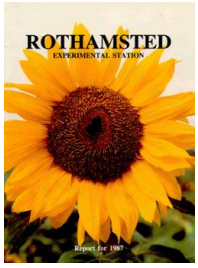
*This work is licensed under a Creative Commons Attribution 4.0 International License.*

Thank you for using eradoc, a platform to publish electronic copies of the Rothamsted Documents. Your requested document has been scanned from original documents. If you find this document is not readible, or you suspect there are some problems, please let us know and we will correct that.

ROTHAMSTED RESEARCH

# Rothamsted Experimental Station Report for 1987

Full Table of Content




## Crop Science Division

### Professor K. J. Treharne

Professor K. J. Treharne (1988) *Crop Science Division ;* Rothamsted Experimental Station Report For 1987, pp 81 - 91 **- DOI: https://doi.org/10.23637/ERADOC-1-28**

*This work is licensed under a Creative Commons Attribution 4.0 International License.*

# CROP SCIENCE DIVISION

## STAFF

**Head of Division Professor K. J. Treharne, Ph.D.**

## Biochemistry Department

*Head of Department*
P. R. Shewry, Ph.D.

*Principal Scientific Officers (UG7)*
B. G. Forde, Ph.D.
M. G. K. Jones, Ph.D.
A. J. Keys, Ph.D.
M. Kreis, Ph.D.
G. Ooms, Ph.D.
W. S. Pierpoint, Ph.D.

*Senior Scientific Officers*
J. B. W. Hammond, D.Phil.
Angela Karp, Ph.D.
M. A. J. Parry, Ph.D.
P. Rea, D.Phil.
A. S. Tatham, Ph.D.
R. M. Wallsgrove, Ph.D.

*Higher Scientific Officers*
Shirley R. Burgess, Ph.D.
M. J. Cornelius
Janice Forde, Ph.D.
J. Franklin, B.Sc.
Alison Frend, Ph.D.
P. Gallois, Ph.D.
Ruth Gordon-Weeks, Ph.D.
N. P. Hall, Ph.D.
H. Jones, Ph.D.
A. C. Kendall, B.Sc.
B. T. Lee, Ph.D.
K. Lindsey, Ph.D.
Philippa J. Madgwick, D.Phil.
Renee Malone, Ph.D.
A. L. Phillips, Ph.D.
Jennifer Topping, B.Sc.
D. Twell, Ph.D.

*Scientific Officers*
E. Almond, M.Sc.
Bernadette Buxton, H.Tech.
M. Cannell, B.Sc.
Caroline A. Catton, B.Sc.
Joanna R. Clark, B.Sc.
N. Halford, M.Sc.
Alison P. Hopley, B.A.
Gillian A. Hull, M.Sc.
Catherine Kettleborough, B.Sc.
Claire Marris, B.Sc.
Kathleen M. Murdoch, B.Sc.
J. Phelpstead, B.Sc.
Jacqueline Pywell, B.Sc.
Susan J. Smith
Penny J. Tillson, B.Sc.
Janice C. Turner

*Assistant Scientific Officers*
Patricia C. Brooks
M. J. Coldwell
A. J. Cowland
M. Dorling
Rachel Dunckley
I. Major
Saroj Parmar
Ruth Risiott, B.Tec. H.N.C.
Susan H. Steele
Debbie Unsworth

*Visiting Scientists*
Y. Altschuler, B.Sc.
Penelope Bebeli, Ph.D
D. Bechtel, Ph.D.
M. Bianchi, Ph.D.
D. Dymock, Ph.D.
R. Faber, B.Sc.
J. Guerra-Sanz, Ph.D.
H. Hasegawa, Ph.D.
N. J. Kruger, Ph.D.
A. J. Marquez, Ph.D.
Fiorella Mellini, Ph.D.
N. Morris, B.Sc.
D. Peterson, Ph.D.
Q. S. Wu, Ph.D.

*Students*
Alison Alderson, B.Sc.
Clair Baynton
M. T. J. de Both, M.Sc.
Melody Clark, B.Sc.
C. Eady, B.Sc.
N. Fish, B.Sc.
C. Healy
Kathryn Pratt, B.Sc.
R. Potter, B.Sc.
Rachel Teverson, B.Sc.
S. Trevanion, B.A.
P. Urwin
Ruth Warman
N. Yeboah, M.Sc.

*Personal Secretary*
Susan C. McCartney

*Specialist Typist*
Jeane E. Hutchins

*Laboratory Attendants*
Pamula Everitt
Janina Jones
Eleanor Weaver

## Introduction

We were pleased to be awarded two new permanent posts to initiate research programmes in the field of plant biotechnology. The appointment of a membrane biochemist at the project leader level represents an extension of our long-standing interests in plant nitrogen metabolism into elucidating and manipulating the mechanisms of nitrate uptake and transport. This appointment is particularly timely in view of the current concern about nitrate losses and contamination of drinking water. The second post for an enzymologist will allow collaboration with members of the Insecticides and Fungicides Department on a new project on the biosynthesis of the terpenoid insect-antifeedant, polygodial. The water-pepper (*Polygonum hydropiper*), a rich natural source of polygodial, will be used as experimental material to



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



establish the pathway of biosynthesis, and to isolate key enzymes of this pathway and the genes that encode them. These genes will then be used to produce polygodial by fermentation, or inserted into crop plants to confer resistance to aphids and other insects.

