Project title:	Genetic characterisation and improvement of plants (apples)
Project number:	TF 58 [Previously APRC SP 58]
Report:	Annual report 1994/1995
Project leader:	D J James, HRI East Malling
Key words:	Apples

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ANNUAL PROJECT REPORT 1994/95

[Note for APRC: APRC contributes £15,000 per annum to this project which runs for three years and started October 1994].

Customer, Project Code and Title: MAFF , IAS MAFF 30890 Genetic characterisation and improvement of plants.

TITLE: Isolation and characterisation of tissue-specific promoters from the developing tissues of the commercial apple.

Project Leader: James, D.J.

Department: Plant Pathology and Weed Science

Target Spend:

Commodity Area: Top and soft fruit

Publications

In Preparation:

Gittins, J.R., Hiles, E.R. & James, D.J. - "Vascular-specific gene expression in transgenic apple"

Contract Reports, presentation to conferences, Grower Groups

Seminar presentation to EEC Concerted Action Group on 'Genetic Transformation of Fruit Trees'
(Contract AIR3-CT 93-0847) entitled 'Tissue specific expression studies in apple'. Alnarp Swedish University, Malmo, Sweden. March 17 1995.

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Transgenic apple plantlets have been produced by *Agrobacterium* mediated transformation using a construct in which the GUS-Intron gene is under the control of the vascular-specific Commelina Yellow Mottle Virus (CoYMV) promoter. Similar transformations have been performed using another vascular-specific promoter *rolC*-GUS-Intron construct but we have been unable to isolate healthy transgenic plants. Both CoYMV and *rolC* plants (since died) demonstrated vascular-specific expression of GUS in preliminary staining experiments. A few CoYMV clones have been examined using fluorimetry, and the level of GUS activity in one of the plantlets is greater than that produced by the constitutive CaMV 35S promoter in a B series control plant selected for high level expression. This result indicates that the CoYMV promoter may be applied for high level vascular-specific expression of an insecticidal protein such as *Bt* toxin, which could be useful in combatting sap-feeding insects like woolly aphid.

A genomic clone isolated from an apple DNA library by screening with a digoxygenin (DIG)-labelled cDNA fragment of the apple ß-galactosidase ABG1 gene (ripening-specific) has been characterised by Southern blotting, restriction mapping and DNA sequencing. A 2.6kb promoter fragment was then subcloned into pSCV1.6 and introduced into *Agrobacterium tumefaciens* (EHA101) by electroporation, ready for plant transformation.

Using DIG-labelled gene probe fragments prepared from apple, pear and potato cDNAs respectively, genomic clones of apple ACC synthase (ripening-specific), polygalacturonase inhibitor protein (PGIP: early fruit development-specific), and s-adenosyl methionine decarboxylase (SAMDC: putative cell-division-specific) have been isolated by library screening. These clones are in the process of characterisation to determine whether they contain promoter fragments which can be sub-cloned into pSCV1.6.

Leaf-specific promoter fragments from RUBISCO small subunit genes of tomato (*rbcS*3C) and soybean (SRS1) have been subcloned into pSCV1.6 to drive expression of GUS-Intron. Both constructs have been introduced into *Agrobacterium tumefaciens* (EHA101) by electroporation and the *rbcS*3C construct used in an apple transformation experiment, although it is too early to assess the results.

Using consensus sequence oligonucleotide primers in a PCR reaction, gene fragments of apple chalcone isomerase (CHI: putative early fruit development-specific), strawberry ACC synthase and strawberry PGIP have been amplified from genomic DNA. Single bands of apple CHI and strawberry ACC synthase, and a doublet of strawberry PGIP bands were produced by the PCR.

The strawberry ACC synthase and PGIP gene fragments have been cloned into T-vectors to be analyzed by DNA sequencing. Four separate strawberry ACC synthase PCR fragments have been sequenced so far and an alignment demonstrates heterogeneity which suggests a gene family.

When apple (cv. Queen Cox) was flowering in the early summer, blossom at "balloon" stage was collected and dissected into the component parts of: petals, stamens, pistils, and ovaries. These were frozen in liquid nitrogen and have been stored at -80°C to be used in mRNA preparation for the future isolation of genes that are specific to the different components of the flower e.g. stamens, pistil, petals etc.

Future work

1. A sample of at least 10 CoYMV apple plantlets will be isolated from transformation experiments performed so far, and subsequent studies will be limited to this small population. The transformed character of the CoYMV plantlets will be confirmed by PCR to demonstrate the presence of the GUS and nptII genes, and the GUS activity quantified by fluorimetry. The level of expression will be correlated to transgene copy number as determined by Southern blotting. The plantlets will also be rooted and the expression pattern of GUS examined in the tissues of the developing apple plant using histochemical and fluorimetric methods. The pattern of expression can then be compared with the constitutive expression produced by the CaMV 35S promoter.

2. Further attempts will be made to produce stable transgenic plants with the *rolC*-GUS-Intron construct, so that the nature of vascular-specific expression can be compared with that of the CoYMV plants.

3. Because the ß-galactosidase ABG1 promoter is ripening-specific it will only be possible to confirm whether it is active once the first fruit have been produced, which will take a minimum of 2 years. In the mean time it may be possible to determine whether the promoter is active by using a biolistics gun to fire the ABG1P-GUS-Intron construct into ripening apple tissue and then stain for GUS activity. Funding for a Dupont helium gun has been obtained and one will soon be available for use at East Malling.

Another alternative, to give a more rapid indication of ripening-specific promoter activity might be to transform a heterologous species. Strawberry transformation is established at East Malling and as it is a Rosaceous species, like apple, the ABG1 promoter could well be active in fruit which could be obtained within a year.

4. Because some ripening-specific tomato promoters require up to 4kb of upstream flanking sequence to produce full activity, a larger ABG1 promoter fragment is being sought. Further restriction analysis and Southern blotting of the ABG1 genomic clone are being used to identify a fragment which would also be sub-cloned into pSCV1.6.

5. If suitable promoter fragments can be obtained from the isolated genomic clones of ACC synthase, PGIP and SAMDC, they will be sub-cloned into pSCV1.6 and introduced into the apple transformation programme. If further genomic clones are required to cover the promoter region, fragments of the characterised clones can be used as gene probes for library screening.

6. When a small population (approx. 10) of transgenic plantlets have been isolated carrying the *rbCS*3C-GUS-Intron and SRS1-GUS-Intron transgenes, leaf-specific GUS expression will be analyzed histochemically and fluorimetrically. The level and pattern of expression directed by the two rbcS promoters can be compared with one another and with the CaMV 35S control plants.

7. CHI, an enzyme involved in flavonol biosynthesis is thought to be expressed in early fruit development. This will be confirmed by RT-PCR using the CHI oligonucleotide primers, and then the PCR fragment can be used as a probe to isolate a genomic clone carrying the promoter.

8. The PCR amplified strawberry PGIP fragments will be sequenced and compared with those published for the PGIPs of bean, tomato, pear, kiwi fruit, and with the apple genomic clone.

The strawberry ACC synthase and PGIP PCR fragments may not be of direct use in this project but may prove invaluable in supporting future grant applications or to stimulate collaborations.

9 Over the next few months, polyA+ RNA will be prepared from the frozen store of apple flower tissues using oligo dT Dynabeads. The mRNA will then be used in differential display RT-PCR using arbitrary random decamers to identify differentially expressed cDNAs. These can then be used as gene probes to isolate tissue-specific promoters. This same technique could also be used to isolate specific promoters from other tissues such as fruit skin.