ROTHAMSTED RESEARCH

# Rothamsted Experimental Station Report for 1986

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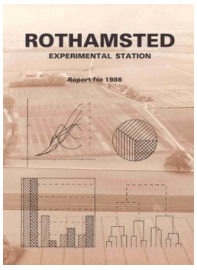


## Molecular Sciences Division

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## BIOCHEMISTRY DEPARTMENT

### Introduction

Despite the uncertain future discussed in the Introduction to last year's Report, the Department is set to become an integrated part of the new Institute of Arable Crops Research during 1987. Although the departure of several senior staff and the loss of their positions has necessitated some administrative reorganization, the range of research interest and approaches has not altered. The emphasis remains on providing basic or strategic information that can be applied to improving the yield, quality or efficiency of production of the major arable crops (wheat, barley, oilseed rape, sugar beet and potatoes) with supporting studies on some model systems. The projects fall broadly into four research areas, formed by



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consolidation of those initiated in 1982. General accounts of the work in these areas and detailed discussions of specific aspects are given below.

Total staff numbers have continued to rise, due to increases in the numbers of staff on fixed-term appointments and visitors. However, further reductions in the permanent staff complement could eventually limit our ability to carry out the wide range of studies which is an integral part of our interdisciplinary approach to crop improvement. We have been awarded two further contracts from the EEC Biomolecular Engineering Programme and a third AFRC New Initiative grant in Food Science. In addition, further funding from the Agricultural Genetics Company and its investors (Ciba-Geigy, De Danske Sukkerfabrikker) has been obtained.

## The biochemistry, chemistry, genetics and molecular biology of carbon and nitrogen metabolism

A major feature of this work is the selection of mutants *in vivo*, and the construction of mutant genes *in vitro*, to further our understanding of intermediary metabolism and to investigate specific ways in which crop plants might be improved. Mutant forms of ribulose bisphosphate carboxylase have been produced through the manipulation of the appropriate genes *in vitro*. Studies of the properties of these modified carboxylases have confirmed the features of protein structure essential for catalysis. More detailed studies should show how the protein needs to be changed to increase its capacity to catalyse carboxylation and to decrease its capacity to catalyse oxygenation of ribulose-1,5-bisphosphate. A Link Award has been initiated with Prof. N. G. Carr (Warwick University) to develop strains of cyanobacteria which lack ribulose bisphosphate carboxylase activity and the $CO_2$-concentrating mechanism. These mutants will be used for the *in vivo* expression of manipulated forms of ribulose bisphosphate carboxylase.

The oxygenation reaction of carboxylase initiates the wasteful process of photorespiration in leaves. Mutants with defects in photorespiratory metabolism, selected from the progeny of chemically mutagenized barley seed, continue to be studied. These help in the understanding of photorespiratory metabolism and are now being studied at the molecular level. Special interest has centred on mutants with defects in the recycling of photorespiratory nitrogen; substituting alternative mechanisms may increase efficiency. Other mutants that have been selected in barley are still being characterized and further selections for specific mutations are in progress. Molecular analyses of the structure and regulation of the key enzymes of ammonia assimilation are also continuing, with emphasis on their expression in developing nodules of legumes.

In addition to our continuing studies on amino acid biosynthesis, a start has been made on the selection of barley mutants with defects in nitrate uptake. Nitrogen is a major limiting factor to crop growth and yield, and extensive use is made of nitrate fertilizers. This may lead to problems when excess nitrate leaches into water supplies with adverse consequences for the environment and for public health. Our goal is to understand the biochemistry and genetics of root nitrate uptake. Improved efficiency in this process in crop plants would reduce the required rate of fertilizer application.

Carbon metabolism in the potato tuber is being studied in an attempt to improve quality for crisp and chip manufacture. The approach is the manipulation of genes for phosphofructokinase to decrease cold-induced sweetening. (Project leaders: Burrell, B. Forde, Keys, Phillips, Wallsgrove. Other staff: Avila Saez, Brooks, Catton, Burton, Cook, Day, Franklin, Hall, Hammond, Hasegawa, Hopley, Keddie, Kendall, Kettleborough, Kruger, Marquez, McArdle, Millard, Parry, Pywell, Quidiello, Relton, Rintamaki, Trevanion, Turner)



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**Dark regulation of ribulose bisphosphate carboxylase.** The inhibitor isolated from potato leaves (*Rothamsted Report for 1985,* 143) has been identified as 2-carboxy-D-arabinitol-1-phosphate. The molecular weight was established in collaboration with A. Mudd (Insecticides and Fungicides) using the gas chromatography/mass spectrograph equipment newly installed at Rothamsted to analyse trimethylsilyl derivatives of the dephosphorylated inhibitor and its lactone. The structure of the inhibitor was deduced from nuclear magnetic resonance (NMR) spectra (with Dr J. Feeney, National Institute for Medical Research, Mill Hill, London) using related synthetic compounds for comparison (with Dr J. Pierce, E. I. Du Pont Company, Delaware, USA) and is fully consistent with its chromatographic properties. Not all species show decreased activity of carboxylase at night, but this does not appear to be related to their taxonomic relationships. Furthermore, the inhibitor is active on carboxylases purified from species not showing the diurnal variation in activity. Either the inhibitor is not made in the dark in some species or it is degraded during extraction. These possibilities are being investigated.