Although this injection of new blood is very welcome, it must be viewed against a background of continuing reductions in permanent staff numbers and the research budget. Nevertheless the total staff numbers have continued to rise thanks to increased outside funding, including contracts from Shell, MMG Agriseed and De Danske Sukkerfabrikker.

The following brief account of research progress and achievements is divided into five sections, corresponding to the major research areas.

## Photosynthesis and carbon metabolism

The focus of our Photosynthesis Programme continues to be the structure, regulation and reaction mechanism of ribulose bisphosphate carboxylase oxygenase (Rubisco). This enzyme catalyses the primary assimilation of carbon dioxide, by carboxylation of ribulose bisphosphate. Unfortunately it also catalyses a second reaction, the oxygenation of the same susbtrate, this ultimately leads to the loss of carbon dioxide in the pathway of photorespiration. Up to one third of the carbon fixed is lost in this way. The relative capacity of the enzyme to catalyse the two reactions depends on kinetic constants embodied in the specificity factor. This varies between organisms, the enzyme from flowering plants having a higher affinity for carbon dioxide than those from bacteria, cyanobacteria and lower plants. Protein engineering is being used to explore the reasons for these differences, and to help design a more efficient enzyme for crop plants.

A second project in this research area is to eliminate cold sweetening of stored potatoes (which currently results in losses estimated at £1m a year) by manipulation of the pathway of glycolysis. (Project Leaders: Burrell, B. G. Forde, Keys, Parry, Phillips. Other staff: Burton, Catton, Coldwell, Cornelius, Cowland, Hall, Hammond, Kettleborough, Kruger, Major, Quidiello, Trevanion, Warman)

**Engineered Rubisco.** Mutant forms of Rubisco are being generated by manipulating the cloned genes for the enzyme from a cyanobacterium *in vitro* and expressing them in *Escherichia coli* (*Rothamsted Report for 1986*, 127). When the 11 amino acids at the N-terminus of the large subunit polypeptide were replaced by the corresponding sequences of amino acids from the enzyme of wheat or maize, the kinetic parameters were similar to those of the unmodified cyanobacterial enzyme. However, when they were replaced with an unrelated sequence of five amino acids the affinity of the enzyme for ribulose bisphosphate was decreased by a factor of 16 and that for $CO_2$ was halved; the maximum rate of carboxylation was also halved. There were no changes in the specificity factor. Therefore, although the N-terminal region is essential for effective catalysis, it is not responsible for the greater specificity towards $CO_2$ that is exhibited by the cereal enzyme.

Cyanobacterial genes were also manipulated to replace other long and short sequences of amino acids in the enzyme with corresponding sequences from wheat and maize. The large subunit polypeptides produced were difficult to assemble into active holoenzymes consisting of eight large and eight small subunits ($L_8S_8$), indicating that their ability to self-assemble is easily disturbed. Although we are still trying to create chimaeric large subunits to identify the sequences that are responsible for the different specificity factors of the wheat and cyanobacterial enzymes, a strategy is also being developed to explore the reasons for failure of assembly. Plasmids have been constructed which allow large and small subunit polypeptides to be made separately in different cultures of the *E. coli* cells. Extracts of such cells have



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



no Rubisco activity. However, when extracts of the two bacterial cell types are mixed, Rubisco activity occurs. The cultures expressing the large subunit contain the polypeptide in a complex with a molecular weight corresponding to an $L_8$ form. Thus assembly of the $L_8S_8$ holoenzyme may proceed by way of an octamer of large subunits to which the small subunits bind.

**Matching Rubisco activity to environmental constraints.** Evidence is accumulating that the activity of Rubisco in leaves is regulated in response to light intensity and temperature. Previous reports described a naturally occurring inhibitor found in potato leaves, and the marked effects of orthophosphate on the activity of the enzyme. Evidence arising from the study of a mutant form of *Arabidopsis thaliana* in the USA (*Photosynthesis Research* (1985) **7**, 193-201) shows that a protein, presently referred to as Rubisco activase, is involved in the regulation of Rubisco in response to light intensity at ambient $CO_2$ and $O_2$ concentrations. In collaboration with staff at the Research Institute for Photosynthesis in Sheffield an investigation was started into the operation of the activase system. Difficulty was experienced in confirming the system's operation in lysed spinach chloroplasts until the requirement for ribulose bisphosphate (RuBP) was provided by RuBP synthesized in the laboratory rather than by a commercial sample. Furthermore if the synthetic RuBP was purified extensively before use in the lysed chloroplast system, activation of the carboxylase in the light was not observed. However the system would function with the purer RuBP if ADP and inorganic orthophosphate or fructose 1,6-bisphosphate were also added. Even with such additions, commercial RuBP could not be used in the lysed chloroplast system to show light regulation of Rubisco activity. The American researchers have since recognized the requirement for ATP. The results described suggest that other impurities in RuBP samples are also needed.

