Project title:	Genetic characterisation and improvement of plants (apples)
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Project leader:	D J James, HRI East Malling
Key words:	Apples

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ANNUAL PROJECT REPORT 1995/96

[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. Isolation and characterisation of tissue-specific promoters from the developing tissues of the commercial apple.
Project Leader(Department):	James, D.J.(Plant Pathology and Weed Science)
Target Spend:	£98
Commodity Area:	Top and soft fruit

Report 1995/1996

Transgenic apple plants have been produced by *Agrobacterium* mediated transformation using a construct in which the GUS-Intron gene is under the control of the vascular-specific promoters of the *rolC* gene (3 plants) and of Commelina Yellow Mottle Virus (CoYMV - 10 plants). Both CoYMV and *rolC* plants demonstrate 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 is greater than that produced by the constitutive CaMV 35S promoter in a B series control plant selected for high level expression.

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 the binary vector pSCV1.6 and introduced into *Agrobacterium tumefaciens* (EHA101) by electroporation. This strain has been used in transformation of apple and strawberry.Transgenic plants are expected soon.

With the assistance of a visiting worker (Stefano Biricolti, University of Firenze, Florence), a genomic clone carrying part of a polygalacturonase inhibitor protein gene (PGIP: early fruit development-specific) has been isolated from the apple DNA library using a DIG-labelled pear cDNA probe.

Using DIG-labelled gene probe fragments prepared from apple and potato cDNAs respectively, genomic clones of apple ACC synthase (ripening-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 permit isolation of promoter fragments which can be sub-cloned into pSCV1.6. It has been confirmed that sufficient sequence is present within both the ACC synthase and SAMDC genomic clones to contain the promoter regions.

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) and these strains used in apple transformation experiments. Six GUS positive *rbcS*3C plantlets have been isolated so far.

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. The strawberry ACC synthase and PGIP gene fragments have been cloned into T-vectors and analyzed by DNA sequencing. Four separate strawberry ACC synthase PCR fragments have been sequenced and an alignment demonstrates heterogeneity which suggests a gene family. The two strawberry PGIP fragments show regions of homology with other PGIP sequences but also contain stretches of additional non-homologous sequence which may indicate that they are members of a separate 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.

Publications

In Preparation:

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

Presentations

Seminar presentation to HRI East Malling: - "Isolation of promoters from the developing tissues of the commercial apple" April 1995.

Future programme 1995/96

1. A number (approx.10) of apple plantlets in which GUS-Intron expression is driven by the CoYMV and *rolC* promoters will be isolated from transformation experiments for use in further studies. The transformed character of the plantlets will be confirmed by PCR to demonstrate the presence of the GUS and nptII genes, and 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. Because the ß-galactosidase ABG1 promoter is ripening-specific it will only be possible

to confirm whether it is active in transgenic apple plants 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 assay for GUS activity. Funding for a Dupont helium gun has been obtained and one will soon be available for use at East Malling.

A more rapid indication of ripening-specific promoter activity might be obtained by examining transgenic strawberry plants. As strawberry is a rosaceous species, like apple, the ABG1 promoter may well be active in fruit which could be obtained within a year.

3. The sequence data obtained from the genomic PGIP clone will be used for the isolation of the cDNA for this fungal resistance factor in collaboration with Murray Tarvis.

4. Promoter fragments obtained from the isolated genomic clones of ACC synthase and SAMDC, will be sub-cloned into pSCV1.6 and introduced into the apple transformation programme.

5. 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.

6. CHI, an enzyme involved in flavonol biosynthesis is thought to be expressed in early fruit development. The CHI primers will be used in an RT-PCR reaction with early apple fruit RNA (prepared by Andrew Passey) as the template to confirm this tissue specificity and to generate a specific probe which can be used to isolate a genomic clone carrying the promoter.

7. The strawberry ACC synthase and PGIP PCR fragments may not be of direct use in this project but will support work in programmes 30066 and other MAFF work on the isolation of temporally regulated genes from apple fruit. They may prove invaluable in supporting future grant applications or to stimulate collaborations.

8. Over the next few months, polyA+ RNA will be prepared from the frozen store of apple flower tissues. 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.