***In vitro* manipulation of genes for ribulose bisphosphate carboxylase.** The genes for the large and small subunits from *Synecococcus* have been expressed in *Escherichia coli* as a model system to investigate the role of specific regions of the polypeptides comprising ribulose bisphosphate carboxylase on its enzymic properties. The construct previously reported (*Rothamsted Report for 1985,* 144) has been manipulated so that translation of the mRNA is correctly initiated and the large subunit polypeptide produced has no N-terminal extension. The bacteria containing this construct (pAnUC92) have been grown in a fermenter to obtain large amounts of the enzyme. A rapid purification scheme has been developed involving sonication of the cells, fractional precipitation of protein from the extract with polyethyleneglycol, and ion-exchange chromatography on 'Fast-Flow Sepharose Q.' A 5 litre culture of bacteria yields up to 0.8 g of pure ribulose bisphosphate carboxylase which appears to be identical in structure and properties to the enzyme purified from *Synecococcus*.

There is extensive sequence homology between the ribulose bisphosphate carboxylases from *Synecococcus* and higher plants. To a lesser degree, this homology extends to the DNA sequences of the genes and consequently there is some conservation of restriction endonuclease cleavage sites. These sites have been exploited to create novel genes for the enzyme, by inserting portions of the maize and wheat large subunit genes into the homologous *Synecococcus* gene. In one experiment, DNA encoding the 11 N-terminal amino acids of the *Synecococcus* large subunit was replaced by DNA encoding the same region in wheat and maize. This region has been shown to be important for enzyme catalysis by proteolytic cleavage (Gutteridge, Millard & Parry, *FEBS Letters* (1986) **196,** 263–268). When expressed in *E.coli,* these chimaeric genes produced active enzyme. In contrast, when this region was replaced by 'nonsense' DNA encoding amino acids from $\beta$-galactosidase, the enzyme produced was inactive. The catalytic properties of these mutant enzymes and the contribution of the N-terminal amino acids to catalysis are being investigated. A conserved AvaIII site was used to construct chimaeric genes encoding the N-terminal half of the maize or wheat large subunit and the C-terminal half of that from *Synecococcus* and *vice versa*. The enzymes produced from these constructs are not active, possibly because the individual polypeptide subunits do not assemble into the holoenzyme. Work is also in progress to determine the role of particular amino acids by *in vitro* mutagenesis of the cloned genes using mis-matched priming by synthetic oligonucleotides. We have isolated five such mutants, including changes to His-295 and Lys-336, and are engaged in identifying any changes in catalytic properties.

**Monoclonal antibodies to carboxylase.** A range of monoclonal antibodies has been raised to ribulose bisphosphate carboxylase from wheat leaves (in collaboration with the Monoclo-



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nal Antibody Centre at Babraham). The antibodies have been characterized, and will be used to develop immunoaffinity adsorbents for the rapid purification and characterization of the enzyme produced by *in vitro* mutagenesis.

**Photorespiratory mutants.** Mutants are available with lesions affecting seven steps in photorespiratory metabolism (*Rothamsted Report for 1985*, 142–3). Genetic analysis of the mutants indicated that all of the enzymes affected are encoded by single nuclear genes. Some double mutant combinations have been constructed for futher studies of metabolism. The glutamine synthetase/glutamate synthase double mutant retains the gas exchange characteristics of a glutamate synthase mutant, while accumulating ammonia at the high rate typical of a glutamine synthetase mutant.

Further studies were made of the mutant RPr 84/29 which is deficient in ribulose bisphosphate carboxylase. The ratio of incorporation of [$^{35}$S] methionine into the large and small subunit polypeptides in leaves was 1.7 for the mutant enzyme compared to 4.9 for the enzyme in the parent line (Maris Mink). The soluble amino acid content of leaves of the mutant was some threefold higher than in Maris Mink. Differences in leaf composition were more marked in young leaves than in old leaves, suggesting a defect in protein synthesis. Levels of messenger RNA for the large subunit polypeptide in the leaves of RPr84/29 were similar to or slightly higher than in Maris Mink. A DNA probe will be used to make similar measurements of the amounts of mRNA for the small subunit polypeptide.

A novel selection procedure is being used to identify new photorespiratory mutants. Seeds of a catalase mutant, RPr79/4, were treated with nitrosomethylurea, a mutagen known to produce a high frequency of mutations in the chloroplast genome. When grown in air, seedlings of Rpr79/4 show silvering of the leaves which is highly characteristic of catalase deficiency. Plants without such symptoms should either have mutations affecting enzymes upstream from catalase in the photorespiratory cycle (glycollate oxidase, phosphoglycollate phosphatase, or ribulose bisphosphate oxygenase) or be catalase revertants. One plant has been selected for further study.

