Plant biochemistry and cell biology

Howard V. Davies & Karl J. Oparka

Institute restructuring has resulted in the formation of four scientific research divisions. The Department of Cellular and Environmental Physiology, which has evolved into the Division of Biochemistry and Cell Biology, houses two research units – Plant Biochemistry and Cell Biology. Following the retirement of Dr W.W. Christie M.B.E., as Head of the Chemistry Department, the Chemistry Group has been incorporated into the Plant Biochemistry Unit. These structural changes, together with a revised SCRI Corporate Plan and science strategy, will form the basis of new research alliances and programmes, further integrating our project portfolio.

Plant Biochemistry

Metabolism and developmental processes The functions of two novel plant α-glucosidases genes, both cloned at SCRI, have been studied in detail using transgenic potato plants as experimental tools. Clear-cut evidence demonstrates that one of the α–glucosidases is required for glycoprotein processing. Under field conditions, this gene is essential for normal plant development. The second α–glucosidase gene contributes relatively little to total enzyme activity and there appears to be little impact on the plant of down-regulating its expression. In light of this evidence, the role of this class of enzyme in carbohydrate metabolism must now be re-assessed. Also in potato, biochemical markers of dormancy break in tuber apical buds have been identified. The markers have been exploited to define the stages of meristem activation to be used in a gene discovery programme with the objective of dissecting the signal transduction processes involved in dormancy break. Differentially expressed genes are being identified and microarray technology will be employed to assess the sequence of events that occur at the molecular level.

Tuber formation is characterised by a switch from apoplastic to symplastic unloading of assimilates in the sub-apical stolon region. The results are enhanced sink potential of stolon tips and marked changes in the compartmentalisation of sucrose. The latter may

be directly responsible for the co-ordinated up-regulation of genes involved in storage metabolism and in the marked changes in invertase activities during tuberisation. Data indicate that the mechanisms responsible for the induction of symplastic unloading during tuber formation are central to the regulation of resource allocation and sink-source relationships in potato. Also highly relevant to tuber development are the findings that over-expressing S-adenosylmethionine decarboxylase in tubers modifies both tuber numbers and size grade distributions (larger number of smaller tubers produced).

Detailed analysis of the role of the urease enzyme in nitrogen metabolism in potato is underway. Urea can be used as a foliar crop spray to meet nitrogen requirements whilst preventing extensive losses to, and leaching from, the soil. However, urea accumulation in the leaf can produce leaf scorch and reduce photosynthetic efficiency. This programme aims to modify endogenous urease activity and to determine the impact on nitrogen metabolism. The potato urease gene has been cloned and a range of transgenic plants has been produced to probe the function of this gene. The urease accessory proteins, essential for optimal urease function, are also under study and several novel genes in this class have been cloned. Functional analyses are underway involving transgenic potato plants as experimental tools.

Variability in the rheological properties of potato starch has been examined. Potato starch granules are distinct from cereal starch granules in that they contain covalently-bound phosphate ester groups that effect the properties of the starches, particularly their viscosity. The peak viscosity and viscosity profile are dependent on the potato genotypes from which the starches have been isolated. In general, the higher the phosphate ester content, the higher the peak viscosity at equivalent concentrations. The viscosities of starch granules are also affected by the presence of ions in the medium. Both monovalent cations, such as sodium, and divalent cations, such as calcium, have an effect but the effects are different. The peak viscosity is higher for sodium than for calcium. The temperature programme used in the Rapid Visco-Analyser has a profound effect on the viscosity profile. These differences can have a major effect on the viscosity of starch granules derived from starches that have an inherently low viscosity, especially those that have a low phosphate ester content. Using a maximum temperature of 65°C, that still allows the gelatinisation temperature to be reached, the viscosity profiles of these starches do not give a peak viscosity in the early stages of the profile and the highest viscosity coincides with the final viscosity.

From the developments outlined above it is evident that the Division continues to play a role in developing the potential of GM technologies applied to crop plants. EU funding has been obtained to assess the impact of genetic transformation on potato tuber composition and the potential for unintended effects on nutritional and anti-nutritional components. The EU consortium will use state-of-the-art technologies (microarrays, proteomics and metabolomics) to assess the extent to which metabolic processes are perturbed.

