encode the protein plus transit peptide, it does not encode an initial Met and therefore is not full length. However, we have identified the start of the mature protein by comparison with the published N-terminal amino acid sequence and this starts about 130 bp from the 5' end. The predicted sequence of the first 43 residues resembles a chloroplast transit peptide library, and so the protein encoded by this cDNA is targetted to the chloroplast. We are now in the process of studying its expression in different tissues of the plant by northern blot analysis and re-screening the library to find a full-length clone.

Unfortunately, news about the *Arabidopsis* cDNA clones is less good. All the putative clones isolated from Christine Raines's library using the pea probe turned out to be due to hybridisation of the vector. We do not know why these clones were identified, but the hybridisation of the probe (presumably contaminated with the plasmid vector) occurs both to plaque filters and to Southern blots of the DNA purified from the clones -- any ideas?

Anyway, we hope we have now overcome the problem by screening the library again including in the hybridisation unlabelled vector along with the radioactive probe to act as a cold competitor. Plaque lysates from the positives we have obtained act as templates in PCR reactions with HMBS primers, and produce the same-sized products as when DNA from the whole cDNA library is used as template. Furthermore, the products hybridise with the pea cDNA clone and with the PCR product (Ag1) generated using *Arabidopsis* genomic DNA as a template. Ag1 has been sequenced partially and show to encode part of HMBS, with an intron of about 500 bp in the middle. Assuming that these new cDNA clones are genuine they will be used to localise the coding region for HMBS within the inserts of the *Arabidopsis* genomic clones we isolated earlier.

On a completely different note, I have been working in Paris at the Institut Jacques Monod for the past two months, attempting to rescue mutants of yeast defective in haem synthesis with *Arabidopsis* cDNA. We have an *Arabidopsis* cDNA library in a yeast expression vector, kindly made available to us by Francois Lacrute, who works in Gif-sur-Yvette, just outside Paris. The vector uses URA3 as the selectable marker for transformation, and since most of the yeast haem synthesis mutants available are uracil prototrophs, I have been busy generating new mutants by gene disruption, which are also ura-. However, there is one strain which is suitable, which is defective in ferrochelatase (the last enzyme of protohaem synthesis) and I am currently in the middle of a series of daily transformations of it. It is realistic to do 20 individual transformants in one go -- each producing about 1000 transformants -- and it is necessary to screen about 200,000 in total to have a reasonable chance of finding the clone of interest. It is unlikely that I will be able to screen all the mutants in the month I have left here, but I am gradually acquiring the *coup de main* (knack!) and will hopefully be able to continue when I return to Cambridge.

AS25@UK.AC.CAMBRIDGE.BIOLOGY

From Mandy Walker...

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

To clone the gene ttg, which is involved in trichome development as well as other pathways such as anthocyanin biosynthesis, I have been using recombinants between ttg and ms1 (6 map units distal to ttg on chromosome 5) to map the RFLPs available in this region. I have recently been generating recombinants proximal to ttg using the genetic markers ga3 and ch5. These recombinants will be used to map the RFLPs 4556 and 6843 accurately in relation to ttg to provide a starting point for a chromosome walk to ttg.

"Has anyone managed to get very purple plants *in vitro*?"

The other aspect that I have recently been working on, is to establish a transient assay system for anthocyanin genes so that the region containing the ttg locus can be identified quickly. Using a triple enhanced CaMV-GUS reporter gene as a control, I have found that the best method of introducing DNA into *Arabidopsis* is by particle bombardment (thanks to Tapas Ghose and David Lonsdale, Cambridge Lab., Norwich). *Arabidopsis* seedlings do not withstand the conditions used for electroporation of intact monocot tissues. I am still looking for the perfect tissue in which anthocyanin biosynthetic genes are expressed. Has anyone managed to get very purple plants *in vitro*?

Mandy Walker & John Gray; Botany School, University of Cambridge.
ARW13@UK.AC.CAMBRIDGE.PHOENIX

From Zoe Wilson...

Genetic male sterility in *Arabidopsis.*

We are building up a collection of tester male sterile lines which are normal in general plant morphology and female fertility. Allelism tests have so far confirmed six complementation groups:

- msA (allelic to ms1) Pollen decays at late stage.
- msZ Pollen staining reactions as w.t, but pollen doesn't germinate in vitro.
- msH Pollen appears normal, even germinates *in vitro* (anthers do not dehisce?).
- msK Pollen shows reduced viability by FDA staining, doesn't germinate *in vitro.*
- msW Very little pollen produced and none viable.
- msY Like an extreme version of msA, no normal pollen grains produced. Abnormalities apparent in anthers prior to meiosis.

Allelic series of both msA and msZ have been obtained in the irradiation work and in EMS mutagenesis. For example, msF is allelic to msA and appears to be leaky. Preliminary evidence suggests tha this is due to some effect of humidity on male fertility. Similarly, msX is allelic to msA and while totally ms up to 6 weeks becomes male fertile thereafter. We are still in the process of making and using end-specific probes from the YAC colonies that have been identified by the RFLP probes close to ms-1. The primarily approach that we have adopted is one of IPCR. This seems to be routinely working to give amplification products of the expected sizes. However, problems are occurring when these probes are used in library screening, particularly with the starting colony failing to light up. In addition to IPCR, we are also trying to use vectorettes for end-probe production (Riley *et al.* 1990 **NAR** 18). Vectorettes have been constructed which incorporate a T7 RNA promoter site and these are currently being used for probe production to compare to results generated by IPCR. The search for recombinants around ms-1 still continues! Screening and DNA preps are currently in progress from potential recombinants lines.

We are still looking for a replacement Post-Doc for this project. If you would like more info, please ring or e-mail us!

Bernard Mulligan, Zoe Wilson, Janet Fuller & Greg Briarty; Dept. of Botany, Nottingham University. Tel: 0602-484848 ext. 3467.
PBXZW@UK.AC.NOTT.CCC.VAX

Guest Summaries

From Mark Aarts...

Enhancer detection with the *Enhancer-Inhibitor* (*Spm*) transposon system.

After half a year of gathering experience in growing *Arabidopsis* in Zoe Wilson and Bernard Mulligan's lab in Nottingham, I started last April with an EC funded project at the Centre for Plant Breeding and Reproduction Research (CPRO) at Wageningen. The project forms part of the BRIDGE *Arabidopsis* 'T' program. Together with Andy Pereira, I am developing a way to use the maize transposon *En* and its defective derivative *I* in an efficient system for gene tagging. The *En/I* system can be an alternative to the more frequently used *Ac/Ds* element system for gene tagging. Since *En* resembles in some aspects the P-element of *Drosophila* (such as low transcription level and position-effect susceptibility of transcription), we are currently modifying *En/I* to use it as an enhancer activity detector, analogous to what has been described for the P-element of *Drosophila*". Enhancer activity detection has an advantage for gene tagging if compared to the "classical" transposon tagging. It facilitates cloning of genes expressed at embryonic stages of development, a mutation of which would normally lead to embryo lethality.