More detailed studies have been made of two photorespiratory mutants. Analysis of leaf extracts from lines deficient in glutamine synthethase (GS) showed that all except one contained little or no protein immunochemically related to the $GS_2$ isoenzyme. The exception was RPr84/12, which had the normal amount of the protein but less than 3% of the catalytic activity. The mRNA for $GS_2$ is being extracted from mutant lines as a first step to investigating the lesions at the gene level.

A rapid purification procedure has been developed for ferredoxin-glutamate synthase (Fd-GOGAT) from barley leaves. An apparently pure protein was obtained after affinity chromatography on ferredoxin-Sepharose. It is, like other Fd-GOGATs, a single polypeptide of $M_r$ 156 000, with an absorption spectrum characteristic of an ion-sulphur flavoprotein. The affinity of the enzyme for the non-physiological electron carrier methyl viologen is very low ($K_m$app=1.9 mM:$K_m$app. (ferredoxin) 2$\mu$M) but at saturating concentrations the activity of the enzyme is the same with either carrier.

**Studies on enzymes of amino acid biosynthesis.** Acetohydroxyacid synthase catalyses the first step in the common pathway leading to leucine, valine and isoleucine. Two alternative reactions are catalysed, leading to either acetolactate or acetohydroxybutyrate. The standard assay used can only measure the first of these reactions, and we have attempted to devise methods of monitoring the second. Oxybutyrate, the substrate for this reaction, strongly inhibits acetolactate synthesis. Some progress has been made in following acetohydroxybutyrate production in crude barley leaf extracts (with Prof. D. Crout, University of Warwick), but more highly purified enzyme is required for detailed kinetic studies.

Acetohydroxyacid synthase from carrot suspension cultures has been used to study the



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kinetics of inhibition by sulphonylurea and imidazolinone herbicides. Both classes of compound were found to be potent, slow-binding, reversible inhibitors (sulphonylureas being competitive and imidazolinones non-competitive) with respect to pyruvate.

Work has continued on the purification of aspartate kinase from carrot cell cultures (in collaboration with the University of Lancaster). A sensitive staining procedure was developed for the visualization of enzyme activity on polyacrylamide gels, after non-denaturing electrophoresis. Using this the $M_r$ of the native enzyme was estimated as 250 000. A similar value was given by gel filtration. Current work is aimed at developing affinity absorbents for use in preparing highly purified enzyme for detailed kinetic studies.

**Nitrate uptake mutants.** Two screening methods have been employed for selection of barley mutants with defects in nitrate uptake. In the first, seedlings are grown in liquid media with limiting nitrate. Plants showing symptoms of severe nitrogen stress are recovered and transferred to high-nitrogen medium. The second screen uses a nitrate electrode to directly monitor nitrate uptake rates in young seedlings. Some 10 000 $M_2$ seedlings have so far been screened, and several potential mutants selected and recovered. $M_3$ seed of some of these lines have been re-screened, and shown to retain the 'low uptake' phenotype. Further characterization of these lines is in progress.

**Molecular biology of key enzymes of ammonia assimilation.** The major pathway of ammonia assimilation in higher plants is catalysed by glutamine synthetase (GS) and glutamate synthase (GOGAT), working in conjunction. Previous studies have shown that GS in *Phaseolus vulgaris* exists as a number of isozymes and that the subunits of these isozymes are specified by a small multigene family. One GS polypeptide (the $\gamma$-subunit) is specific to nitrogen-fixing root nodules and appears to be coded by a gene that is expressed only during nodule development. The nodule-specific gene has been cloned and its 5′-flanking region sequenced as the first step in the identification of *cis*-regulatory sequences that may be responsible for nodule-specific expression. A second GS gene has been identified 3–4 kb downstream from the nodule-specific gene and in the same orientation. Sequence analysis shows it to be a previously unidentified member of the GS multigene family. A constitutively expressed GS gene has also been cloned and its 5′-flanking region sequenced for comparison with the nodule-specific gene.

**Decreasing cold-induced sweetening in potato.** Phosphofructokinase (PFK) purified to virtual homogeneity (*Rothamsted Report for 1985*, 150) has now been analysed in some detail. Tubers of potato contain four forms of the enzyme (called I, II, III and IV) which are probably all heterotetramers. Four polypeptides (1, 2, 3 and 4) with $M_r$s between 45 000 and 54 000 have been resolved by SDS-PAGE. Cyanogen bromide and chlorosuccinamide digests of the polypeptides indicate that they are all closely related. Antibodies have been raised in rabbits to these polypeptides which recognize the proteins on 'western blots'. Each form of the enzyme has a different polypeptide composition. We are currently screening cDNA libraries to isolate clones for PFK and making constructs to reintroduce modified genes for PFK into potato with *Agrobacterium*.

