Plant biochemistry and cell biology

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The key disciplines within the Division i.e. phytochemistry, cell biology, molecular biology and biochemistry, are central to the delivery of SCRI's goals. The alliances being fostered within the Division and across Divisions are now providing real added value in terms of publication quality, scientific depth and opportunities for income generation. Sustaining and developing further these collaborations will be an important consideration in coming years to achieve SCRI's primary goals – the delivery of novel science of relevance to the consumer and to industry.

Developmental processes

Meristem activation The life cycle of the potato tuber is being used as a model developmental system to study the activation/deactivation phases of shoot apical meristems. The mechanisms controlling the meristem's activation status play a fundamental role in the establishment of dormancy and apical dominance. Physiological studies have indicated that the activation and deactivation status of meristems correlate with the presence and absence, respectively, of molecular trafficking between the meristem and the rest of the plant. Biochemical markers of tuber apical meristem activation status have been identified and have been used to guide the generation of gene libraries, enriched in sequences that are up-regulated in the early stages of dormancy release. Over 300 different sequences have been obtained. *In situ* hybridisation techniques have enabled the precise patterns of gene expression to be determined. A transgenic approach is being applied for the functional characterisation of candidate genes.

Fruit ripening The biochemical characterisation of carbohydrate metabolism in ripening strawberry fruits has been completed. A key role for mitochondrial citrate synthase and mitochondrial malate dehydrogenase in the regulation of fruit acidity, and hence flavour, has been established and the corresponding cDNAs have been cloned. Additional studies have focused on the effects of ethylene upon the quality of ripe fruit and ethylene has been shown to accelerate fruit deterioration. A productive collaboration initiated with the Molecular LASER Physics Department of Nijmegen University (the Netherlands), has provided unique insights into patterns of ethylene evolution

from ripening strawberry fruits. This work has proceeded in parallel with a search for novel genes involved in the ethylene-sensitive deterioration processes. To identify these genes, Suppressed Subtractive Hybridisation (SSH) has been successfully employed and *c.* 800 clones have been isolated for direct sequencing.

Biochemistry

Leaf urea metabolism Urease and urease genes have been cloned from potato and urease activity successfully up- and down-regulated using a transgenic approach. ^{15}N labelled urea has been used to determine nitrogen turnover and partitioning in control and transgenic lines. The outcome is that the rate of metabolism of foliar applied urea can now be manipulated in both directions. Opportunities now exist to test the impact of variable urease expression on N use efficiencies. The literature indicates that N losses due to volatilisation can be significant using foliar applied urea. However, data obtained with the transgenics indicate that losses due to volatilisation are relatively small (10-15%) and do not correlate with urease activity, which itself generates the ammonium prone to volatilise.

Regulation of barley malting quality A project has been initiated aimed at defining the role of specific enzymes and enzyme inhibitors involved in the dissolution and breakdown of starch, cell wall and protein components of barley grains during the malting process. The activity of the starch debranching enzyme, limit dextrinase, has been defined during the malting process in two good quality and two poor quality cultivars and evidence for the presence of specific limit dextrinase inhibitors has been obtained. The nature of these inhibitors is being investigated. The timescale and global levels of arabinoxylan-degrading activities (endoxylanase, exo-xylosidase and arabinosidase) have been surveyed. The solubilisation of arabinoxylans during malting appears to be unrelated to the total endoxylanase activities of the malts. This may be due to the expression of endoxylanase isoforms with different kinetic properties or different levels of specific endoxylanase inhibitors in the good and poor quality varieties. The global proteolytic enzyme activities of the good and poor quality malts were qualitatively and quantitatively similar. Thiol and aspartic proteases contributed the majority of the total proteolytic activity and similar patterns of protease expression were noted during the malting of the good and poor varieties. The global proteolytic activity also matched the profile of protein solubilisation in the different malts. Polypeptide profiling of malting barley has been undertaken. Initial pilot studies on cell wall-associated proteins have been carried out and stage- and cultivar-specific proteins have been identified.

Phytochemistry

Glycoalkaloids The effects of post-harvest stress and light exposure on the glycoalkaloid content of long-day adapted tubers have been investigated. The expected increases in solanidine-based glycoalkaloids, α-solanine and α-chaconine occur in *Solanum phureja* but, in contrast to the behaviour of *S. tuberosum* cultivars, a number of *S. phureja* lines accumulate an additional glycoalkaloid. A combination of liquid and gas spectrometric techniques, together with traditional thin layer chromatographic methods, were used to identify this compound as α-solamarine, a tomatidenol-based glycoalkaloid with solatriose sidechain. Little information is available on the toxic properties of this compound but it has been reported to occur in a limited number of *S. tuberosum* cultivars.

Volatiles A method, utilising porous polymer entrainment combined with thermal desorption-gas chromatography-mass spectrometry, has been developed for the identification of volatile compounds which are likely to be a major source of flavour compounds. Preliminary results utilising this method have shown consistent results. The majority of products identified are derived from lipids and/or amino acids. This methodology is currently being used to compare *S. tuberosum* and *S. phureja* cultivars which differ distinctly in organoleptic properties.

Antioxidants and lipids Research into plant antioxidants is continuing apace as part of the core research programme and in conjunction with a SEERAD grant and funds from a European Northern Periphery Programme (NORTHBERRY project). Studies on the soluble antioxidants in raspberry, strawberry and blackcurrant have shown that the derivation of antioxidant activity is distinct for each species. In blackcurrant, vitamin C is the dominant antioxidant whereas in strawberry, the polyphenolic compounds, catechins and anthocyanins, predominate. The main soluble antioxidants in raspberry remain uncharacterised but experiments suggest that they are glycosylated phenols. Extensive studies have revealed limited variation in soluble antioxidant potential between strawberry genotypes but significant variation in raspberry and even more so in blackcurrant. Examination of wild blackcurrant and raspberry species indicates even greater variation. This could be utilised to boost the

antioxidant status of cultivated germplasm. Investigations into non-soluble, or cell wall-associated antioxidants, suggests that a significant proportion of the antioxidant potential of soft fruit, in particular blackcurrant, has been unaccounted for. These antioxidants interact with the wall through both covalent and non-covalent binding. For a full report see p. 94.