**Properties of Rubisco from crop plants.** Among $C_3$ crop plants, the rate of photosynthesis per unit leaf area is most rapid in sunflower and a fresh attempt was made to characterize the Rubisco from this species. Browning of the leaf extracts was prevented by using high concentrations of sulphydryl reductant and polyvinylpyrollidone. The carboxylases purified from such extracts of two sunflower varieties were compared with respect to their specificity factors to the purified Rubisco from wheat. Two methods of measuring specificity factor were used and the measurements replicated but the values obtained for the Rubiscos from sunflower were not significantly different from that for wheat, indicating that the faster rates of photosynthesis shown by sunflower are not associated with a more efficient Rubisco.

**Low temperature sweetening in potato tubers.** The accumulation of sugars in potato tubers stored at low temperature becomes an important economic problem during their processing into crisps. Current evidence suggests that inactivation of the glycolytic enzyme phosphofructokinase (PFK) at low temperature may be a major cause of such sweetening. The aim of our work is to reduce this sugar accumulation by genetic manipulation of PFK.

We have compared the stabilities at low temperatures of PFK from various sources. The four isoforms of PFK have been partially purified from leaves and tubers of potato cultivars and related wild species. Their activities have been determined over the range 2−25°C and compared with those from Record, which is the dominant cultivar used by processors but is prone to low temperature sweetening. Tubers from a potato line supplied by Dr. G. McKay (Scottish Crop Research Institute) accumulate less sugar than those of Record during storage at 4°C. Three of the four PFK isoforms isolated from this clone are more active at low temperature than the corresponding isoforms from Record. Partial amino acid sequences have



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



been obtained for the four PFK isoforms isolated from Record tubers. Oligonucleotide probes derived from these sequences are being used to identify and isolate cDNA clones coding for the enzyme.

## The biochemistry and molecular biology of nitrogen metabolism

We are studying the uptake, transport, assimilation and metabolism of nitrogen in the plant to understand the factors that determine the efficiency of utilization of applied fertilizer nitrogen and, in the case of legumes, nitrogen fixed by symbiotic *Rhizobium*. Induced mutants of barley are being used as tools to study the metabolic role and regulation of the photorespiratory nitrogen cycle, and to aid the identification and characterization of the nitrate uptake system of roots. In addition, our studies of the regulation of expression of genes encoding constitutive and nodule-specific isoenzymes of glutamine synthetase of *Phaseolus* are progressing well. (Project Leaders: B. Forde, Rea, Wallsgrove. Other staff: Day, Hasegawa, Hopley, Kendall, Marquez, Pywell, Teverson, Turner)

**Photorespiration.** Studies on barley mutants with defects in photorespiratory metabolism continue. No new classes of mutant have been selected, but analysis of the biochemistry, physiology and genetics of existing lines has provided more insight into photorespiration.

A rapid and simple leaf disc assay has been developed to monitor the activities of phosphoglycollate phosphatase, glycollate oxidase, catalase, and peroxidase. The basis for the assay is the reaction between $H_2O_2$ and 4-aminoantipyrene in the presence of peroxidase. By varying substrates or assay components, any of the above enzymes can be assayed. Using this method as a primary screen, six new catalase and two new phosphoglycollate phosphatase mutants have been identified. The photosynthetic and chlorophyll fluorescence characteristics of all mutant lines have been studied (with Dr M. Sivak and Dr J. Rowell, Research Institute for Photosynthesis, Sheffield). The methods used were not able to distinguish between all the mutant classes, but some common characteristics were observed that confirmed earlier hypotheses. The data were consistent with rapid depletion of Calvin cycle intermediates in the mutants, in air and light, and no evidence was found for uncoupling of photophosphorylation in those mutants that accumulate ammonia. The quantum requirement of many of the mutants was sensitive to the growth conditions: even limited pre-exposure to air significantly raised the value. The phosphoglycollate phosphatase mutants had quite distinctive characteristics, related to their low photosynthetic rates even in high $CO_2$. Saturation at low light intensities, and marked stimulation of photosynthesis by exogenous phosphate, indicated severe phosphate limitation in these plants.

A study has started of the uptake characteristics of the mutants defective in chloroplast dicarboxylate transport (with Dr A. Tobyn and Dr M. Proudlove, University of Sussex). Preliminary work suggests that the uptake properties of the barley chloroplast may be quite different from those of pea or wheat.

Further studies have been made on barley Fd-glutamate synthase, the purification of which was reported last year (with the Universities of Malaga and Seville, Spain). Antibodies raised against the pure barley enzyme do not react with the enzyme from *Chlamydomonas reinhardii* or tomato. However, partial sequencing of the enzymes from barley and *C. reinhardii* reveals some highly conserved regions, notably at the N-terminus. Homology with flavin-binding sites of other enzymes has also been found.