## Manipulation and analysis of genetic material in arable crop plants

A major aim is to develop efficient systems for the regeneration of the major arable crop plants (wheat, barley, potato, oilseed rape, sugar beet) from isolated protoplasts, cells or tissue using *in vitro* culture. These systems are being applied to transfer or introduce useful genetic information into and between plant cells, with the regeneration of intact modified plants. The methods of gene transfer used include *Agrobacterium* and its engineered



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derivatives as vectors, direct gene transfer (particularly electroporation) into protoplasts and tissues, and various methods of protoplast fusion (especially electrofusion). The expression of genes isolated and used in this programme is examined, with special emphasis placed on the control of plant development. Changes induced by culture processes themselves are also being evaluated to determine their origins, whether they can be controlled and their potential applications to crop improvement.

The techniques are also useful in analysis of plant metabolism to identify reactions limiting crop production, and work is closely integrated with other projects within the Department. (Project leaders: M. G. K. Jones, Karp, Ooms. Other staff: Baynton, de Both, M. Clark, Cooper-Bland, Eady, Fish, Foulger, H. Jones, Lee, Lindsey, Murdoch, Potter, Risiott, Steele, Symonds, Tillson, Twell, Wu).

**Potato.** A wide range of studies continues to be carried out on potatoes. Comparison of effects of different culture media on chromosome stability of regenerated plants indicate that hormone composition and concentration in the callus stage of regeneration are most important. The genomic stability of cultured potato plants is also being studied using DNA restriction fragment length polymorphisms. Plants have also been regenerated successfully from tuber protoplasts. Novel somatic hybrids between both tetraploid and dihaploid potato and the wild species *Solanum brevidens* have been produced using chemical and electrofusion of protoplasts, and characterized at the biochemical and molecular levels. Similarly, tetraploid hybrids have been synthesized from two selected dihaploid clones (material provided by the Scottish Crops Research Institute, Pentlandfield). Work on partial genome transfer following irradiation of donor protoplasts is in progress. Two approaches are being used to transfer DNA into potato plants. Electroporation of DNA constructs into protoplasts is being used to study both transient expression and stable integration. We are also trying to improve the efficiency of specific gene transfer into tissues by *Agrobacterium*. A clear polarity effect associated with rapid shoot formation was observed when tuber tissues and basal and apical stem tissues were infected with mutant *A. rhizogenes* strains. This could lead to a very rapid (two to three weeks) regeneration system of transformed plants with minimal somaclonal variation.

Potato plants have also been transformed with *Agrobacterium* binary vectors with the natural T-DNA cytokinin gene (T-cyt) or each of four T-cyt genes with promoter mutations. 'Southern blot' analysis of transformed regenerated plants showed the presence of T-cyt DNA sequences. The plants also showed changes in growth and development, which were probably due to expression of the introduced T-cyt genes. Further analysis of growth and T-cyt DNA gene expression in this material is in progress.

The expression of $R_i$ T-DNA genes (from *A. rhizogenes*) in four previously isolated transformed potato lines is also being examined. Preliminary results indicate limited variation in the steady state levels of RNAs transcribed from these genes in the roots, shoots and tubers.

**Sugar beet.** Reliable systems for production of dividing protoplasts have been established. The conditions for high efficiency electrofusion of these protoplasts have been determined, and the protoplasts have been surface labelled with a specific glycolipid in preparation for directed electrofusion. Electroporation techniques are being investigated for inducing direct gene transfer into protoplasts. The effects of electrical pulses on the permeability of membranes to dye and DNA molecules have been examined, and electroporation is being used to introduce DNA for studies of transient expression and stable integration. The regeneration of plants from protoplasts and tissues is also being studied.

**Cereals.** Work on the tissue culture and transformation of cereals is continuing. The bread wheat suspension culture (C82d) that yields dividing protoplasts has been recycled three



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times to select lines with a higher division frequency (called $C82d^2$, $C82d^3$, $C82d^4$ respectively). The improvement is about tenfold. The cytology of wheat suspension lines, recycled lines, and protoplasts derived from them have been examined in detail to reveal numerous cytological changes. Various gene constructs with chloramphenicol acetyl transferase (CAT) reporter genes have been introduced into wheat suspension culture protoplasts by chemical and physical (electroporation) methods of direct gene transfer to develop methodology for examining expression of cereal gene constructs in cereal tissues. Transient expression of CAT genes has been obtained two to seven days after their introduction into protoplasts. Selection systems to obtain stably transformed cereal tissues have been examined, and emphasis is now on cereal transformation.

Plants have been regenerated from immature embryos of barley, and examined cytologically and biochemically for somaclonal variation. Considerably less variation is observed than in wheat. Plants regenerated from *Hordeum spontaneum* (with Dr A. Breiman, Tel Aviv University, Israel) were stable with 2n=14 chromosomes.