To date, most of the work is being done in adapting *En* and *I* elements in a way to use them in a multi-element system, comparable to how most *Ac/Ds* transposon tagging systems are working. For *En/I* this is a three-element system, since there are two gene products coding for the transposase functions that are required to activate an *I* element. A whole array of *I* elements have been made, with different promoter-markergene combinations. These elements are currently used to be introduced into *Arabidopsis thaliana* Landsberg *erecta* by root transformation. These tranformed lines will eventually be crossed to a line transformed with the transposase genes. The resulting F_2 progeny will be examined for specific enhancer activity.

"Wilson *et al.* (1989) *Genes Dev.* 3,1301-1313.

Mark Aarts; CPRO-DLO, Postbus 16, 6700 AA Wageningen, The Netherlands.
AARTS@ITAL.AGRO.NL

From David Bagnall...

Late flowering and light.

Initial experiments looked at how photon flux density (PFD), light quality and daylength affect time to flower and leaf number in a range of late flowering ecotypes and mutants. Low PFD (less than 100 $\mu mol^{-1}m^{2}s^{-1}$) substantially delayed flowering in soil grown plants both in Landsberg (36 days at 50 $\mu mol^{-1}m^{2}s^{-1}$) and in fca, which did not flower after 120 days at 50 $\mu mol^{-1}m^{2}s^{-1}$. Growing the plants asceptically in test tubes of Noble agar (with MS salts) which included 1.5% sucrose, made flowering time independent of PFD, such that fca flowered in about 40 days over 50-200 $\mu mol^{-1}m^{2}s^{-1}$. Consequently, experiments with test tube grown plants have substantially less experiment to experiment variation in time to flower.

"Light quality...markedly affected time to flower and leaf number."

Light quality, measured as the ratio of spectral intensity at 660nm and 730nm, markedly affected time to flower and leaf number in late flowering ecotypes and mutants. Standard Canberra phytotron cabinets with metal arc, quartz halogen and incandescent lamps have spectral ratios in the range 1.3 to 2.4, depending on the mix and intensities

of lamps, whereas cabinets with fluorescent (cool, white) tubes have a ratio of 5.8. In the glasshouse, under sunlight the ratio was measured as 1. In cabinets with supplementary in incandescent lamps and a spectral ratio of 1, late flowering mutants and the vernalisation-responsive ecotypes Eifel, Innsbruck, Pitztal and Kiruna all flowered quicker and with fewer leaves, than when they were grown under fluorescent lamps. These results imply that late flowering in both *Arabidopsis* mutants and ecotypes involve, in part, a phytochrome mediated response, as well as sometimes vernalisation and photosynthetic responses.

David Bagnall, Jo Burn & Liz Dennis; CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.
BURN@AUOZ.PICAN

From Fatima Bano...

Glucosinolates in *Arabidopsis*.

I have recently finished the HPLC analysis of about 70 ecotypes of *Arabidopsis*. Identification and quantitation of glucosinolates in thses lines is currently underway.

As reported earlier (see last Newsletter), leaf wounding in Landsberg *erecta* caused induction of glucosinolates. But an elaborate time course study of leaf wounding has failed to confirm the previous finding. It seems that very minor variations in growing conditions cause quantitative changes in leaf glucosinolates. Further experiments regarding the effect of environmental stress factors (*e.g.*, light, temperature, water regime) on glucosinolate content are now being carried out in an attempt to interpret this problem.

I am currently looking at glucosinolate variation in four isolates of Columbia, since whilst analysing Landsberg *erecta* and Columbia and their F1 and F2 progenies, I discovered qualitatively differing glucosinolate profiles between the parental stocks of Jonathan Clarke (who kindly provided the seeds) and those of mine.

There are still a few glucosinolates that I have not been able to identify in *Arabidopsis* lines, but within the next few months I hope to confirm their structure either by using Liquid Chromatography/Mass Spectrometry or by isolating and purifying the glucosinolates using a large number of plants.

The final part of my project involves a couple of crosses between lines having homologous glucosinolates and studying their segregation in F1 and F2 generations. This classical genetics work may enable us to gain some insight into the intricate mechanism of glucosinolate biosynthesis.

Fatima Bano & John Turner, School of Biological Sciences, University of East Anglia, & Roger Fenwick, Institute of Food Research, Norwich Laboratory.

From Michel Delseny...

Seed formation and germination.

Our group has screened an *Arabidopsis* genomic library with an Em-like cDNA isolated from a radish dry seed cDNA library. Two different genes have now been sequenced by Monique Raynal, Gordon Huestis and Pascale Gaubier. Several cDNA clones have been obtained from a library made by Jerome Giraudat in GIF and partially characterised. Our current data indicate that the two genes are expressed in maturing *Arabidopsis* seeds. Gillian Hull, who joined us as a post-doc from Rothamsted, has now prepared chimeric constructions for one of these genes and transferred them to tobacco and *Arabidopsis*. Hopefully, we will be able to study the regulation of this gene by ABA using a homologous system and the mutants described by Korneef in Wageningen. The BRIDGE meeting in Perpignan on 7 & 8 March allowed fruitful discussion with all the European participants in the ABA-regulated genes sub-project and collaboration between our respective groups should develop.

Additional news from Perpignan is the arrival of Martine Devic from the Sainsbury lab in Norwich as a post-doc to join Patrick Gallois and Keith Lindsay's project of T-DNA-tagging genes expressed during embryogenesis. The first transformants have now been regenerated in Perpignan and many other transformations have been carried out during the last two months.

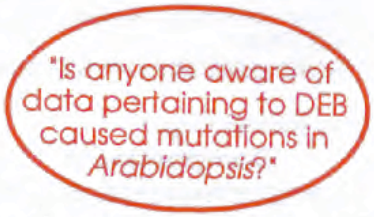
Michel Delseny; Laboratoire de Physiologie et Biologie Moleculaire Vegetale, Universite de Perpignan, 66860 Perpignan, France. Tel: 33.68.66.88.48 Fax: 33.68.66.84.99
DELSENY@EARN.FRPERP51

From Roger Innes...

Genetic characterisation of disease resistance in *Arabidopsis*.