Antioxidants and free radical processes Antioxidants are a diverse set of molecules, which are able to donate hydrogen atoms to reactive free radicals, negating the damage that these cause through interaction with cellular components. This work is currently conducted in the core program of the Unit, along with a SERAD Flexible Fund grant and a grant from the EU Northern Periphery Programme. The aims of the programme include the identification of nutritionally-relevant antioxidants in soft fruit, their bio-availability and accumulation in tissues and the correlation of uptake with biomarkers of disease risk. It is hoped that the results will provide the initial information required to improve the antioxidant content of red berry fruits, by conventional or biotechnological means. The ultimate aim is the establishment of recommended intake of antioxidants for optimal health.

An important dietary antioxidant is vitamin C (ascorbic acid). The biosynthesis of L-ascorbic acid (L-AA) by yeast (*Saccharomyces cerevisiae*) has been examined. Under 'natural' conditions (i.e. when grown in the presence of D-hexoses), *S. cerevisiae* cells synthesise D-erythroascorbic acid (D-EAA) but not L-AA. D-EAA is a five carbon L-AA analogue with similar redox properties and is thought to perform similar antioxidant functions in yeast to those performed by L-AA in other eukaryotes. However, yeast cells can be induced to synthesise L-AA when grown in the presence of L-galactose (L-Gal), L-galactono-1,4-lactone (L-GL) and L-gulono-1,4-lactone (L-GuL). Studies with radiolabelled substrates showed that yeast cells lack the pathway of L-AA biosynthesis present in plants but can be made to synthesise L-AA via the pathway naturally used for D-EAA biosynthesis. This finding has potential biotechnological applications.

A multi-disciplinary EU-FAIR project, involving the

Division of Biochemistry & Cell Biology and the Division of Pathology, continues to increase our understanding of the oxidative stress imposed upon host plant tissues by the necrotrophic fungal pathogen, *Botrytis cinerea*. EPR spectroscopy and LC-MS quantitation of key analytes indicate that the pathogen brings about pronounced changes in the redox state of the surrounding tissues. The implications of this shift to more oxidising conditions are poorly understood at present, although redox state is increasingly becoming recognised as having a major influence upon gene expression.

Lipids, waxes and lectins The complex lipids of blackcurrant leaves have been examined for the first time and contain α–linolenic acid, hexadecatrienoic acid and stearidonic acid. This would appear to be the first report of these three acids being found together in the complex lipids of leaves from the same plant. All previously studied plants have fallen into one of three groups according to the presence of α–linolenic acid only (18:3 plants), α–linolenic and hexadecatrienoic acids (16:3 plants), or α–linolenic and stearidonic acids (18:4 plants).

The physiochemical nature and physiological function of plant and insect cuticular waxes varies between different parts of the organism. For example, in several brassica species studied, waxes from lower (abaxial) leaf surfaces had proportionally more wax esters, secondary alcohols hydroxy ketones and aldehydes, whereas the upper (adaxial) leaf surface had more ketones and amyrins. Such differences are of importance to herbivorous insects such as aphids, which feed on the lower surface and may require specific chemical cues to identify the correct host plant. Aphids in turn have their own characteristic suite of cuticular chemicals. These include aphid-specific triacyglyerols (see review article) and complex mixtures of alkanes. The distribution of individual members of both classes of compound appears to be species-specific, differences having been found between raspberry aphid and pea aphid. In addition, alkanes from raspberry aphid were found to differ in composition between the surface and interior of the cuticle.

Studies on plant waxes have also been extended to include the characterisation of flower waxes. Initially, these have been concentrated on faba bean flowers, where two new classes of epicuticular wax esters based on phytol and cinnamyl alcohol have been identified. The high relative concentration of cinnamyl alcohol esters would appear to reflect the high concentration of phenyl propenoids previously reported in the volatiles released by these flowers.