**Oilseed rape.** Progeny analysis of cv. Jet Neuf transformed with Ri T-DNA is in progress and considerable variation is apparent in expression of introduced genes. This may be caused by Ri T-DNA methylation.

### The chemistry, genetics and molecular biology of plant storage proteins

The emphasis of research on cereal seeds remains firmly on studies which will enable us to understand and ultimately manipulate the factors that determine cereal grain quality. Two aspects of quality are of particular interest—the nutritional quality of barley as feed for non-ruminant livestock (pigs and poultry), and the quality of home-grown wheat for breadmaking. Both are determined, to a large extent, by the composition and properties of the grain proteins. The low contents of two essential amino acids (lysine and threonine) in the major storage protein fraction (hordeins) of barley grain limit the nutritional quality of the whole grain. We are therefore studying the structure and control of expression of these proteins and their genes. Similar studies are also being made of several proteins that are unusually rich in lysine, notably two inhibitors of chymotrypsin and $\beta$-amylase. In addition to studies of the classical genetics of these proteins, work has started on mapping their genes using DNA restriction fragment length polymorphisms and by *in situ* hybridization using probes labelled with biotin. The breadmaking properties of wheat are determined to a large extent by the gluten fraction. This is a visco-elastic mass, comprised predominantly of storage proteins. The structure of gluten is being studied using a range of approaches including spectroscopic analysis, amino acid sequence determination and cloning and sequencing of gluten protein genes. This project has been strengthened by the award of a New Initiative grant to explore the molecular basis of gluten properties by protein engineering.

A related project is to improve the content and composition of the proteins present in potato tubers. Genes for the major tuber storage protein (patatin) have been isolated and their structure, organization and control of expression is being studied. (Project leaders: Karp, Kreis, Ooms, Shewry. Other staff: Burgess, Buxton, J. Clark, M. Clark, Copley, J. Forde, Franklin, Gallois, Halford, Healy, Henderson, Hull, Hutchinson, Johari, Lyons, Madgwick, Marris, McArdle, Parmar, Peterson, Pratt, Silva Rosales, Smith, Tatham, Twell, Whittington-Smith, Williamson)

**Isolation and expression of barley seed protein genes.** Studies of the regulation of cereal seed protein genes are of value not only in relationship to grain quality, but also because they give an insight into possible mechanisms involved in the regulation of grain development. We are interested in the structure and the regulation of expression of genes for the hordeins, and for the lysine-rich proteins $\beta$-amylase and chymotrypsin inhibitors (CIs) 1 and 2. A



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number of cDNA clones for these proteins have been sequenced, enabling the first complete amino acid sequences of $\beta$-amylase and of two isoforms of CI-1 to be deduced. These cDNAs have been used as probes to determine gene numbers and to estimate the abundances of mRNA in shoots, leaves and endosperms of normal and mutant (high lysine) barleys. Although CI-2 is expressed in all three tissues, the mRNA transcripts are much more abundant in the endosperm and 300 bases shorter than in the other tissues, indicating that they may be transcribed from different genes. $\beta$-Amylase is also expressed in all three tissues but most abundantly in the endosperm. In contrast, no transcripts for CI-1 are present in leaves or shoots. The high-lysine genes *lys* and *lys3a* only affect the expression of these genes in the endosperm. The cDNAs have also been used to identify genomic clones for $\beta$-amylase and CI-2 and these are currently being characterized.

We have used two approaches to study the control of expression of cereal protein genes. Firstly, a functional analysis of the 5′-flanking region of the B1 hordein gene is being made using a heterologous tobacco transformation system. A 2.9 kb *EcoRI* fragment containing a complete B1 hordein gene and five deletion mutants of this fragment have been inserted into the Bin 19 and Bin 6 vectors. These constructs were transformed into the tobacco genome using the leaf disc/*A. tumefaciens* transformation system. A 600 bp sequence from the 5′-flanking region of the B hordein gene was also fused to the coding region of the bacterial reporter gene, chloramphenicol acetyl transferase (CAT), in Bin 19. About 150 transgenic plants were regenerated. In these the B hordein gene was expressed only in the tobacco seed using its own promoter. Furthermore, the 600 bp sequence from the 5′-flanking region of the B hordein gene contained all the information necessary to direct the expression of the CAT gene in the tobacco seed. The second approach is to study the interaction of transacting protein factors and the 5′-flanking region of the B hordein gene. The method of Miskimins *et al.* (*Proceedings of the National Academy of Sciences, USA* (1985) **82,** 6741–6744) has been used to identify several nuclear proteins that appear to interact specifically with this region. Also, some of these proteins are present in nuclei from endosperms but not those from shoots.