I have now moved to Indiana University, but I will provide an update for developments of the last few months in the Staskawicz laboratory, as well as a few comments on what we will be focusing on in Indiana.

In the March Rabido newsletter, we reported on a putant of Col-0 that was no longer capable of responding to bacterial pathogens expressing the avirulence gene avrRpt2. This "susceptible" phenotype was faithfully transmitted to the M3 generation, and the Staskawicz group (Andrew Bent & Barbara Kunkel) is now analysing backcrosses and crosses to both resistant and susceptible ecotypes in preparation for RFLP mapping. As this putant is derived from a DEB mutagenised population, we are hoping that the mutation is the result of a deletion, which would allow Andrew & Barbara to identify the mutated DNA by genomic subtraction. Is anyone aware yet of data pertaining to DEB caused mutations in *Arabidopsis*?



The other hot result of the last three months is the identification of a well characterised avirulence gene that induces a resistance response in *Arabidopsis* ecotype Ws-0 (of T-DNA tagging fame). The gene is avrB from *Pseudomonas syringae* pv. *glycinea* (*Psg*). When expressed in virulent *Psg* strains, this gene induces a resistance ("hypersensitive") response in some, but not all cultivars of soybean. The resistance response segregates as a single dominant gene in crosses between resistant and susceptible cultivars of soybean. By extrapolation, one would predict the resistant response in *Arabidopsis* ecotype Ws-0 will also be under control of a single resistance gene. We have found that the Col-0 »

putant described above that no longer responds to avrRpt2, still responds to avrB. Thus the mutation seems to be specific to avrRpt2, rather than in a gene required for a "generic" resistance response. We are currently screening for ecotypes and mutants of *Arabidopsis* that do not respond to avrB.

In Indiana, we are continuing to screen for mutants of Col-0 that fail to respond to either avrRpt2 or avrB. We are hoping to identify mutants that are specific to each avirulence gene, and mutants that fail to respond to either gene. Identification of the latter class would suggest that the resistance response triggered by these two different avirulence genes shares a common pathway. We are currently screening several populations that were mutagenised by different agents (*e.g.*, gamma-ray, EMS, DEB, *etc.*) so as to maximise the chance of hitting a specific gene. Along these lines, we are also re-vitalising our efforts towards developing an efficient transposon tagging system for *Arabidopsis*. We are continuing to focus on a *Ds* base system, and are currently making new *Ac* transposase constructs that fuse an *Ac* cDNA to floral meristem specific promoters.

Roger Innes; Department of Biology, Indiana University, Bloomington, IN 47405 U.S.A.
RINNES@EDU.INDIANA.BIO.IUBIO

From Graham King...

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

As part of our programme to compare the genome architecture of *Arabidopsis* and *Brassica* genomes, I have calculated thermal stability maps from all published sequences. These display interesting features such as stability jumps associated with intron/exon boundaries and 5' signal regions. The thermal stability profiles are calculated using software from Larry Lerman and William Fripp at MIT, using thermodynamic considerations to take into account base-stacking interactions as well as overall base-composition. The resultant stability maps display considerably more uniformity in co-operatively melting regions than is accounted for by plots of GC content. Current work is aimed at looking at local codon usage in such uniform regions to determine how much stability constraints contribute to codon choice. It is hoped that in future we may be able to correlate sequence stability of alternative alleles, or members of multigene families, with timing and degree of transcriptional activation.

"I would be very interested in obtaining non-published sequences."

In order to build up a better idea of stability variation in non-coding and flanking regions I would be very interested in obtaining non-published sequences. Since the sequence information will only be used for input into statistical or thermodynamic programmes, confidentiality of such sequences need not be compromised. In addition I am sure many labs have 'useless' *Arabidopsis* sequence data generated in walking or cloning experiments, which I am keen to include in our survey of *Arabidopsis* sequence characteristics.

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

From Maarten Koornneef...

ABA in Wageningen

From the revived *Arabidopsis* group in Wageningen (thanks to the EC Bridge Programme), I want to report our efforts to isolate and characterise new abscisic acid (ABA) and seed-maturation mutants. We define these as mutants with abnormal embryo development, but with a disturbed last phase (approximately the last week) of seed development. In this period; dormancy is induced, many seed proteins are synthesised, and desiccation tolerance develops. We know from our earlier work that ABA is very important for many of these processes.

Karen Leon is the Ph.D student working on this project. At the moment, she is studying the genetics of a non-dormant mutant, which is very different from our ABA-deficient and -insensitive mutants isolated earlier. In addition, several mutant-isolation schemes are being tested, which have already yielded several interesting putants. Our collaboration with other laboratories within our University and with the international *Arabidopsis* community, provides us with the materials and know-how to characterise many aspects of the morphology, physiology and biochemistry of these mutants. We received probes of seed-specific genes from Elliot Meyerowitz and will soon receive probes of other "seed" and ABA-induced genes from the groups of Delseny in France and Pages in Spain. We have already received transgenic seeds with gus controlled by an ABA inducible promotor. In Wageningen, we collaborate with both the Karsen's, group who is performing a detailed analysis of our previously isolated mutants and double mutants, and the Sacco de Vries's group who have started to study the *Arabidopsis* homologues of their carrot-embryogenesis genes by *in situ* hybridisation.

"...choose the right organism for your problem and collaborate as much as possible."

I want to emphasize these collaborative aspects because for progress in science, two aspects are very important: choose the right organism for your problem and collaborate as much as possible. The latter aspect is especially crucial for small groups like ours.

From José Martínez-Zapater...

Genetic and molecular analysis of floral induction.

Hello from Spain! Although we have been receiving issues of the AFRC PMB *Arabidopsis* Newsletter, we have not yet comunicated a report of the research interests and progress in our lab. This time we could not stand any more the pressure from David Flanders, and here, at the last minute, is our report.

We are interested in the analysis of the process of floral induction and, more specifically, in the mechanisms of cold induction of flowering and the control of phase change. Several

projects related with this topic are currently running in our lab.

Cloning the *fve* locus

The *fve* locus has been characterised by mutations that produce a delay in flowering time that can be rescued by vernalization. Two different recessive mutations were isolated in the laboratory of Maarten Koornneef that produce slightly different phenotypes. This locus maps to chromosome 2 between loci *cp2* and *fti*. We are currently integrating these markers within the RFLP map to find the closest linked RFLPs on either side of the gene. Using these markers, we will select the YAC clones overlapping the genetic locus. The corresponding DNA sequences will be identified by complementation of the mutant phenotype.