**Amino acid biosynthesis.** The simultaneous assay of both activities of acetohydroxy acid synthase has been achieved, using NMR spectroscopy (with Prof. D. Crout, University of Warwick). In the presence of 2-oxobutyrate, acetolactate synthesis is suppressed in favour



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



of acetohydroxybutyrate synthesis. Stable reaction conditions have been achieved, such that the reaction rate can be maintained for up to 24 hours. A rapid partial purification of the barley enzyme has been developed, using Q-Sepharose chromatography, and the enzyme can be satisfactorily stored at −70°C for several weeks. An apparent Km for pyruvate of 2 mM has been determined, which is very much lower than the values reported by earlier workers.

**Nitrate uptake.** Screening for low uptake mutants of barley has continued. Some 100 000 $M_2$ seedlings have now been screened, resulting in 60 'low uptake' lines. Many of these lines failed to survive, and not all of the others transmitted the mutant phenotype to their progeny. A continuing problem is the segregation of mutant and wild-type phenotypes, in both $M_3$ and $M_4$ generations. In the absence of a stable mutation, only limited physiological characterization is possible. Some of the mutant lines do not grow on nitrate as a nitrogen source, but only on urea or ammonia. Others will survive in the presence of nitrate. A procedure for the isolation of tonoplast and plasma membranes from barley has been developed and these membranes will be employed to determine the effects of nitrate induction on membrane function and composition with the specific goal of identifying and isolating nitrate porters.

**Glutamine synthetase genes.** Further progress has been made in characterizing the gene family that specifies glutamine synthetase (GS) in *Phaseolus vulgaris*. It has now been established (in collaboration with a Link Group at the University of Warwick) that there are a total of five GS genes, which have been designated *GlnA*-$\alpha$, GlnA-$\beta$, GlnA-$\vartheta$, GlnA-$\delta$ and *GlnA*-$\epsilon$. Three of the genes (*GlnA*-$\beta$, GlnA-$\vartheta$ and *GlnA*-$\epsilon$) have been cloned and their promoters are now being analysed to investigate the *cis*- and *trans*-acting factors that regulate their expression during plant development. Of particular interest is the differential expression of *GlnA*-$\beta$ and *GlnA*-$\vartheta$: the former gene is apparently expressed constitutively, while the latter is under stringent regulation, being expressed only in root nodules, in the symbiotic association with *Rhizobium*.

The 5′-flanking region of each of the three cloned genes has been fused to the coding sequence of a bacterial reporter gene, $\beta$-glucuronidase (GUS), to provide a simple and sensitive assay for promoter activity. The chimaeric genes have then been introduced into the forage legume, *Lotus corniculatus*, using a transformation system based on *Agrobacterium rhizogenes*. A method has been developed for obtaining nodules on the transformed 'hairy roots' without having first to regenerate whole plants. Thus the time taken to test initially for gene expression in transgenic roots and nodules is reduced from five to six months to five to six weeks. First results from these experiments indicate that a 2 kb fragment from the 5′-flanking region of *GlnA*-$\vartheta$ is sufficient to direct nodule-specific expression in *L. corniculatus*.

In order to obtain a specific hybridization probe with which to study GS-minus photorespiratory mutants of barley at the molecular level, we have attempted to clone the cDNA for the chloroplast form of barley GS. A barley leaf cDNA library has been constructed in pUC9 and screened with a 50mer oligonucleotide probe. Since no GS sequences in monocotyledons have been cloned previously, the oligonucleotide was designed from a consensus sequence of the most strongly conserved region of a variety of published dicotyledonous GS cDNA sequences. Two cDNA clones were identified, pcHvGS6 and pcHvGS14, which have inserts of *c* 1500 bp and 1150 bp, respectively. Restriction analysis indicates that they are identical, except that the larger one extends 350 bp further at the 5′ end of the coding strand. When a Northern blot of RNA from wild-type and mutant barley leaves was probed with pcHvGS6, several of the GS-minus mutants were found to differ from the parental line in the abundance and/or the mobility of the hybridizing mRNA species, suggesting that the cDNA clone is detecting transcripts of the mutated GS gene.



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



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

Since the inception of the AFRC Genetic Manipulation Programme, the remit of work in this area has been to develop efficient systems for tissue culture and regeneration of the major arable crop plants—wheat, barley, potato, oilseed rape and sugar beet. The *in vitro* systems are then applied to the transfer or introduction of useful genetic information into and between plant cells, with the regeneration of whole modified plants. The methods of gene transfer used include *Agrobacterium* and its engineered derivatives as vectors, direct gene transfer into protoplasts and tissues, and protoplast fusion. These procedures are not only being used to transfer agronomically-important traits, but also to explore the regulation of gene expression, and the control of plant development. Changes induced by the culture processes themselves are also being evaluated to determine their origins, whether they can be controlled, and their potential application to crop improvement. The programme is closely linked to other projects within the Department involving molecular biology. We have also initiated a new project, supported by Shell Research Ltd, to develop a map of the barley genome based on DNA restriction fraction length polymorphisms. (Project leaders: M. G. K. Jones, Karp, Ooms. Other staff: Baynton, Bebeli, de Both, Cannell, M. Clark, Cooper-Bland, Dunckley, Dymock, Eady, Faber, Fish, Gallois, Guerra-Sanz, H. Jones, Lee, Lindsey, Malone, Murdoch, Phelpstead, Potter, Risiott, Shewry, Steele, Symonds, Tillson, Topping, Twell, Unsworth, Wu)