**The presence of $\alpha$-type prolamins in wild species of the Triticeae.** The $\alpha$-type gliadins are a group of prolamin storage proteins found only in seeds of wheat and wild relatives of the *Triticum*/*Aegilops* group, and are encoded by genes on the group 6 chromosomes. Other prolamins present in these species are encoded by genes on the group 1 chromosomes, and the $\alpha$-gliadin genes almost certainly also originated on these chromosomes but were moved by translocation.

We have shown that $\alpha$-type prolamins are also present in two other genera of the Triticeae, where they are encoded by chromosomes homologous with the group 6 chromosomes of wheat. The species analysed were *Haynaldia villosa* (in collaboration with Dr D. Pappin, University of Leeds), *Elytrigia pontica* and *Elytrigia elongata* (with Dr. J. Dvorak, University of California, USA and Dr D. D. Kasarda, USDA, California, USA). The $\alpha$-type prolamins were identified by N-terminal amino acid sequencing and their structural genes located by electrophoretic analysis of addition lines of individual chromosomes of *H. villosa* and *E. elongata* into wheat. The presence of these prolamins in *H. villosa* was unexpected as this species is thought to be closely related to rye. Rye does not contain $\alpha$-type prolamins, but a derived form of $\gamma$-type prolamin (the $M_r$ 75 000 $\gamma$-secalins) encoded by genes on chromosome 6R. The absence from *H. villosa* of proteins homologous with the $M_r$ 75 000 $\gamma$-secalins indicates that this group is a recent evolutionary development, probably only present in the genus *Secale* (wild and cultivated ryes).

**The structures of wheat gluten and related proteins.** Analysis of the high molecular weight (HMW) subunits of glutenin, which have been implicated as determinants of breadmaking



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quality, remains a major priority of our Gluten Chemistry programme. A further HMW subunit gene has been isolated from the cultivar Cheyenne and its nucleotide sequence determined. This is the first subunit gene from chromosome 1B to be isolated, and the encoded protein is closely related to subunits encoded by chromosomes 1A and 1D.

A second subunit encoded by chromosome 1B, derived from the durum wheat cultivar Bidi 17, has been the subject of a detailed conformational study using viscometric analysis and circular dichroism spectroscopy (with Dr J. M. Field, Miln Marsters Group, Docking). This showed that the molecule is rod-shaped with dimensions of about 350–400Å×17–20Å. These dimensions are consistent with the central repetitive domain forming a coiled structure composed of repetitive $\beta$-turns. A similar structure, called a $\beta$-spiral, is formed by the synthetic polypentapeptide repeat motif of the elastomeric mammalian protein elastin. This conformation is unusually stable, being only partially unfolded by 5.9M guanidine hydrochloride. The repetitive domains of HMW subunits are flanked by non-repetitive N- and C-terminal domains. These contain most or all of the cysteine residues which may cross-link the polypeptides to form large elastomeric polymers. Similar studies of C hordein of barley indicate that a $\beta$-spiral may also be present, but the absence of cysteine residues precludes the formation of elastomeric polymers.

We have also made preliminary conformational studies (also with Dr J. M. Field) of the second and quantitatively major group of aggregated gluten proteins. These are present in alcohol-soluble and alcohol-insoluble aggregates, where they have been called aggregated gliadins and low molecular weight subunits of glutenin respectively. Comparison of available amino acid sequences indicate that these groups are closely related to each other and to the monomeric $\alpha$-, $\beta$- and $\gamma$-gliadins. They also have similar secondary structure contents, with 31–37% $\alpha$-helix, 18–24% $\beta$-sheet and 41–49% $\beta$-turn and random coil in 50% (v/v) aq. propan-1-ol at 20°C.

**Analysis of patatin genes of potato.** The structural organization of ten genomic clones for patatin has been determined for the cultivar Désirée. On the basis of restriction endonuclease maps and partial nucleotide sequences they have been grouped into at least six classes. The 5′-end of one clone (pPOT1) was fused to the reporter gene chloramphenicol acetyl transferase (CAT) and reintroduced into cv. Désirée. Expression only occurred in tubers and the level was, to a large extent, independent of the site of integration in the genome. A similar transcriptional gene fusion was made between the 5′-end of pPOT1 and the coding region plus its control sequences of the T-DNA cytokinin gene from *Agrobacterium*. Plants transformed with this construct formed roots, and their tuberization properties are currently being examined. Translational gene fusions between the 5′ control regions of the remaining isolated patatin genes and the reporter gene $\beta$-glucuronidase have also been made and reintroduced into Désirée.