Genetic and physiological analysis of vernalization

Mutants that respond to vernalization are useful tools for the characterisation of this process. In collaboration with C. Dean we are constructing double mutants of *fca* and *fve* with mutants that are either deficient or insensitive to ABA and GA. The goal of these double mutant constructions is to elucidate the role of those plant hormones in the vernalization process. We are also interested in the analysis of the interactions between acclimation and vernalization. Both responses are induced in plants by low temperatures and they could share common steps in the recognition of the cold stimulus or in the early steps of their signal transduction pathways. We are currently investigating this possibility by selecting mutants that are unable to acclimate or vernalize in an *fve* mutant background, and testing their responses.

Phase transition mutants in *Arabidopsis*

So far, late flowering mutants isolated in *Arabidopsis* show a delay in flowering time, but no morphological abnormalities. We have recently isolated a late flowering mutant, in the Rschew ecotype, that shows an interesting phenotype. Mutant plants are very delayed in floral induction and, when they start to flower, the transition from vegetative to reproductive development is not complete. In the initial stages of the reproductive growth, vegetative organs differentiate in place of reproductive organs along the inflorescence. Later the development, inflorescences and flowers are completely normal. We think this mutant can be useful to elucidate the interactions between the floral inductive mechanisms and the genes that control organ identity. Its genetic, morphological and physiological analysis is currently underway.

"This mutant can be useful to elucidate the interactions between the floral inductive mechanisms and the genes that control organ identity."

José M. Martínez-Zapater, José A. Jarillo, Marylin Cruz-Alvarez & Antonio Leyva. Departamento de Protección Vegetal, CIT-INIA, Ctra. de La Coruña Km7, 28040 Madrid, Spain. Tel: +34-1-3572286; FAX: +34-1-3572293.

From Javed Mirza...

Arabidopsis research in Pakistan.

While I was a Ph.D. student at Aberdeen University in E.P. Maher's lab (1978-82), not many people in the U.K. were working with *Arabidopsis*. From your exciting newsletter, it appears that *Arabidopsis* is now a favourite organism to study plant molecular biology. Here I give a brief report of *Arabidopsis* research in this part of the world.

In my lab, we are at present involved in sceening a new generation of auxin-resistant mutants from EMS-treated M2 populations. So far, we have isolated several putative NAA-resistant mutants with distinct phenotypes. These mutants, together with the the already identified mutants of genes AUX1, DWF, AXR1, and AXR2 (screened for resistance to 2,4-D, IAA and ethylene), would hopefully broaden the spectrum of auxin-resistance loci in *Arabidopsis*. Apart from these mutants, we have also isolated putative mutants affected in flowering time, senescence, and silique dehiscence.

Our research programme also includes study of high temperature stress in *Arabidopsis*. At present, I have a got a post-graduate student, Saiqa Rasheed, and a graduate student, Mazhar Iqbal, working with me.

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

From Stephen Ohl...

Leiden proudly presents...

As announced by Peter Sijmons in the previous issue, we finally got our new '*Arabidopsis*-lab" running and can start with new enthusiasm, equipment and money to gain some more insight into the biology of our lovely little weed.

Eric van der Graff is working on a shotgun-cloning approach for phytochrome and morphogenesis-related genes by protoplast transformation with a construct containing a strong plant-promoter/enhancer. Transformed calli/plants will be screened for phenotypes related to overexpression or disruption of these genes. The vector will offer a simple way of cloning back adjacent plant DNA. Eric is now in the final stages of his vector construction and will soon find himself amidst real plants (and protoplasts).

Dianne van der Kop is studying the regulation of an auxin-inducible gene from tobacco, GNt103, and will be setting up a selection system in transgenic *Arabidopsis* to identify mutants in auxin regulation (upregulation of the auxin-inducible promoter). At the moment, she analyses her *Arabidopsis* plants transformed with the auxin-inducible promoter hooked up to GUS and observes blue staining with much excitement.

Annette Vergunst and Stephen Ohl are extending the ongoing project on homologous recombination (studied in tobacco by Remko Offringa and Marcel de Groot) to the plant system relevant for this newsletter. Our first attempt will be to target a defective hygromycin resistance gene (HPT) stably integrated into the genome (for thi spurpose we obtained some transgenic lines from L. Willmitzer's group in Berlin) with the intact HPT coding region lacking the promoter. Our specific aim is to investigate the suitability and efficiency of *Agrobacterium*-mediated DNA transfer to protoplasts for high frequency transformation via *Agrobacterium*. She will be happy for any suggestion that might help her to crack this nut.

Stephan will extend this study to ➤

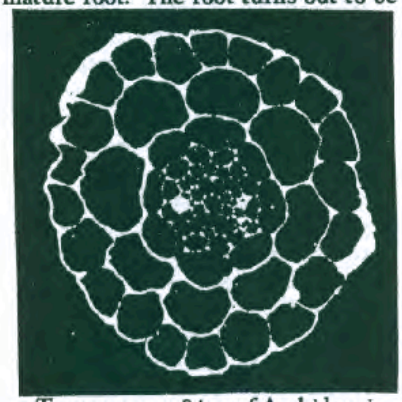
the targetting of endogenous genes. As a first model-system, he is trying to target the gene for the large submit of the *Arabidopsis* RNA polmerase II (AtrpII, kindly obtained from C. Koncz, MPI, Köln) with a 5' truncated fragment of the gene carrying a single basepair exchange, which confers resistance to α-amanitin upon homologous recombination. Stephan is optimistic to have his first test-constructs containing the α-amanitin resistant AtrpII gene and its truncated form ready soon to work out selection conditions.

From Keith Roberts...

The root apical meristem: development and patterning.

Dave Flanders has bullied me into describing what we are struggling to do with part of our PMB grant and as it seems the only way in which we can guarantee delivery of the *Arabidopsis* Newsletter, I guess I submit!

Paul Knox, who was working on our PMB project looking at the developmental patterns of various cell surface arabinogalactan proteins (AGP) in carrot, has recently left to take up a lectureship at Leeds. He will take much of that work with him, but we are now changing tack slightly and beginning to look at development in the *Arabidopsis* root apical meristem. Essentially we are doing two classes of experiment. The first is to try to describe the exact anatomy of the root apical meristem and how that relates to the emerging pattern of cells in the mature root. The root turns out to be remarkably determinate (although not quite as spectacularly as with *Azolla*). There are almost invariant cell numbers, for example, in the cortex (eight cells) and the endodermis (eight cells) with apparently two phloem sieve tube elements and two primary xylem vessels (see Figure). We have been mapping some of our monoclonal antibodies that recognise cell surface AGPs onto this emerging anatomy. One antibody recognises an AGP epitope on a single cell in the centre of the root meristem. This epitope is then later switched on in adjacent cells as the cells age until eventually a complex pattern, which includes xylem parenchyma and endodermis, is established. We will continue to map some of these pattern-specific or position-specific markers onto the meristem. Paul Linstead had been instrumental in doing most of the detailed anatomy.