**Potato.** Many plants transformed by *Agrobacterium* have been obtained following optimization of the conditions for transformation of stem segments using a two-step procedure. Two days after infection, stem segments were cultured on a callus medium for two to four days, followed by a second regeneration medium for four weeks. Varieties transformed include Désirée, Pentland Javelin, Record, Maris Bard, Estima, Wilja and Maris Piper. The highest transformation frequencies were obtained for Désirée, Maris Bard and Estima.

Molecular analysis of DNA sequences important in directing tuber- specific expression in whole transformed plants has continued. The aim is to make transformation more specific by directing expression in particular organs or cell types. This work involves analysis of the 5' control sequences of members of the patatin gene family. Following the demonstration that 5' sequences adjacent to the coding region of one gene family member (LPOT1) directed tuber-specific expression of the reporter gene chloramphenicol acetyl transferase (CAT), the control sequence of a second gene family member (LPOT2) was found to direct root and tuber-specific expression, with further control exerted by light.

Analyses of the effects of introducing Ti plasmid cytokinin genes with mutated promoter regions and Ri T-DNA genes on whole plant development have continued. Several examples of unstable transformation have been observed, the instability occurring at different stages to give different morphological patterns.

The conditions for introduction of DNA reporter gene constructs into protoplasts by electroporation have been optimized, using CAT and GUS reporter genes, and related to the biophysical properties of the protoplasts. Detailed studies of the transient expression of such constructs have been made, and the emphasis is now on obtaining stable integration.

Studies of protoplast fusion have continued, with the first UK field experiment using somatic hybrid plants, between *S. tuberosum* and the sexually incompatible *S. brevidens*. Analysis of hybrids, (with Gibson of Plant Pathology), has shown the transfer of resistances to potato leaf roll virus and to potato virus Y from *S. brevidens* into the hybrids. Although the hybrids are male sterile they are female fertile, and crosses with *S. tuberosum* are in progress to allow exploitation of the novel germplasm.

**Sugar beet.** Work on electrofusion of, and direct gene transfer into sugar beet protoplasts has continued. The conditions for the production of heterokaryons by electrofusion, with



https://doi.org/10.23637/ERADOC-1-28 

This work is licensed under a Creative Commons Attribution 4.0 International License.



subsequent production of hybrid callus, have been optimized, and the effects of applied electric fields on plasma membrane permeability have been quantified using the membrane-impermeable marker phenosafranine. This provided information of the dynamics of membrane breakdown and recovery under a range of electroporation conditions. The level of transient expression of the reporter gene CAT, electroporated into protoplasts either by rectangular pulses or by capacitor discharge pulses, depended on two types of parameter: physical (electrical conditions, plasmid concentration and protoplast density) and physiological (the state of differentiation of cells from which protoplasts were obtained). Evidence was obtained for the introduction of DNA into intact cells. Work is also in progress to obtain stable transformation, and to study size, copy number and fate of the DNA introduced into protoplasts by electroporation.

**Cereals.** Work on cereal tissue culture and transformation has continued with green shoots being obtained from cultured protoplasts of bread wheat. Techniques have been developed for isolating protoplasts from specific cell types (aleurone, endosperm), and a range of protoplasts is being used to study transient expression of specific gene constructs introduced by electroporation. Various techniques for the production of stably transformed tissues are being evaluated. For example, approximately 15 000 seeds of wheat have been screened for stable integration of kanamycin resistance genes following 'macroinjection' of DNA into developing inflorescences. Although this has not yet yielded transformed plants, work is still in progress. New molecular constructs are also being made to give expression of reporter genes introduced into protoplasts.

Plants regenerated from immature tissues of rye and triticale differing in heterochromatin content have been produced to study the origin, cause and control of somaclonal variation. Certain rye genotypes have proved to be particularly responsive to culture and embryogenesis, with the production of embryogenic cell suspension cultures.

Protocols for the detection of repetitive sequences of barley chromosomes by *in situ* hybridization using biotin and tritium labelling have been optimized. Experiments are now in progress to detect low copy number sequences using a B hordein probe. B hordein genes are present on chromosome 5 at 20–30 copies per genome.

**Tobacco, sunflower.** Tobacco has been used as a model system for direct gene transfer by electroporation, both for optimization of the conditions for transient expression of marker genes with different promoters, and to obtain stable integration. Transformed tobacco plants resistant to kanamycin have been produced with good efficiency. Initial work on tissue culture of sunflower has begun, with production of protoplasts and selection for mutants resistant to herbicides.