Large scale screening of 50 species of plants has identified 14 species that produce mannose-specific lectins. These have monomeric molecular weights of 11-13 kD, which suggests that they are part of the superfamily of mannose-specific lectins. Detailed glycosidic linkage analysis has been undertaken using simple and complex oligomannosaccharides. This showed that, in general, the lectins displayed a preference for α(1-3) and/or α(1-6) glycosidic linkages. However, one of the lectins, (from *Beta vulgaris*) did bind α(1-2) linkages. The lectins have been screened for Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV) binding ability using ELISA systems. In general, HIV and SIV binding activities co-eluted during HPLC separations. However, binding specificities were separable in some species. *In vivo* testing showed that selected mannose specific lectins were very effective at inhibiting Feline Immunodeficiency Virus (FIV) infection and syncitia formation. The mannose specific lectin (*Hippeastrum* Hybrid Agglutinin [HHA]) from *Amaryllis* was the most effective, reducing FIV viral production by 85% at a lectin concentration of 160 ng/ml. This out-performs the current pharmaceutical candidate compound, a cyclolactone derivative.

Cell Biology

Plasmodesmata Recent research on the structure/function relations of plasmodesmata, the small pores that interconnect higher plant cells, has shown that discrete classes of plasmodesmata behave differently with respect to their capacity to traffic macromolecules. Simple plasmodesmata can traffic relatively large proteins while branched plasmodesmata allow the passage of only relatively small solutes. Simple plasmodesmata predominate in sink (unloading) tissues, suggesting that the increase in molecular size exclusion limit of these plasmodesmata may accommodate the rapid fluxes of proteins and solutes required for growth in rapidly expanding sink tissues. Significantly, viral movement proteins (MPs) target only branched plasmodesmata and do not associate with the simple plasmodesmata in sink tissues. This unique feature of MPs has been used to study the progression of plasmodesmal development in leaf tissues undergoing the sink-source transition. Using transgenic tobacco expressing a MP-green fluorescent protein fusion, it was possible to follow the distribution of simple and branched plasmodesmata non-invasively during leaf development. The results revealed an enormous loss of simple plasmodesmata during the

sink-source transition, suggesting that this class of plasmodesmata has a transitory existence in sink tissues. In contrast, branched plasmodesmata persist throughout leaf development and predominate in source tissues. The formation of branched plasmodesmata occurs asynchronously in different cell layers and is correlated with the duration of cell division in a given leaf tissue. It would appear that the relative distribution of simple versus branched plasmodesmata in a given tissue governs the ability to traffic macromolecules, including systemic RNA signals, between cells.

Virus movement and gene silencing Basic studies have continued on the cell-cell and long-distance movement of plant viruses. Previous studies concentrated on the movement of a range of viruses that infect dicotyledons. Recently, attention was turned to monocotyledonous-infecting viruses. In collaboration with Biosource Genetics Corporation, the movement of *Barley stripe mosaic virus* (BSMV) expressing green fluorescent protein has been examined. These results confirmed previous studies on dicotyledonous species and established that only major leaf veins are used in the unloading of solutes and viruses. In contrast to previous published reports, the data show that barley utilises a symplastic phloem unloading mechanism.

A well-reported feature of virus infection is the inability of viruses to invade meristematic tissues. Using a range of GFP-tagged viruses, the ability to invade shoot and root meristems was studied non-invasively using confocal microscopy. In the case of Tobacco Mosaic Virus (TMV), it was shown that this virus is able to infect lateral root meristems. However, following the activation of the lateral root meristems, a wave of viral suppression occurs that is initiated in the meristem and progresses basipetally along the root. A detailed report on this topic appears on page 132. In contrast to the above observations, it was found that Tobacco Rattle Virus (TRV) effectively invaded root meristems and persisted in dividing cells of the meristem. This feature makes TRV a particularly valuable vector in virus-induced gene silencing (VIGS) studies in which a range of meristem-specific genes can be silenced using virus-based vectors. Using transgenic *Arabidopsis* plants expressing meristem-specific GFP constructs, it was shown that TRV expressing a truncated GFP sequence was capable of initiating GFP silencing throughout the shoot apical meristem.

The TMV-induced hypersensitive response Using a combination of molecular and cell biology, the events underpinning the hypersensitive response (HR) have been studied using TMV tagged with the green fluorescent protein. These studies have revealed some of the very early events associated with the HR and have identified several novel physiological effects induced during the HR. A detailed report on this study appears on page 136.

SCRI-Biosource collaboration The first year of the above collaborative research project has been completed. Basic studies underpinning virus movement have been combined with more applied projects examining the potential of viral-based vectors in the production of novel proteins. To date, considerable progress has been made in the construction of stable viral vectors for therapeutics and vaccines.