Transverse section of Arabidopsis *root stained to reveal the two phloem sieve elements. Eight cortical and endodermal cells are clearly seen.*

In a parallel series of experiments, which Nicola Stacey has been doing, we are trying to use a tissue printing technique for screening for a variety of cell surface mutants, including those that show altered expression of these AGP markers. The work is at a very early stage, but initial results seem moderately encouraging. So the protocol is included with this newsletter (*see attachments – ACM*). We would be very interested, incidentally, if anybody has earlier references, or indeed data, on root anatomy in *Arabidopsis* or if anyone has interesting root developmental mutants which we might collaborate on from the structural point of view. We have already established a link with Phil Benfey (Rockefeller, NY) who has a variety of insertional mutants in root development, but we would be very interested in looking at any other root variants. We shall shortly be advertising the remaining 1½ years of Paul Knox's grant with the emphasis on cell surface markers and *Arabidopsis* root development, so if anyone knows any likely applicants....

From Peter Sijmons...

Worms on weeds!

The number of pathogens on *Arabidopsis* is again expanding and now includes plant-parasitic nematodes. We have developed monoxenic culture conditions for infection and development of a range of economically important nematodes on roots of *Arabidopsis*. Full life cycles were obtained with cyst-, root-knot- and migratory nematodes. The thin and transparant roots allowed *in vivo* studies in unprecedented detail. The availability of this model system will be welcomed by plant-nematode researchers and may attract newcomers to this fascinating parasitic interaction. Full details on experimental procedures will be published in the September issue of The Plant Journal. This work is done in close collaboration with Urs Wyss & Florian Grundler in Kiel (Germany, FAX +49-431-880 1583) and Nicola von Mende & Paul Burrows in Rothamsted (e-mail: Vonmende@RESA.AFRC.AC.UK).

"The thin and transparent roots allow studies *in vivo* in unprecedented detail."

Special request to readers of this Newsletter: we are of course interested in root-, hormone- or metabolic mutants as well as transgenic *Arabidopsis* with altered metabolism. Especially those plant lines that are not yet available through the common *A. thaliana* seedbanks. Anyone who is interested in such a co-operation (*i.e.*, who has potentially interesting plant lines) should contact either of the Worms-on-Weeds groups mentioned here.

Peter Sijmons; MOGEN, Einsteinweg 97, 2333 CB, Leiden. MPKDEKKE@RULGL.LEIDENUNIV.NL

From Billy Sinclair...

The characterisation of gravitropic mutants of *Arabidopsis thaliana*.

Work is being carried out on a range of gravitropic mutants of *Arabidopsis* that have altered responses to gravity and which map to one of three unlinked genetic loci. The severity of the lesion varies from a complete loss of response in roots and shoots to a normal response in shoots and a partial response in roots. By comparison of the growth characteristics and biochemical constituents of normal and mutant seedling roots, we are investigating the stages in gravitropism from the initial perception of the stimulus to the growth response.

To date, we have: quantified the various responses of the mutants to gravity stimulation, begun to characterise calmodulins from normal and mutant plants, and studied the effects of the calmodulin inhibitor, Trifluoperazine, on root growth and gravitropic response (which has enabled us to mimic the response of one of the mutants using the normal seedlings). Studies on the production of mRNA species in normal and mutant plants are under way.

W.T. Sinclair (1,2), A.J. Trewavas (1) & E.P. Maher (2); 1 Institute of Cell and Molecular Biology, University of Edinburgh, Kings Buildings, Mayfield Road, Edinburgh, EH9 3JH; 2 The Open University in Scotland, 60 Melville Street, Edinburgh, EH3 7HF.

From the Two Brians (Thomas & Jordan)...

Photoperiodic induction and UV-B responses.

We have continued our characterisation of the photoperiodic responses of the Landsberg and Columbia strains. One of the most interesting findings is that the sensitivity of plants to the inductive effects of long days can be varied and with the right conditions we can induce Landsberg with a single long day. Screening for mutants that do not distinguish between long and short days in very young seedlings is now well under way and looking promising. We will soon be starting to screen for mutants with altered sensitivity to numbers of long days using protocols derived from our characterisation studies.

"We can induce Landsberg with a single long day."

In the UV-B project, the response of *Arabidopsis* to supplementary UV-B is being studied at the whole plant and gene expression level. *Arabidopsis* appears to be less sensitive than species such as pea in terms of visual signs of stress. Preliminary results indicate that cab and rbcS RNA transcripts are reduced in response to UV-B. Current work involves further characterisation of the down-regulation and additional work on chalcone synthase expression.

With that its goodnight from me and goodnight from him.

Brian Jordan, Brian Thomas, Dave Mozley, Pat James & Richard Anthony; HRI, Littlehampton.
THOMASB@UK.AC.AFRC.GCRI

From Richard Williamson et al...

Arabidopsis root mutants.

We have been successful in isolating further developmental mutants which we will be testing for allelism with our existing cell shape mutants. The first three mutants that we tested proved to be non-allelic and mapping to the chromosome should be finished next month. Tobias Baskin has obtained some very interesting data showing striking differences in the time course with which shape changes are induced by a temperature shift in our temperature-sensitive mutants and these results will greatly assist working out the cell-biological basis of the shape changes by providing us with knowledge of the critical times at which changes must begin if they are to be causally involved.

Quite a few changes have occurred around the place regarding funding. The Australian National University, CSIRO and the biotechnology company, Biocem have been successful in their application for the funding of one of the first 15 Cooperative Research Centres. Unfortunately, the funding is only half that sought so that a lot of trimming of programs and expectations of new appointments is in store before the July 1 start-up date. *Arabidopsis*, however, features quite prominently in the program of the new centre. The centre programme was one of the Government's promises at the last election and is designed to encourage cooperation between researchers in government, CSIRO and industry.

Andreas Betzner has been back in Germany for the last couple of months, but will be returning shortly to work for Biocem in Canberra having refreshed his taste buds with German beer.

Richard Williamson, Tobias Baskin, Jacek Plazinski & Ann Docherty; Plant Cell Biology Group, RSBS, Australian National University, PO Box 475, Canberra, ACT 2601, Australia.
WILLIAMSON@RSBS0.ANU.EDU.AU

From Mark Young...