## Seed biology

In the present situation of excess cereal production it is increasingly important to optimize the quality of the crop for its end use. Since high proportions of the barley and wheat grown in the UK are used for livestock feed and breadmaking respectively, we are identifying the factors that limit grain quality for these end uses and exploring approaches to quality improvement. The quality of barley as feed for monogastric livestock is limited by the low contents of two essential amino acids (lysine and threonine), which itself results from the unusual amino acid composition of the major grain storage proteins (called hordein). Other grain proteins, such as the chymotrypsin inhibitors CI-1 and CI-2, are rich in lysine and threonine, but are only present in the grain in low amounts. We have isolated genes encoding hordein and CI-2 and are studying their control of expression. The information gained from this study should facilitate the manipulation of the protein composition, and hence nutritional



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



quality, of the grain by genetic engineering. Although there has been a large increase over the last decade in the proportion of home-grown wheat used for breadmaking, it is still necessary to supplement with either hard wheats from North America or with gluten isolated by an industrial separation process. The breadmaking quality of wheat is also determined by the seed storage proteins (called gliadins and glutenins), but in this case by their physico-chemical properties. They are the major components of gluten, which has a unique combination of elasticity and viscosity (or extensibility). The poor breadmaking performance of UK wheats is related to a low degree of gluten elasticity. Our studies of the structures and interactions of gluten proteins are directed towards elucidating the molecular basis of gluten viscosity and elasticity, and manipulating protein structure to optimize the properties for traditional (i.e. breadmaking), and novel (e.g. in the food industry) end uses. They are collaborative with Institute of Food Research, Norwich, and we are pleased that renewals were approved for three fixed-term posts in the Department funded by AFRC Food Division. (Project Leaders: Karp, Kreis, Shewry. Other staff: Alderson, Almond, Altschuler, Bechtel, Bianchi, Brooks, Burgess, Buxton, Dorling, J. Clark, M. Clark, J. Forde, Franklin, Frend, Gallois, Halford, Hull, Hutchinson, Madgwick, Marris, Mellini, Parmar, Peterson, Pratt, Smith, Tatham, Topping, Urwin, Whittington-Smith, Williamson, Yeboah)

**The chromosomal locations of genes for β-amylase in wheat, barley and related species.** The β-amylase cDNA clone described in last year's report (*Rothamsted Report for 1986*, 137) has been used to determine the chromosomal locations of β-amylase structural genes in wheat, barley, rye and *Aegilops umbellulata* by 'Southern blot' analysis of wheat aneuploids and the available whole chromosome and telocentric chromosome addition lines of the other species into wheat (in collaboration with Dr P. Sharp and Dr M. D. Gale of Institute of Plant Science Research (IPSR), Division of Cytogenetics and Plant Breeding, Cambridge). This confirmed and extended the previous reports, based on analyses of the major seed isoenzymes, of genes on chromosomes 4L of barley, 4Aβ, 4DL and 5AL of wheat, 5R of rye and 5U of *Ae. umbellulata*. In addition, all species also had additional loci on the group 2 chromosomes: 2S of barley, 2AS, 2BS, 2DS of wheat, 2R of rye and 2U of *Ae. umbellulata*. The identities of the proteins encoded by these loci, if the genes are expressed and not inactive pseudogenes, are not known, although they could correspond to minor isoforms of the enzyme present in seeds and/or vegetative tissues.

**Analysis of the genes for barley seed proteins.** In order to study the control of expression of the lysine-poor B1 hordein gene a 549 bp sequence from the 5' flanking region was linked to the CAT reporter gene and transferred into the genome of tobacco. Expression of CAT activity was only observed in the endosperms (not the embryos or testas) of the developing seed from about 15 days after flowering, indicating that the sequences responsible for developmental and tissue-specific expression are present in the 549 bp sequence.

A gene for the lysine-rich chymotrypsin inhibitor CI-2 has also been isolated and its nucleotide sequence determined. Unlike the hordein genes, it contains an intron (of 90 base pairs). It will now be possible to explore the regulation of expression of this potentially valuable gene using the approach outlined above.

**Studies of cereal protease inhibitors.** The major grain trypsin and α-amylase inhibitors of rye have been isolated and their complete and partial amino acid sequences determined (in a joint project with Dr M. Richardson of the University of Durham, support by an SERC/CASE Studentship). They are both members of a seed protein superfamily that includes other cereal protease-inhibitors, 2S storage globulins of a range of dicotyledonous species (castor bean, lupin, brazil nut, oilseed rape) and specific domains of the major prolamins of the



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



Triticeae and the minor prolamins of maize. An oligonucleotide probe based on the directly determined amino acid sequence of the trypsin inhibitor has been used to identify a putative clone encoding the protein in a cDNA library constructed in the phage vector λgt 10 using an mRNA fraction from developing rye endosperms as a template.

