Plant biochemistry and phytochemistry

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The furore over genetically modified foods has accelerated since the turn of the year and must have an impact in the short- to medium-term on all concerned with research and development in plant biotechnology and related sectors. The public should be made aware of the fact that molecular sciences now provide the indispensable tools with which to dissect plant form and function, tools which in the hands of professionals add considerably to the basic knowledge required for crop improvement at many levels. Part of the GM debate centres around potential impacts on the environment, and on the chemistry and composition (safety) of the end products, whether destined for human or animal consumption. The SCRI's skill base facilitates high quality research in all of the above areas, as exemplified by activities in the biochemistry and phytochemistry programmes. This applies to plants whether genetically modified or not. The following overview gives examples of progress in some key areas over the past year.

Carbohydrate research Sink-source relationships play an important role in regulating carbon partitioning to commercially important storage organs such as potato tubers. When developing tubers are detached from the mother plant, there is a significant reduction in the starch synthetic capacity of the tubers, accompanied by a rapid reduction in ADP glucose and an increase in hexose phosphates. The results indicate that one of the first responses to tuber detachment is a reduction in carbon (or ATP) import into the amyloplast. It is speculated that the rate of metabolic exchange between the cytosol and the amyloplast in storage parenchyma cells of developing tubers, depends upon a continuum between the storage organ and the photosynthetic machinery. However, results to date indicate that sucrose is not directly involved in

this process. Also related to sink-source interactions, a programme on the metabolism of tubers during dormancy break (EU-funded) has shown that initial bud growth is sustained with 'soluble' carbon reserves present in the tuber throughout the dormancy period. It is speculated that activation of transport mechanisms and channels from the tuber storage parenchyma to the buds, is a key process in dormancy break.

A transgenic approach is exploited to investigate the role of a range of target genes on carbohydrate metabolism in potato. For example, two α-glucosidases have been cloned, and the effects of down-regulating these genes, using an antisense approach, is well underway. Alkaline invertases from potato and sugar beet have also been cloned and an investigation of the function of these genes is in progress. Maize genes involved in the biosynthesis of starch have also been expressed in potato, and the effects on starch determined. Genes from micro-organisms are also under evaluation with a view to generating novel starch types. A SOAEFD Flexible Funded programme on the processing potential of starches from Scottish-grown crops has been completed and the report submitted. One of the outcomes of the programme is a database containing information on the composition and properties of 619 samples of starch. While all analyses were not carried out on all samples, it is probably the largest single database available on starch composition and properties. The report allows a comparison of the properties and composition of many industrially-produced cereal and potato starches with those produced in the lab from equivalent plant sources. In addition, the scale of the material collected allows many comparisons of the influence of genotype, as well as of genotype x environment interactions, to be derived. The programme also dealt with the relationships between physical and chemical composition of starch, with a view to predicting more complex behaviour from simpler analyses. Considerable use was made of statistical and neural network analyses.

Mathematical biology and biochemical systems Theoretical work aimed at understanding the regulation, dynamics and thermodynamic properties of biochemical systems has progressed, with a major focus on the ability of these systems to adapt to different types of applied stresses. Hierarchical levels of organisation exist in living systems and a full understanding of biochemical functioning must account for the interactions within and between these different levels, and with heterogeneous and stochastically fluctuating environments. A central challenge is to understand how the complex reactions taking place inside cells remain co-ordinated under different environmental conditions and how they respond to changing conditions. Although biochemical systems are generally complex, all have two main features. One is that the activity of any enzyme species becomes saturated at some concentration, irrespective of any additional regulation. The other is that the changes in flux catalysed by any enzyme are not simply proportional to changes in substrate concentration, i.e. enzymatic kinetics are always non-linear. A linear simplification would require very restrictive conditions, which cannot generally be validated for biochemical systems. It is the interactions of these two main features that determine whether or not biochemical systems can adapt to different environmental conditions. Non-linearity may result in different types of temporal and spatial organisation in biochemical systems, and the simple fact that enzyme activity saturates, imposes conditions on these states in order for the reactions to remain co-ordinated. If these conditions are not satisfied, then the cell cannot function. Importantly, the conditions which are suitable for the co-ordination of biochemical systems under different environmental conditions can be determined, and collaborative research in this area is progressing.