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

I have recently moved to the Department of Biological Sciences, Purdue University USA from CSIRO Division of Plant Industry, Canberra Australia. One of the projects that I started at CSIRO and that I am continuing at Purdue is the use of *Arabidopsis* alcohol dehydrogenase as a model target for inactivation by catalytic RNAs (ribozymes). Arabidopsis ADH was chosen as a model system because it is a well characterised, inducible,single gene, with excellent quantitative assays. Various ribozyme constructions which employ different expression and stability strategies are being introduced into C24 *Arabidopsis*. Seeds from primary transformants are currently being screened for reduced ADH activity. The long term goals of this project are to, (i) design more active ribozymes for *in vivo* activity and (ii) to define host components (genes) involved with modulating ribozyme activity. If you have any helpful comments or wish further information on this project, please feel free to contact me.

Mark Young; Department of Biological Sciences, Purdue University, West Lafayette, IN 47907 USA.
Tel: +1-317-4946 453; Fax: +1-317-494 0876
YOUNG@ACLCB.PURDUE.EDU

PMB Flower Development Workshop Report

➳ *continued from the next page (24)...*

The other major highlight of Day 2 was Dave Twell's (now at Leicester) presentation on his work carried out in Sheila McCormick's lab. This included information on the late pollen specific LAT genes with a very impressive set of deletion mutational analysis experiments Though there were murmurings of discontent from the audience as he ran 20 minutes overtime into dinner time (a hungry scientist is not a happy one).

And this, basically, was it for the first PMB Flower Development Workshop.

From Gary Foster...

PMB Flower Development Workshop: A Brief Report

University of Leicester, 19&20 March, 1991

(May it be known, that the author of this report claims no copyright and no responsibility for the report outlined below, all names and events have been changed to protect the innocent.) On 19th and 20 March, a historic event took place which saw the coming together of two communities which till then had hardly acknowledged the others existence. What was this great event I hear you cry? Was it the demolition of the Berlin wall and East meeting West? No! Was it the start of Peter Brookes peace talks in Northern Ireland? No! Was it even the meeting of the casts of Eastenders and Coronatio Street? No; it was none of these, it was in fact even more momentous, it was the joint meeting of the AFRC Plant Molecular Biology with the AFRC *Arabidopsis* programme under the daunting title of, "The Plant Molecular Biology Flower Development Workshop".

The whole event took place at Beaumont Hall. This is situated in the picturesque Botanical gardens, which would have been very appropriate for a flowering meeting – if it hadn't been in the middle of winter. Professor Harry Smith introduced where the conference was being held and John Draper introduced why it was being held (promoting closer links between the two groups on this important subject and other boring stuff like that).

Hugh Dickinson (Reading) gave a perfect start to the conference by giving an overview of flowering (quite appropriate) and made a good point that molecular biologists must still use a microscope every now and again. He showed that most of the genes isolated to date are from the tapetum and more importantly, why and included one of his many famous quotes by describing the tapetum as the "Cinderella of flowering." It does all the work, but never goes to the ball".

Rod Scott (Home team), reviewed the work so far on the genes isolated and showed very well that what we have studied so far is just a drop in the ocean! He then went on to describe his own work in which *B. napus* bud lengths have been connected with their developmental stage, allowing a number of specific libraries to be made. A more detailed report of the genes and promoter analysis from these libraries was later given by Wyatt Paul *et al* (tapetum specifics) and Mike Roberts & Gary Foster (microspore specifics).

Andy Greenland (ICI Seeds), gave us an insight into the workings of industrial research in relation to engineered artificial male sterility. He stated during the course of his talk that by the end of the conference we would all be sick to death of tapetum specific promoters (and he was right). Outlined briefly the advantages and disadvantages of using transgenics and the score for each still appears to be even. At the end of the talk, Hugh Dickinson described the killer genes used in these sorts of systems as "Molecular Rottweillers" (or was it pitrbull terriers?).

George Coupland (IPSR). gave an excellent talk on the two main approaches used to tag and isolate genes involved in flowering *i.e.*, chromosome walking and transposon tagging. He described the fine tuning that is currently going on with the *Ac* and *Ds* systems to create the ultimate tagging mean machine.

Bernard Mulligan and Zoe Wilson (Nottingham), performed their well polished double-act of two talks in one slot. These two talks followed on well from G. Coupland, Esq. by outlining some more gene isolation methods; namely, mutagenesis, chromosome walking, and subtractive PCR library production.

Keith Lindsey (Leicester), tried to confuse us all by inserting (pun!) a new word into our vocabulary. The word being INTERSPOSONS, which I won't try and explain, but see previous newsletters for details. He did, however, show us some very nice pictures of a remarkably high number of tagged genes using this system. Watch this lab. for a lot of interesting results on the flanking regions in the future.

This rounded the first day off nicely and everyone retired to the bar (conveniently located in the next room) for some informal discussions (some more informal than others). The conference dinner followed which took place in the presence of ample wine (Leicester University vintage!) and good company. After this a series of short presentations took place, again with ample wine, under the inspiring chairmanship of Keith Lindsey. The following sensible passage was composed by that same chairman:

The evening of the first day provided an opportunity for an informal discussion session. A small number of participants were asked to give 10-15 minute summaries of their research interests as a focus for wider discussions. Julie Gray (Nottingham), described some work she had carried out in Adrienne Clarke's laboratory in Australia on the possible role of ribonuclease activity in the self-incompatibility response. Sue Albini (Birmingham) discussed a cytological approach to studying meiosis and recombination. Luisa Janniche (Norwich) introduced some of the work from Keith Robert's laboratory on *in situ* localisation of the JIM8 monoclonal antibody in *Brassica* flowers, and Tina Barsby (Nickersons, Cambridge) completed the session by reviewing the cybridization approach to engineering CMS in *Brassica*, and imparted a commercial perspective to the discussion.

The ensemble then retired to the bar (again) for further refreshments, with the last person (who shall remain nameless) seen struggling round the corner towards the halls of residence at 3:00 o'clock in the morning.

The next morning was started off bright and early with a couple of talks from Rosemary Carpenter and Enrico Coen. That was enough to impress even the worst hungover scientist. Rosemary presented her few results (!) on the mutations caused by Tam3 in Antirrhinum-snap dragons, in relation to the genes controlling flower development. The awesome number of plants that have been analysed over the years was an inspiration to us all. Rico followed on from Rosemary's talk and quite eloquently described the scheme for homeotic genes controlling flower development.

Cathy Martin (IPSR, again), presented a series of results on the genes controlling petal pigmentation in snap-dragons. She showed some beautiful pictures of flowers, backed up with some excellent biochemistry and molecular biology, presented in a clear and concise way (even the author of this report grasped some of it).

continued at the foot of the previous page (23)... »

From Pat Heslop-Harrison & Jola Maluszynska...