A similar approach has been used to identify a cDNA clone for a related barley protein in a library constructed in the plasmid vector pUC9. The insert of the clone has been sequenced and the deduced amino acid sequence is almost identical to directly determined N-terminal and peptide sequences of the chloroform/methanol-soluble protein CMd. The biological role of this protein is not known as, unlike several closely related proteins, it has no known inhibitory activity.

**NMR spectroscopy of wheat gluten.** We have used nuclear magnetic resonance (NMR) relaxation techniques to study the dynamics of dry wheat gluten from breadmaking wheat (hexaploid), pasta wheat (tetraploid) and commercial glutens (in collaboration with Dr P. S. Belton, IFR-N). A correlation was found between the type of gluten and its transverse relaxation characteristics. The relaxation times, which indicate the mobility of the system, were in the order commercial gluten > pasta gluten > breadmaking gluten > lipid extracted gluten. The results also indicate that the lipid component acts as a plasticizer to some of the gluten proteins, its removal greatly reducing the mobility of the gluten mass. Further studies on gluten subfractions are being made, to determine in which groups the mobility resides.

**Structural studies of synthetic peptides corresponding to the repeat motifs of cereal prolamins.** Structural studies, using circular dichroism and Fourier-transform infrared spectroscopy, of synthetic peptides corresponding to the repeat motifs of C hordein and the HMW subunits of wheat have confirmed earlier studies, showing the presence of different $\beta$-turn types. This indicated the presence of type I (or III) $\beta$-turns in the octapeptide repeat of C hordein (consensus sequence PQQPFPQQ). In the HMW subunits type II $\beta$-turns were present in the hexapeptide repeat (consensus sequence PGQGQQ) and type I (or III) $\beta$-turns in the nonapeptide repeat (consensus sequence GYYPTSPQQ). Further studies are underway using NMR spectroscopy to obtain interatomic distances for computer modelling. The peptides are also being used to raise monoclonal antibodies (in collaboration with Dr C. Mills, IFR-N) as probes for conformational epitopes and to study protein deposition during endosperm development.

## The relationships between plants, pathogens and phytophagous insects

Studies of the response of tobacco leaves to virus infection are continuing with progress being made in identifying the elicitor molecules that are transported and induce the synthesis of pathogenesis-related (PR) proteins in neighbouring leaves. In addition the new project on the biosynthesis of polygodial has started with studies of the site of synthesis in water-pepper (with the Insecticides and Fungicides Department). (Project Leader: Pierpoint. Other staff: Gordon-Weeks)

**Characterization of the pathogenesis-related (PR) proteins of tobacco.** The PR proteins accumulate in the intracellular spaces of tobacco leaves that have been infected with a virus or other pathogen that induces a hypersensitive response, or treated with chemical elicitors, for example salicylic acid. One specific group (the PR-I proteins) have been studied in detail, although their function is unknown. Other components have been identified as chitinases and glucanases with antifungal activity. We have focused our attention on two other components of this mixture. PR protein R has an amino acid sequence very similar to the sweet protein



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



thaumatin and to an inhibitor of α-amylase and trypsin from maize seeds (see Richardson *et al.*, *Nature* (1987) **327**, 432-434). Like thaumatin it probably has a compact structure stabilized by eight intra-molecular disulphide bonds. For instance, the native molecule moves faster on high performance gel permeation chromatography than would be expected from its molecular weight. Antibodies raised against PR-R and thaumatin do not cross-react with the native proteins, but do when the proteins are reduced and denatured (with Miss M. Cusack, University of Liverpool). This presumably results from the exposure of otherwise inaccessible shared epitopes.

The other protein investigated, PR-R′, has been shown to have a region which contains repeats of the tripeptide sequence variable-leucine-proline (with Dr D. J. Pappin, University of Leeds). This sequence could form a structure similar to that of collagen, a structural protein of mammalian connective tissue.

**Elicitors of the synthesis of PR proteins.** Grafting scions of uninfected xanthi nc tobacco on to the root stock of an interspecific tobacco hybrid that produces PR proteins constitutively resulted in the production of PR proteins in the xanthi leaves. These experiments, which confirm the earlier ones of Gianinazzi and Ahl (*Netherlands Journal of Plant Pathology* (1983) **89**, 275-281), strongly suggest the presence of mobile elicitor(s) of PR proteins in the hybrid. Extracts from the intercellular fluids of the hybrid, like those from the intercellular fluid of one of its parents (*Nicotiana debneyi*) following infection with tobacco mosaic virus, did indeed elicit the production in xanthi leaves of PR-Ia, which was identified by electrophoresis and immunoassay.

Experiments are now underway to purify and characterize the components responsible for this activity and to eliminate the possibility that it is artefactual.

## Glasshouse services

During 1987 the glasshouse section was reorganized and now services, with five staff and 1500 m² of glass, the Crop Science and Soils and Crop Production Divisions at Rothamsted. The section provided some 16 000 pots and trays of plant material during the year. A new $CO_2$ chamber (12 m²) for raising plants under higher than ambient $CO_2$ has been built. Work has been started on four chambers (at 4.5 m²) suitable for raising genetically manipulated plants. (Services coordinator: Franklin. Other staff: Weaver (Biochemistry); Plumb (Crop Production); Bird and Reason (Soils))

## 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, Directorates DG XII (Education and Science) and DG VI (Agriculture) of the EEC, The International Potato Centre, The Potato Marketing Board, Shell Research Ltd. and MMG Agriseed.