## Disease resistance

Our joint programme with the Plant Pathology Department has continued, with studies on the response of the plant (notably the production of pathogenesis-related, or PR, proteins) to virus infection. A new project, supported by a grant from the Cell Signalling and Recognition programme, is to identify the molecules that migrate from infected tissues to elicit PR protein production and a resistance response in nearby uninfected parts of the plant. (Project leader: Shewry. Other staff: Antoniw, Buxton, Gordon-Weeks, Pierpoint, Tatham; Gunn, White (Plant Pathology))

**Discovery of PR proteins in other plants.** PR proteins ($M_r$ 14000–18000) serologically related to the PR1a protein from *Nicotiana tabacum* cv. Xanthi-nc were detected by 'western



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blotting' in infected or salicylic acid-treated plants of tomato, potato, *Solanum demissum*, sugar beet, *Chenopodium amaranticolor*, *Gomphrera globosa*, barley and maize (with Drs E. P. Rybicki, M. B. von Wechmar and J. L. Dekker, University of Cape Town, S. Africa). No such proteins were found in extracts from healthy untreated plants except *G. globosa*. The amount of this protein in *G. globosa* increased substantially on treating plants with salicylic acid. The existence of serologically-related PR1-type proteins in both monocotyledonous and dicotyledonous plants indicates that they may have an important, if as yet undefined, role in pathogen-infected plants.

**Induction of PR proteins.** Pierpoint *et al.* (*Physiology and Plant Pathology* (1981) **19,** 85–97) found that detached leaves of tobacco plasmolysed in mannitol usually produced small amounts of PR protein. We have found that mannitol induction of PT-1a protein in leaf discs is suppressed in the presence of antibiotics indicating that their induction is not due to mannitol but to the growth of contaminating bacteria. Increasing concentrations of mannitol progressively inhibited PR-1a protein accumulation induced by salicylic acid and 0.7M mannitol inhibited over 95% of both PR-1a and total protein synthesis. Salicylic acid-treated leaf discs exported a high proportion of newly synthesized PR-1a through the cut edge of the leaf into the surrounding medium, consistent with the extracellular nature of these proteins, and secreted up to 90% of their PR-1a into the culture medium.

**Characterization of PR proteins.** The PR proteins occur in the intracellular fluids of tobacco leaves and can be readily extracted by gentle infiltration techniques. This has facilitated the purification and characterization especially of those PR proteins (N to R) which have slow mobilities on electrophoresis. We have not yet succeeded in separating them from peroxidase activity, and the possibility exists that one or two of the minor components are enzymes involved in the lignification of cell walls, a process that accompanies the localization of infections.

However, most attention has been devoted to PR protein R, which has a large (8 mol%) content of cysteine and an $M_r$ near 23 000. Its properties resemble those of a protein recently predicted (Cornellisen *et al.*, *Nature* (1986) **321,** 531–532) by analysis of m-RNAs induced by virus (TMV)-infection of tobacco leaves, and which has a high (65%) sequence homology with the sweet-tasting protein thaumatin that occurs in the fruits of the West African shrub *Thaumatococcus danielli*. Determination of the N-terminal amino acid sequence of R (with Dr D. J. Pappin, University of Leeds) shows the presence of two isoforms in approximately equal amounts. One of these is almost certainly the thaumatin-like protein, whereas the other is very similar but the product of a separate gene. The CD-spectra and chemical properties of R indicate that, like thaumatin, it has a structure consisting mainly of $\beta$-sheets stabilized by disulphide bonds. However, it does not react with an antibody to thaumatin and it is not sweet; thus it apparently lacks the specific '$\beta$-ribbon' that is believed to contain the major antigenic determinant of thaumatin and which also confers the intense and persistent sweet taste to the protein.

**Identification of elicitors.** Intracellular fluids from TMV-infected leaves will induce some measure of virus-resistance when injected into healthy leaves (Modderman *et al.*, *Phytopathologische Zeitschrift* (1985) **113,** 165–170). We have therefore started to examine these fluids for the presence of mobile elicitors ('wound-hormones') which will also induce the synthesis of PR proteins in healthy leaves. We have overcome the initial problem that intracellular fluids already contain the proteins whose induction is under test, and are now studying fluids from an interspecific tobacco hybrid (*Nicotiana glutinosa*×*N. debneyi*) in which PR proteins, and presumably their inducers, are expressed constitutively.



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## Glasshouse services

During the year the interdivisional (Molecular Sciences and Agronomy and Crop Physiology) glasshouse service has grown some 10000 pots and trays of material. Development has continued on hydroponic systems, particularly for growing sugar beet and screening for nitrate uptake mutants.

The computer logging and monitoring system has been extended to cover two further glasshouses. (Services coordinator: Franklin: Other staff: Weaver (Biochemistry); Plumb and Ward (Physiology and Environmental Physics)

## Staff and visitors

**Outside support and collaboration.** The Department gratefully acknowledges the financial support that has been provided by the Agricultural Genetics Company; De Danske Sukkerfabrikker; The Gatsby Foundation; Ciba-Geigy; Limagrain; ICI Plant Protection; Sigma Chemical Company; Directorates DG XII (Education and Science) and DG VI (Agriculture) of the EEC; The International Potato Centre; Rank-Hovis McDougall; The Potato Marketing Board and The University of Leiden.