Arabidopsis my Arabidopsis

NOW THAT WE are finding that other species in the genus *Arabidopsis* are useful, we would like to suggest that we use Arabidopsis (not in italics or underlined) as a common, English or vernacular name for *Arabidopsis thaliana* L. (Heynh.). Publications can then correctly talk about "screening an Arabidopsis library", (i) without falling foul of the instructions to authors about nomenclature, (ii) without using the name *A. thaliana*, and (iii) with everybody knowing what plant is being worked on. We have approval for the use of the non-italicised genus as an

> "We suggest that we use Arabidopsis (not in italics or underlined) as a common English name for *Arabidopsis thaliana*".

English name on taxonomic grounds from Gren Lucas, the Keeper of the Herbarium at Kew. Thale Cress, Common Wall Cress and such like would remain as alternative English names, but as now I would not expect many publications to use them! Any comments?

The ACM fully supports Pat's idea, but for the more romantic among you, the following suggestion also landed on the doormat of the Arabidopsis *Newsletter's office...*

From Bill Bailey...

What, or Who, is a Thale?

IT CAME AS A surprise and shock to me, to find out that there was no such thing, or person even, as a thale, after which the famous crucifer appears to be named. My hopes for finding a romantic folklorish origin for the name have now been dashed.

Before being aware of this however, I had in my imagination, pictured the source of the name as a rough wall a "thale or theyl, the outer rampart of a Viking fortress, often constructed of rude masonry or rubble, upon which the wild cress, so essential a component of the Norse diet, flourished" (The Alternative Oxford Dictionary). I had also speculated about the preserved bodies of bog men from Jutland, who were "thaled" (garotted with plaited horsehair) and whose stomach contents were found to contain *A. thaliana*. It is not known whether this is evidence of its use in regular diet or as a component of the ritual pre-execution meal or Thale-fare, food given to condemned men in pre-Christian Scandinavia " (Eoanthrobotany 1987 4,iii, pp 23-7).

I had even fantasised the Thale himself, splendid in his buskins and torc, striding over the sandy heath of northern Europe, bidding his wives and children to gather the dainty vegetable in osier "wort-wickers" (Beowolf, the Clacton fragments).

I do not know why I am drawn to the Northman image, but such is the evocative nature of words. Perhaps I am reminded of *thegne* (Old Norse). But alas, no thale can be traced. There hasn't ever been such a person, place or thing.

"Thale cress" seems to be a nineteenth-century, made-up name, not a good old traditional country one. There was a vogue for inventing English names for plants in Victorian times as it was thought that ordinary folk were incapable of learning or pronouncing Latin ones. Some of them are gems of our language *e.g.*, Flexible Naiad, English Sticky Eyebright and Lady Larpent's Leadwort. It appears that *Arabis thaliana* (as it was in those days) was somehow transliterated as an anglicisation of "Thal's cress" to "thale cress" and thus only obliquely commemorates the learned Dr. Johann Thal (1542-83) after whom the plant was named by Linnaeus. Was it the difficulty of pronouncing "Thal's" that persuaded the nineteenth century English botanists that they had to provide an alternative name? You would have thought that with a semi-Hanoverian Queen and a Saxon Prince Consort there would have been no prejudice against speaking German in Victorian England, but I must admit we English still make a sorry attempt at pronouncing *Fuchsia* and *Kochia*. I have not been able yet to find the earliest reference to the use of the name "thale cress" but I plan to check back in the literature and may be able to find the culprit. I hope to find out something about Dr. Thal as well, who also has a plant genus, *Thalia* (tropical aquatics in the Marantaceae) named after him. Incidentally, Thalia was the Greek muse of comedy and pastoral poet and she has no connection with this discussion, except in a purely inspirational way. But *thallein* (Gr) does mean to bloom, which *A. thaliana* is not loth to do, thank goodness.

I therefore propose that the use of the name "thale cress" is abandoned and the plant be referred to henceforth as "Thal's cress" -- if a common English name is needed. There are several precedents for using a commemorative specific name in the common name in this way. In the British flora we already have for example: Don's Twitch. Forster's Woodrush and Graham's Sedge.

On second thoughts, there's a lot to be said for "sand rocket" (ref. Peter Sijmonds in *Rabido*), which would be a pleasant name to say, as well as being more descriptive. It would be interesting to have a list of the vernacular names of Thal's cress from other countries. The plant is not "pretty" enough to get itself into foreign wild-flower books and I have not come across any other names so far but will look out for them in future.

Bill Bailey, 17 Faraday Drive, Milton Keynes, MK5 7DD.

From Elucius...

Arabidian Kitchen

(second of an occasional series)

Well after the huge feedback from the last issue, I have been slaving once again over a warm hotplate to dredge up more Arabidian delicacies for you all. Since the summer is upon us, it seemed right to experiment with more light weight summer snacks:

Seed sprout mix.

Try a mix of *Arabidopsis*, alfalfa and fenugreek seeds sprouted in an upturned jar with a muslin lid for 5-6 days. Rinse daily and leave to drain. About a week should give you a good mix of sprouted seeds which are an absolute must heaped into peanut butter sandwiches made with wholemeal bread. (Alfalfa and fenugreek seeds from any good whole food shop.)

An-arab-hada-salad.

This one is free ranging and open to customisation. Chop finely a good handful of parsley, green onion and *Arabidopsis* shoots. Use a mix of *Arabidopsis* leaves and flower stalks. Add two finely chopped carrots and two roughly chopped courgettes. Mix well and add a dressing of 2/3 olive oil: 1/3 (by volume) lemon juice well seasoned with 1-2 crushed garlic cloves. An addition of some chopped tofu (soya bean curd; buy it in Sainsbury's) marinated in soy sauce with chopped fresh ginger is a possibility. Happy cooking. Elucius.

From Polly...

Alternative Arabidian Kitchen

Aribo Bars

When you are caught in the greenhouse with that in-between meals feeling, take a break, take an ARIBO BAR. These tasty morsels can be created in the comfort of your own home and are a must for all those chocoholics out there. These health bars do a body good and keep you going whilst you do your crossings, count your leaves, look for mutants, or do whatever you do in your intimate moments with the little weed. So this is what you do.

Take: 4oz margarine, 3oz golden syrup, 1oz cocoa powder, 8oz digestive biscuits (crushed), 2oz walnuts, 1oz *Arabidopsis* at any stage of development (I prefer a mixture of leaf colour, but just go with what you have to hand), 4oz plain chocolate for topping.