We have shared SERC/CASE research students with the Universities of Durham, Kent and Cambridge.

**Visits abroad.** Members of the Department attended the following conferences: American Association of Cereal Chemists, Nashville, USA (P. R. Shewry), Agropolis Meeting, Montpellier, France (P. R. Shewry), EEC Meeting, Louvain-La-Neuve, Belgium (M. T.



https://doi.org/10.23637/ERADOC-1-28 

*This work is licensed under a Creative Commons Attribution 4.0 International License.*



J. de Both, N. Fish, B. G. Forde, J. Forde, P. Gallois, H. Jones, M. G. K. Jones, M. Kreis, K. Lindsey, B. T. Lee, G. Ooms), EEC Meeting, Groningen, The Netherlands (H. Jones, M. G. K. Jones), EEC Meeting, Rome, Italy (G. Ooms), International Botanical Congress, Berlin, FRG (M. G. K. Jones, N. Kruger, B. T. Lee, P. R. Shewry), 3rd International Wheat Gluten Workshop, Budapest, Hungary (P. R. Shewry, A. S. Tatham), 4th Seed Protein Symposium, Gatersleben, DDR (P. R. Shewry), Electronics and the Management of Plants, Monte Carlo, Monaco (M. G. K. Jones), Rubisco Workshop, Tucson, Arizona, USA (A. J. Keys), International Protoplast Symposium, Wageningen, The Netherlands (M. T. J. de Both, C. Eady, H. Jones, M. G. K. Jones, B. T. Lee, K. Lindsey, K. Murdoch), Anglo-French Meeting of the International Association for Plant Tissue Culture, Angers, France (R. Potter), FASEB Meeting, Copper Mountain, Colorado, USA (P. Gallois, K. Lindsey), EMBO Course, *In situ* hybridization, Sofia, Bulgaria (M. Clark), UNEP Course on Techniques in Bioproductivity and Photosynthesis, Lucknow, India (R. M. Wallsgrove), EMBO Course, Zurich, Switzerland (J. Topping), Meeting on Barley, Helsinki, Finland (M. Kreis)

Visits abroad were made to the following institutes to further collaboration or for exchange of information: De Danske Sukkerfabrikker, Copenhagen, Denmark (P. Gallois, M. Kreis, M. G. K. Jones, K. Lindsey), Carlsberg Laboratories, Copenhagen, Denmark (M. Kreis, M. S. Williamson), Risø Laboratories, Copenhagen, Denmark (P Gallois, M. Kreis, K. Lindsey), Free University of Brussels, Belgium (M. Clark, M. G. K. Jones), Friedrich Miescher Institute, Basle, Switzerland (M. G. K. Jones, M. Kreis, B. T. Lee), University of Hohenheim, FRG (B. T. Lee), Ciba-Geigy, Basle, Switzerland (M. G. K. Jones, B. T. Lee), Max-Planck Institute, Cologne, FRG (B. G. Forde, R. Potter), University of Würzburg, FRG (H. Jones), Plant Cell Engineering Laboratory, Beijing and the University of Nanjing, People's Republic of China (A. Karp, A. Tatham), Limagrain, Clermont-Ferrand, France (M. T. J. de Both, M. G. K. Jones), Universities of Malaga and Seville, Spain (R. M. Wallsgrove), University of Dayton, Ohio, USA (M. Parry), Universities of Baarn, Wageningen and Utrecht, The Netherlands (W. S. Pierpoint), University of Paris-Sud, Orsay, France (M. Kreis), University of Ceara, Fortaleza, Brazil (P. R. Shewry) University of Louvain, Belgium (M. Kreis).

**Staff.** During the year M. M. Burrell, S. Burton, Stephanie Cooper-Bland, Hazel Day, N. Fish, C. Healy, Jeanette Hutchinson, Conchita Quidiello, J. Relton, S. Rogers, Corinne Symonds, and Ruth Whittington-Smith left. J. Antoniw transferred to the Plant Pathology Department.

Newcomers to the Department were: E. L. Almond (University College, London), M. E. Cannell (University of Birmingham), A. Cowland, M. Coldwell, Rachel Dunckley, M. W. Dorling, Alison Frend (Flour Milling and Baking Research Association), I. Major, Renée Malone (St. Patrick's College, Co. Kildare), J. Phelpstead (University of Leicester), P. Rea (University of York), Jennifer Topping (University of Edinburgh), Debbie Unsworth.

Janey Henderson, R. Johnson and D. Twell were awarded Ph.D. degrees from the Universities of Durham, Warwick and London respectively, and Jacky Pywell and Corinne Symonds were awarded B.Sc. degrees from Hatfield Polytechnic.



https://doi.org/10.23637/ERADOC-1-28 