Comparisons of the fatty acid composition of 36 SCRI blackcurrant genotypes indicated that the contribution of γ-linolenic acid (GLA) generally varied between 11-19%, although three genotypes had values of 22-24%. Such levels have not been reported previously for blackcurrant oil. The study showed the potential for developing blackcurrant genotypes with 'added value', particularly as blackcurrant seed is a by-product from juice production. GLA is a nutritionally important fatty acid present in the seed oils of a restricted number of plant taxa and is beneficial for a range of conditions including atopic eczema and rheumatoid arthritis.

Rape plants in which β-ketoacyl-acyl carrier protein reductase (a component of fatty acid synthase) is down-regulated, using an antisense approach, have reduced seed and leaf fatty acid contents. The effects on complex leaf membrane lipids were examined in two mutants. Compared to the wild-type, there were significant differences particularly in one mutant, but the nature of the differences depended on leaf maturity. In young leaves, the concentrations of all lipids were reduced. In mature leaves, the concentrations of most lipids were reduced but phosphatidylglycerol was unchanged whereas phosphatidylethanolamine increased. The fatty acid profiles of individual lipids were mainly similar to the wild-type, particularly in mature leaves. However in young leaves, linoleic acid was reduced in all lipid classes and trans-3-hexadecenoate was substantially higher in plastidic phosphatidylglycerol.

Cell Biology

Plasmodesmata and virus movement The structure/function relationships of plasmodesmata continue to form the research focus of the Unit of Cell Biology. Previously, it was shown that plasmodesmata in developing leaf tissues change from simple to branched forms during the sink-source transition, a conversion associated with a massive down-regulation of plasmodesmatal conductance. The basic differences in plasmodesmata continue to be studied to establish the 'division of labour' that occurs among plasmodesmatal types. Techniques have been developed, based on confocal laser scanning microscopy, that determine accurately the size-exclusion limits of plasmodesmata connecting different tissues within the plant. Studies show that plasmodesmata differ in their permeabilities depending on both physiological and developmental cues. An almost universal feature of plant virus movement is the modification of plasmodesmata to allow passage of the viral genome between cells. Two significant features of the movement process are plasmodesmal 'gating' (transitory increase in the plasmodesmal size exclusion limit) and RNA trafficking (passage of the viral RNA as a ribonucleoprotein complex through the plasmodesmal pore). The behaviour of plasmodesmata during virus infection is being studied by reverse genetics and by incorporating Movement Protein-Green Fluorescent Protein (MP-GFP) fusions into viral genomes to study the subcellular behaviour of MPs *in situ*. Biolistic approaches have helped in these studies by allowing the MP-fusions to be introduced into cells in the absence of other viral gene products. Recently, the gene for a red fluorescent protein (DsRed) was cloned from the non-bioluminescent coral, *Discosoma*, increasing the fluorescent 'palette' of proteins available to cell biologists (see below).

Biosource collaboration The major collaborative venture with Biosource Genetics Corporation (now Large Scale Biology) is entering its third year. The major thrust of this programme is to improve the stability and movement of plant viral vectors for novel protein production in plants. As part of the joint venture, the role of MPs in the viral movement process is being studied using a unique combination of molecular virology, cell biology and non-invasive imaging techniques. One aim has been to produce robust viral vectors, based on *Tobacco mosaic virus* (TMV). Enhanced cell-to-cell movement and stability have been specific targets for vector improvement. Recently, the technique of DNA (gene) shuffling, a PCR-based method for *in vitro* recombination of either single randomly mutated genes or gene families, has been used to evolve enzymes and proteins with novel specificities or enhanced activities. As part of the Biosource collaboration, the MP gene of TMV was shuffled to compensate for losses in activity incurred by foreign gene expression. A full report on this novel approach to viral vector improvement can be found on p. 103.

To study the subcellular basis of improved viral vector movement, fluorescent proteins were fused to wild-

type (*wt)* MP and to the improved shuffled (*shuff)* MP. A direct comparison of the *wt*- and *shuff* MPs was made possible by fusing the MPs to alternative fluorescent proteins (DsRed *versus* GFP) for subcellular localisation. A more detailed report can be found on p. 107. Studies of the behaviour of viral MPs also provide important clues as to the endogenous mechanisms of protein trafficking between plant cells. A popular model for macromolecular trafficking (including that of viral MPs) invokes the microtubule cytoskeleton as an intracellular scaffold along which protein-RNA complexes are transported from their sites of synthesis to plasmodesmata. Because the *shuff*MP was able to enhance TMV movement without associating with microtubules, it was decided to examine the movement of a fluorescently tagged TMV through cells treated with the microtubule-depolymerising agent, colchicine. In these experiments, transgenic plants expressing a GFP-tubulin fusion were used to determine colchicine concentrations at which microtubules were completely depolymerised, without affecting other cell functions. TMV moved unimpeded through the colchicine-treated cells demonstrating that microtubules are not required in the viral movement process. These critical observations force a re-evaluation of current models of macromolecular transport in plants, and the Cell Biology/Biosource Groups are currently developing alternative models to explain the targeted movement of macromolecules through plasmodesmata.