A new collaborative project has been established with Strathclyde University and Glasgow University, with support from the BBSRC and EPSERC. A postdoctoral research assistant has been employed to work on the effects of environmental fluctuations on biochemical systems. In addition, collaborative research with University of Laguna, Spain, is underway with the aim of improving the efficiency of citric acid production in *Aspergillus niger* metabolism.

Free radical mechanisms and natural antioxidants in plants An EU-FAIR project has been initiated, involving collaboration between four SCRI departments and seven European and Israeli partners. The programme is investigating the oxidative processes associated with necrotrophic infection. SCRI is utilising both spectroscopy (EPR) and comparative biochemistry to study the complex events associated with pathogenesis. EPR spectroscopy has been used to study apparent shifts in redox status of tissues post-inoculation, whilst characteristic markers of lipid peroxidation have been quantified and correlated with the aggressiveness of the pathogen. EPR spectroscopy also has been used to investigate the effects of abiotic and biotic stresses on free radical processes in cereal

plants. The main applications this year have been on wheat and rice in collaboration with the Austrian Research Centre, Seibersdorf, and the International Rice Research Institute. The work with wheat has concentrated on the detection of free radical damage at relatively low levels of exposure to ozone. The EPR data indicate that there is a threshold exposure level, below which there is no effect on the free radical signal and above which there are major increases in free radical signal with relatively small increases in ozone concentration.

Experiments to identify free radicals generated in plant tissues as a result of physical damage, have been performed using the new spin trap DEPMPO, which has the advantage over its older analogue DMPO in that it is able to discriminate between $O_2^{\cdot -}$ and HO· adducts. Preliminary results indicate that HO· and C-centred radicals are the main products formed in a range of different tissue types. However, the relative intensities of the EPR signals from the two adducts are strongly dependent on the spin trap/tissue ratios and considerably more work is needed in order to understand the relevant reactions involved.

Extending our interests in the nutraceutical status of foods and the role of antioxidants, funding has been gained for work on the potential health-giving properties of the constituents of fruits such as strawberry, raspberry and blackcurrant. The work, funded by a SOAEFD Flexible Fund grant, involves collaborations with the Rowett Research Institute and Glasgow University. Individual antioxidants and natural synergistic combinations of antioxidant molecules will be identified and those with the most potent activities will be assessed in animal models for bio-availability, selective accumulation in tissues, *in vivo* antioxidant properties and their effects on biomarkers of disease risk. The results will provide the initial information necessary to genetically improve the antioxidant capacity of soft fruits, the ultimate aim being to provide an indigenous additional food source for improving antioxidant status *in vivo* - a prerequisite in establishing requirements for optimum health.

Fruit research In strawberry, a wide range of ripening-related genes has been isolated using cDNA AFLP-based approaches. Candidates genes have been selected for inclusion in a transgenic programme with the objective of improving a range of quality traits. As part of a SOAEFD Flexible Funded grant, protocols for two-dimension gel electrophoresis of strawberry proteins have been developed and exploited to sequence proteins which change in relative abundance during ripening. This proteomic-based approach has proved extremely complementary to the programmes on differential gene expression, identifying different targets for crop improvement. In parallel with molecular approaches, the use of modified atmosphere packaging to extend shelf life has been studied. Strawberries have been packaged in sealed plastic punnets using impermeable polypropylene film and three gas mixtures; 20% O_2:80% N_2 (air), 5 % O_2:5% CO_2:90% N_2 and 80% O_2:20% N_2. All gas mixtures dramatically reduced weight loss during storage, with air marginally the best. Maintenance of strawberry juice antioxidant status over relatively longer-term storage was achieved most effectively with high O_2 as the packaging gas. Overall, the fruit stored in the high O_2 mixture proved most successful with respect to maintained firmness, minimised cell wall breakdown and visual appearance. A more expansive article on the fruit packaging research is provided later.

NMR images of fruit infected with fungal pathogens such as *Botrytis cinerea* frequently show high intensity regions corresponding to the fungal lesion. To demonstrate whether or not such contrast is due to the fungal hyphae or to damaged host tissue, blocks of agar gel have been inoculated with spores and imaged by NMR. In the absence of any cell structure to be disrupted by the pathogen, no contrast was visible by NMR in the region infiltrated by the fungus. Thus, the dramatic NMR images of *Botrytis*-infected fruits are due to damaged host tissues. The non-invasive nature of NMR imaging has also proved useful in monitoring the water status of raspberry canes and buds to reveal large changes immediately prior to bud break. The subsequent fruiting of the bud monitored *in situ* is testament to the non-destructive nature of the technique.

Lipid research A new method for the determination of residual solvents in synthetic ^{13}C-labelled triacylglycerols has been developed using thermal desorption. Residual solvents such as acetone, acetonitrile and branched-chain hexanes can be quantified to 0.1 parts per million level. With this information, further purification steps could be performed to reduce solvent residues to levels acceptable for nutritional studies.

Work has continued on developing methods for structural characterisation of plant membrane phospholipids and glycolipids. A novel approach for determining molecular species composition, using liq-

uid chromatography (LC)-atmospheric pressure chemical-ionisation mass spectrometry of diacylglycerol nicotinates, has been developed further to cover a wider range of lipids, and the LC separation has been optimised. A two-dimensional thin-layer chromatographic procedure has also been developed to separate individual phospholipids and glycolipids and has been applied to leaf tissue of *Arabidopsis* wild-type and mutants with altered lipid compositions. The method will be used to isolate individual lipids for subsequent quantification, and fatty acid and molecular species determination.

A novel fatty acid (octadeca-8,10-dien-12-ynoic acid) has been identified in the seed oil of *Tanacetum corymbosum* by chromatographic, spectroscopic and degradative procedures. 4,4-dimethyloxazoline derivatives are widely used for GC-MS analysis of fatty acids, but applications to natural fluorinated fatty acids and synthetic fatty acids, labelled with stable isotopes, have shown that rearrangements can occur in the mass spectrometer that can confuse the interpretation of results.

Compound-specific isotopic analysis of fatty acid methyl esters has yielded promising results, particularly for applications to human lipid metabolism. On-line pyrolysis of methyl esters, separated by GC, to hydrogen, followed by continuous flow measurement of the deuterium/hydrogen ratio, gives reliable results when there is a near natural abundance distribution of deuterium-containing isotopomers. This provides a convenient and sensitive method for measuring deuterium incorporation from labelled body water, and hence fatty acid synthesis *de novo*. With partners in an EU project, the processes of desaturation and chain extension are being studied using uniformly ^{13}C-labelled linoleic acid and following the incorporation into arachidonic acid using GC-combustion-IRMS to detect the low enrichment.

Plant volatiles A range of methods has been evaluated for identifying volatile compounds released by, or present, on the surface of blackcurrant leaves. Analysis of the leaf surface extracts revealed that the main constituents were mono- and sesquiterpene hydrocarbons. Similar results were obtained using solvent elution and thermally desorbed polymer-entrainment, but 'green leaf' volatiles and a homoterpene were also detected. Thermal desorption resulted in the production of at least two artefacts due to heat- or metal-induced rearrangements. Steam distillation was the least satisfactory method, causing rearrangement and oxidation of some terpene constituents. The optimum method for characterising plant-derived odour plumes proved to be solvent elution combined with polymer-entrainment.

As part of an investigation into the ecological chemistry of insect predator/prey interactions, the surface and internal lipids have been analysed from two species of ladybird and their principal food source, the pea aphid. The beetle lipid consisted of hydrocarbons, fatty acids, alcohols and defensive alkaloids (external and internal) and triacylglycerols (internal). Aphid lipid (internal) consisted almost entirely of triacylglycerols, but with shorter acids (C_6, C_{12}, C_{14}) on the glycerol backbone than was found (C_{18}, C_{20}) in the equivalent ladybird compounds. The nature of the ladybirds' internal lipid depends on the type of food source. Insects fed on aphids showed evidence of incorporation of fatty acids derived from aphid triacylglycerols into their own triacylglycerols.

In a collaborative study with the Department of Biological Sciences, Stirling University, the floral volatiles contributing to the aroma of the cut flowers of three varieties of the Sweet Pea (*Lathyrus odoratus*) have been characterised. Major components included (E)-b-ocimene, linalool, nerol, geraniol and α-bergamotene.

Plant fibres research An EU-funded programme on Reed Canary Grass, as a source of pulp for paper and biomass, has been extended due to commercial interest. The plots at SCRI continue to thrive and produce high yields of biomass. However, in common with all the more southerly partners, the lack of a really cold winter with a clear cessation of growth has hindered this programme. The quality of the material produced in all countries, except Sweden and Finland, may be inferior due to too high levels of protein. The group at SCRI has the responsibility for quality assessment over the whole programme in relation to cell wall composition and use of IR spectroscopy to predict quality.

A new initiative is underway into the use of hemp fibre, both untreated and treated, as a source of strength to recycled paper. An inclusion level of 1-2% is likely to be feasible compared with 20% for other fibre sources.

The use of trifluoroacetic acid in the characterisation of plant cell walls is continuing. A fragmentation scheme has been proposed and the fragments are currently being assessed to show how some of the cell

wall macromolecules are connected to build up the three-dimensional network. Improvements in the scheme also are being investigated, with a major advance being the elimination of water during fractionation and hence reducing the opportunities for acid-catalysed hydrolysis of susceptible chemical bonds. Based on these experiments, a simple procedure for the determination of cellulose and total non-cellulosic polysaccharides in cell wall samples is being proposed. Oxone bleaching/delignification studies have now been completed on Jute and Sisal. Oxone proved to be effective at removing lignin and bleaching at low temperature (70°C) and low doses (5% Oxone) without any deleterious effects on the fibre performance. Studies with TAED, a novel environmentally benign delignification/bleaching agent, have been initiated using cereal straw as the sources of fibre. Preliminary results suggest that it is equally effective, if not better than, conventional chlorine-based bleaching strategies.

An initial study into the replacement of PVC/polyester fibres systems with coated plant fibres has been completed and several conclusions drawn. For example, polyurethane coatings are amenable for use with woven cellulosic materials and are capable of inhibiting fungal degradation of matrix flax fibres. However, co-blending of specific high strength synthetic/natural fibres would be required to increase tensile strength to values similar to high strength PVC fabrics.

With respect to the biochemistry of cell walls and lignification processes, oxidase activity has been shown to be ubiquitous in extracts from lignifying, developing xylem tissue and from a taxonomically-diverse range of tree species. This suggests that these enzymes are required for lignification reactions. In addition, the enzymes from angiosperms and gymnosperms have specificities for monolignol oxidation that mirror the monolignol composition of the lignins from these species. A quasi-proteomic approach has been used to identify proteins that are differentially expressed in the developing xylem of compression and normal wood of conifers. A number of candidate cell-wall associated proteins have been identified that may be responsible for the altered cell wall structure and composition characteristic of compression wood. In particular, one compression-wood specific protein yielded an amino-terminal protein sequence that was homologous with known plant laccase genes. This represents the first laccase to be identified from conifers.

Work in research related to the use of stable isotopes is presented in a separate research article.