We have also shared SERC/CASE research students with the Universities of Durham and Warwick. We also wish to acknowledge collaborations with a number of Institutions throughout the world. The contributions of some of the individuals and institutions are acknowledged in the text.

**Vistors.** During the year the Department was pleased to welcome for extended visits: Dr H. Bauwe (German Democratic Republic), Miss Penelope Bebeli (Greece), Dr F. Botha (S. Africa), Dr N. Bulleid (Canterbury), Dr P. Gallois (France), Dr H. Hasegawa (Japan), Dr Maria Hungria Cunha (Brazil), Dr R. P. Johari (India), Dr Paloma Manzanares Secades (Spain), Dr. D. Peterson (USA), Miss Eevi Rintamaki (Finland), Miss Conchita Avila Saez (Spain), Dr Laura Silva Rosales (Mexico), and Dr G. Van der Steege (Netherlands).

**Visits abroad.** Members of the Department attended the following conferences: EEC meeting, Wageningen, The Netherlands (M. G. K. Jones, M. Kreis, C. Marris), EEC meeting, Paris, France (K. Lindsey), International Symposium on Plant Molecular Biology, Strasbourg, France (M. Kreis), Gordon Conference, Rhode Island, USA (M. M. Burrell), Workshop on Inorganic Nitrogen Metabolism, Jarandilla, Spain (B. G. Forde, R. M. Wallsgrove), EEC meeting, Versailles, France (S. Cooper-Bland, H. Jones), Potato Association of America meeting, Ithaca, USA (A. Karp), International Association for Plant Tissue Culture, Minneapolis, USA (S. Cooper-Bland, M. T. J. de Both, M. G. K. Jones, K. Lindsey), FEBS Berlin, FRG (A. L. Phillips), Congress of the Federation of European Societies of Plant Physiology, Hamburg, FRG (M. G. K. Jones), EMBO Workshop, Freiburg, FRG (M. Kreis), EMBO Workshop on Plant Viruses, Wageningen, The Netherlands (J. F. Antoniw), Netherlands Plant Virus Group, Wageningen, The Netherlands (J. F. Antoniw), International Photosynthesis Congress, Rhode Island, USA (A. J. Keys), Fifth International Barley Genetics Symposium, Okayama, Japan (P. R. Shewry), International Grain Forum, Amsterdam, The Netherlands (P. R. Shewry), French Society of Plant Physiologists, Paris, France (R. M. Wallsgrove), and Electroporation meeting, Bielefeld, FRG (M. T. J. de Both).

Visits abroad were made to the following institutes to further collaboration or for exchange of information: Max-Planck Institut, Cologne, FRG (M. Kreis, B. G. Forde, G. Ooms), Vrije Universiteit van Brussel, Belgium (M. G. K. Jones, B. G. Forde, G. Ooms, R. M. Wallsgrove), University of Baarn, The Netherlands (J. F. Antoniw), Ciba-Geigy,



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Basle, Switzerland (J. F. Antoniw), INRA, Dijon, France (J. F. Antoniw), De Danske Sukkerfabrikker, Copenhagen, Denmark (J. F. Antoniw, M. G. K. Jones, K. Lindsey), University of Thessaloniki, Greece (A. Karp), Athens School of Agricultural Sciences, Greece (A. Karp), Texas A & M University, USA (M. M. Burrell), University of Seville, Spain (B. G. Forde), INRA Versailles, France (G. Ooms), Agrigenetics Company, Wisconsin, USA (M. G. K. Jones), Pioneer Hi-Bred Inc., Iowa, USA (M. G. K. Jones), Swedish University of Agricultural Sciences, Universities of Uppsala, Stockholm, Umea and Lund, Sweden (A. J. Keys), Universities of Leiden and Ghent (B. G. Forde, G. Ooms), Universities of Amsterdam and Wageningen (G. Ooms), Universities of Hangzhou, Shanghai, Nanjing, Beijing, People's Republic of China (M. G. K. Jones), INRA, Nantes, France (A. S. Tatham), and Limagrain Biosem, France (M. T. J. de Both).

**Staff.** Marjorie Byers retired after 30 years. During the year S. W. J. Bright, Josie Dunham, D. Foulger, S. Gutteridge, Barbara Hackett, M. Keddie, Christine Leatham, Tish Makin, Barbara Millard, Jane Oliver and Jane Turton also left.

Newcomers to the Department were: Caroline Catton (University of Manchester), Hazel Day (University of Cambridge), Alison Hopley (University of Oxford), Jeanette Hutchinson, Gillian Hull (University of London), B. T. Lee (University of Hohenheim, Stuttgart, FRG), K. Lindsey (University of Edinburgh), Pippa Madgwick (University of Oxford), Kathryn Pratt (University of Durham), Eleanor Weaver and Ruth Gordon-Weeks. J. F. Antoniw returned after spending a year working in the Plant Virology Group at the University of Leiden, The Netherlands.



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