Melt together margarine, syrup and cocoa powder. Remove from heat and stir in the crushed biscuits, walnuts and diced *Arabidopsis*. Press into greased tin and leave to set. Melt the chocolate and spread over the top of the bars. Leave to set. Cut your ARIBO BAR into snack-sized wedges before serving.

Stanzas for Scientists

After the ACM's complaints about a lack of interest in this literary portion of the newsletter, a vigorous debate ensued between David Baulcombe (current leader of the Sainsbury lab triumvirate) and him as to the relative merits of last issue's choice of poem. The result was that the ACM hurled down the gauntlet and challenged the supermarket literator to recommend something he felt worthy of these hallowed pages. His choice is reproduced below. With any luck, this will so affront another reader that he or she (which reminds the ACM that we have yet to have a poem from a female poet -- no Sylvia Plath, though *please*) will suggest a suitable selection for the next newsletter. Send complaints, reviews, even original poems to the usual address.

J.C. Squire
(1884-1958)

The Discovery

There was an Indian, who had known no change,
Who strayed content along a sunlit beach
Gathering shells. He heard a sudden strange
Commingled noise; looked up; and gasped for speech.
For in the bay, where nothing was before,
Moved on by the sea, by magic, huge canoes,
With bellying cloths on huge poles, and not one oar,
And fluttering coloured signs and clambering crews.

And he, in fear, this naked man alone,
His fallen hands forgetting all their shells,
His lips gone pale, knelt low behind a stone,
And stared, and saw, and did not understand,
Columbus' doom-burdened caravels
Slant to the shore, and all their seamen land.

This Issue's Quote

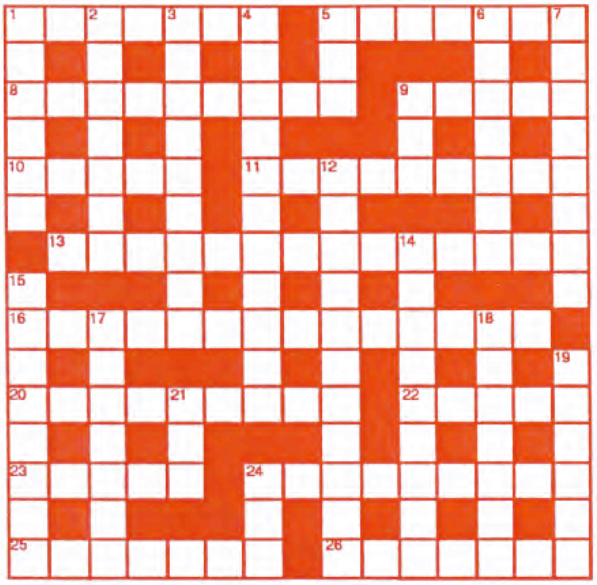
"Few organisms have yielded such a wealth of scientific information as has come from this plebeian weed."

The weed in question: *Arabidopsis* perhaps? Well, the writer was E.W. Sinnott, the year was 1959, and the plant was...? A prize to the first person who can remember the name of this long-forgotten "model" organism.

Thanks to...

- Mary Anderson for her Nottingham ad.
- David Baulcombe for his suggestion of the poem.
- Jon Clarke for photocopying and collating..
- Jeff Dangl for his Köln ad.
- Wim De Waegeneer for his cartoon.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying some of the graphics.
- Anne Edwards for her cartoon.
- Elucius for his cookery column.
- Gary Foster for his conference report.
- Joan Green for the Current Awareness List
- Anita Jellings for the title, "For Thale or Went."
- Polly for her recipe.
- Black Rot for the crossword.
- Renate Schmidt for suggestions & proof-reading.

Arabidopsis Prize Crossword



Sun special

by Black Rot

ONLY TWO correct answers were received to the previous crossword, *Out of molehills*. It thus appears that it was either too difficult, or that most of you out there don't have access to an atlas. In order to try and fill the mail-bag a little more, Black Rot has this time set a really easy, but still highly interesting teaser. To make things as straightforward as possible, virtually all the down clues are *not* cryptic; and those that are, are much less convoluted than previously. All this simplicity means that the usual thematic nature of the crossword has been abandoned, but you can't have everything.

As to last time's prize winners. The two correct entries were both joint ones (reflecting the difficult nature of the task, perhaps?) and from the J.I. site. First drawn out of the very small hat, by Cindy Cooper, who keeps the Molecular Genetics media kitchen as the hub of the department, was the entry from **Lyn Jolliffe** & **Chris Hylton**. They get the £5 book-token to fight over. The runners-up, **Colette** & **Denis Murphy**, thus receive the more-than-consolation, John Innes site-prize of a bottle of the Director's wine. The ACM suspects, however, that the brains behind the entry will be lucky to sample the prize as she is back in Ireland and the shelf-life of cast-offs from the Flavell cellars leaves a lot to be desired.

A book-token for the first correct entry out of the draw. Further prizes may be awarded depending on the number of correct entries.

DON'T FORGET!
The deadline for the next newsletter is: Monday, 7th OCTOBER

Clues Across

1. Illegal, we hear, for me to be on bench when unwell (7)
5. Crop is a trifle dull (7)
8. (&19 Down) Penny Lane at London terminus (9,6)
9. ...to me a _____ in the flesh 2 Cor. 12:7 (5)
10. Pizza-loving Japanese turtle ? (5)
11. Osbert drawing rose red house ? (9)
13. Please look after this GWR arrival (10,4)
16. Cross after 18.15 departure is stuck between Hungerford and Blackfriars (8,6)
20. Policed by FBI--failed appallingly (9)
22. Egyptian president sat around these days (5)
23. Nicked, enamoured (5)
24. Health resort is proud, we hear, to direct submarine movement (9)
25. Clipped grain-bearer in hut (7)
26. Small ring gives one tunnel vision (7)

Clues Down

1. Doing nothing, just ticking over (6)
2. Girl's name (7)
3. Herb (9)
4. Departing (in the vernacular) (8,3)
5. Friend (3)
6. Matthew, Mark, Luke or John (7)
7. Against (8)
9. ___ for two and two for ___ (3)
12. Good thinking always produces this (3,1,3,4)
14. Finally fixes during carpenters strike ? (5,4)
15. Lucky to get both of these from a cherry! (3,5)
17. One of twin demands for small sunshine (7)
18. Kind of duck (7)
19. See 8 Across
21. Charged particle (3)
24. Brick-carrier (3)

If you can still bear to think about it, here are the answers to *Out of molehills*, the crossword in *Rabido*: