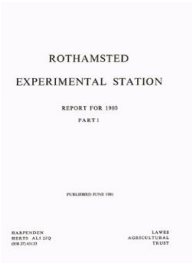
ROTHAMSTED RESEARCH

Report for 1980 - Part 1

Full Table of Content



Biochemistry Department

B. J. Miflin

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Introduction

Expansion of the Department to meet the needs of the programme on the genetic manipulation of crop plants has continued, although slowly because of the current financial situation; at present two posts remain to be filled. The new building which gives an increase of about 25% in our usable laboratory space has been completed and is now in use. This houses those people concerned with the study of seed storage proteins and their genes, and with protoplast regeneration and the selection of biochemical mutants. The scientific content of these programmes remains much as described in previous years.

The Department's interest in one area of host/pathogen relationships has come to an end with the retirement of Margaret Holden. This work on the biology and biochemistry of the *Gaeumannomyces–Phialophora* complex has helped to distinguish more clearly between the various members of this extremely important but poorly differentiated group of fungi.

Work on intermediary nitrogen metabolism has centred largely around the control

https://doi.org/10.23637/ERADOC-1-137

of synthesis of lysine, threonine and methionine in normal and specifically selected mutant crop plants.

Host/plant pathogen relationships

Virus studies. The aims of this work are to identify the nature and function of novel proteins that are found in plant leaves in which the spread of invading virus is restricted, and to describe some of the surface features of potato virus X (PVX) that may affect its behaviour in infected leaves. This work is largely done in collaboration with the Plant Pathology Department and some of it is presented in that Department's report.

Properties of different strains of PVX. Cultivated potatoes contain two major genes which induce a local hypersensitive response to PVX and hence confer resistance against this virus. Two strains of the virus, however, do not interact with either of these genes, and hence systemically infect all potato cultivars. These strains have some surface features in common since, unlike other strains, neither is sensitive to brief exposure to proteolytic enzymes. Although their coat proteins react differently with enzymically generated quinones, this property changes with cultural treatment. As strain X^4 is serially subcultured in tobacco plants, the number of molecules of quinone that each subunit of coat protein binds, rises from 0·5 to 1; when strain X^{HB}, cultured in tobacco, is passed through Pentland Dell potatoes and re-introduced into tobacco, the value rises from 1 to between 2 and 3. These surface changes are not accompanied by changes in the way that the strains interact with the hypersensitivity genes. They suggest that both strains are populations of similar but distinguishable genomes which may be preferentially enriched in different cultural conditions. (Pierpoint, Strowman, with Carpenter, Plant Pathology Department)

Synthesis of pathogenesis-related proteins in TMV-infected plants. Using the semi-quantitative assay described previously, the synthesis of the pathogenesis-related proteins (PR-proteins) has been followed in tobacco leaves reacting hypersensitively to infection with TMV. The amounts of two of them (PR-1a and 1b) increase with increasing concentration, of inoculum, but not with the number of lesions formed per leaf. The time course of their appearance differentiates them from protease-inhibitors which are also formed following infection, and indeed, purified PR-1a, b and c have little or no protease-inhibiting activity.

The production of PR-proteins in detached leaves, that have been treated with extracts prepared from either healthy or infected tobacco leaves, has been confirmed. The production may be a reaction to stress rather than to a specific 'wound-hormone', as some proteins can also be produced by treating leaves with the non-metabolite mannitol. (Leason, Pierpoint and Strowman)

Biochemistry of the fungi of the *Gaeumannomyces–Phialophora* complex. Measurement of colony diameter on oat-leaf agar provides a quick and simple test for helping to distinguish between members of the *Gaeumannomyces–Phialophora* complex. Seventy-two isolates were tested: all 19 European isolates of *Phialophora radicicola* var. *radicicola*, 18 out of 20 *Gaeumannomyces graminis* var. *tritici* (*Ggt*) isolates and a *Gaeumannomyces* isolate from *Deschampsia* failed to grow out from the inoculum plug. Twelve isolates of *G. graminis* var. *graminis* spread over the agar at about the same rate as on potato dextrose agar (PDA) but three did not grow at all. Eight isolates of *Phialophora graminicola*, nine of *G. graminis* var. *avenae* and two oat-attacking isolates that had been identified as *Ggt* all showed some growth, although with most of the isolates this was retarded compared with that of PDA.

https://doi.org/10.23637/ERADOC-1-137

Fifty-seven isolates of fungi of the *Gaeumannomyces–Phialophora* complex that had been maintained in stock culture were tested by host-infection (*in vivo*) and agar plate, filter-paper and flask culture (*in vitro*) methods for production of perithecia. Most isolates of each of the three varieties of *G. graminis* produced perithecia in one test or another (*avenae* (*Gga*), 100%; *tritici* (*Ggt*), 75%; *graminis* (*Ggg*), 71%), but stock cultures of the two varieties of *Phialophora radicicola* did not form perithecia by any method. For *G. graminis*, *in vivo* methods (89, 56–75, 50% of isolates producing perithecia) were generally rather more effective than *in vitro* methods (33, 69 and 43%). No one *in vitro* method was the best for all varieties of *G. graminis*; full strength potato-dextrose agar was the least effective medium for perithecia, but at quarter-strength and supplemented with yeast extract it ranked with malt extract broth agar and a filter-paper method as the most effective *in vitro* methods for *Ggt*, but not *Gga* or *Ggg*.

In host-infection tests using stock isolates those host–parasite combinations that produced most disease or most colonised roots tended also to be those in which the fungus produced perithecia. Perithecia that formed on roots excised from wheat seedlings used to bioassay field soil with little *Ggt* infestation were mostly on roots without take-all lesions. In soils with more infestation there was a positive association between lesioned roots and perithecia. *G. cylindrosporus* perithecia also occurred on these excised roots but not on the same as *Ggt* and concurrence of *Ggt* and *P. radicicola* var. *radicicola* was $\leqslant 30\%$ of isolates. These fungi seemed to have different seasonal occurrences.

Work on the interaction in liquid culture of *Gaeumannomyces graminis* var. *tritici* and *Phialophora radicicola* var. *graminicola* was resumed. For earlier experiments (*Rothamsted Report for 1975*, Part 1, 21) a malt extract broth medium was used; this was replaced by a salts medium. Growth was slower but in general the results were similar to those with the richer medium. Limitation of nutrients was clearly a factor in the inhibition of growth of *Ggt* in a *Prg* medium, but this did not explain why the *Prg* had to be actively growing to have the effect. The possibility that an unstable inhibitor was produced was investigated but the experiments did not give clear-cut results.

The carboxymethycellulase activity of many isolates has been measured to add to the information already obtained on cell-wall degrading enzymes in relation to pathogenicity of fungi in the complex. All isolates of the least pathogenic fungus in the complex *Prg* had very low activity. They had earlier been found to have low polygalacturonase activity. There was a marked difference betweeen the two weakly pathogenic fungi *Prr* and *Ggg*. Nearly all the *Gaeumannomyces* isolates (from Australia) have low activity whereas the *Phialophora* isolates (from Europe) produced moderate to high activity. Virulent isolates of *Gga* had low activity. Isolates of *Ggt* showed considerable variation in virulence and carboxymethycellulase activity. Most of the virulent isolates produced high activity of carboxymethycellulase and polygalacturonase but others had moderate or even low activity of one of the enzymes. There seems to be no obvious correlation between pathogenicity and the production of cell-wall degrading enzymes in this variety. (Boothby, Dodds and Holden)

Intermediary nitrogen metabolism

Ammonia assimilation. Studies have continued on assimilation of ammonia in the root nodules of *Phaseolus vulgaris*. The enzyme glutamine synthetase has been extracted and considerably purified. Future work is planned to produce antisera to the protein to enable the identification of products of *in vitro* protein synthesis driven by messenger RNA isolated from root nodules. In this way it is hoped that the regulation leading to the synthesis of large amounts of this enzyme in nodules, might be better understood.

https://doi.org/10.23637/ERADOC-1-137

Amino acid biosynthesis. Studies have continued on the two key regulatory enzymes of the pathway leading to the biosynthesis of lysine and methionine.

A breakthrough has been the discovery of the involvement of the activated form of methionine, (S)-S-adenosyl-L-methionine (AdoMet), in the regulation of aspartate kinase. Previous evidence suggested that the only mechanism by which methionine could regulate its own synthesis was through the stimulation of threonine synthesis by AdoMet. Studies with barley, maize and pea leaf and bean seed aspartate kinase have shown that although AdoMet has little action by itself at concentrations up to 1 mM there is a marked synergistic inhibition in the presence of lysine, even at levels as low as 25 μM. Equimolar concentrations of AdoMet and lysine caused maximum inhibition at 0·2 mM compared with the 1 mM lysine required to achieve a similar degree of inhibition on its own. The results suggest that the lysine-sensitive aspatate kinase of higher plants also contains a regulatory binding site(s) for AdoMet, and is thus able to modulate the flux through the aspartate pathway at concentrations of AdoMet and lysine of 20–200 μM.

Barley leaf homoserine dehydrogenase has been separated into two isoenzymes by chromatography on Amicon Matrix Gel Red A, an affinity resin. It was confirmed that the chloroplasts contain the high molecular weight (174 000) threonine-sensitive isoenzyme and that the low molecular weight (69 000) cysteine-sensitive isoenzyme is located in the cytoplasm.

Inhibitor studies. Methionine sulphoximine and phosphinothricin were examined as inhibitors of pea leaf glutamine synthetase. Both compounds were competitive with glutamate at low concentrations but at high concentrations showed a mixed type of inhibition, suggesting irreversible binding to the active site. K_i values of 0·073 mM for phosphinothricin and 0·16 mM for methionine sulphoximine were determined.

Photorespiration. Considerable time has been spent examining the enzyme content of selected photorespiratory mutants of barley. The results will be discussed in the Botany Department section.

Chlorophyll biosynthesis. In collaboration with Dr E. Harel, University of Jerusalem, Israel, studies have been carried out on the biosynthesis of 5-aminolevulinic acid (ALA) the precursor of chlorophyll in green plants. Analysis of the $^{15}N/^{14}C$ ratio of ALA after the feeding of ^{14}C, ^{15}N-glutamate suggests that at least part of the ALA is synthesised by a route which involves the removal and replacement of the amino group of glutamate. A possible pathway is via 2-oxoglutarate and 4,5-dioxovaleric acid.

Selection of biochemical mutants. Analysis of R2501, one of the selected barley mutants resistant to lysine plus threonine, has continued. The previously demonstrated accumulation of soluble threonine in young plants was shown also to occur in mature seeds. The soluble content of threonine is increased such that it comprises 9·6% of the total threonine content in seeds from resistant plants as compared with 0·8% in normal plants. This is sufficient to increase the total threonine content of the seed by 6%; methionine content is increased by the same amount. Resistant plants have an aspartate kinase enzyme which is relatively insensitive to feedback inhibition by one of its end product regulators, lysine. This altered enzyme is proposed as the basis of resistance to exogenous lysine plus threonine, and the reason for increased amino acid accumulation. In this case therefore direct selection for an alteration in a metabolic pathway has isolated a barley mutant in which the desired amino acid accumulation occurs in the barley seeds.

We have also selected for resistance to the proline analogue 4-*trans*-hydroxyl-L-proline.

https://doi.org/10.23637/ERADOC-1-137

Barley mutants resistant to 4 mM-hydroxyproline have been selected. In one of them (R5201) resistance is due to the action of a codominant gene. In this mutant there is more free proline in young leaves under normal conditions and proline also accumulates to a greater extent under severe water stress induced by polyethylene glycol. It thus appears that this mutant may be of some use in examining the role of proline in stress metabolism as well as contributing to our scanty knowledge of the regulation of proline biosynthesis.

Amino acid analysis. The amino acid analysers have been used to complete about 1700 analyses during the last year. Tryptophan is destroyed under the conditions we normally use for protein hydrolysis and unless considerable care is used, the recoveries of the sulphur amino acids can be low. We are currently examining methods which may permit the determination of all amino acids, including the sulphur amino acids and trytophan, in one hydrolysate. (Bright, Cullimore, Hill, Kueh, Lea, Leason, Mayne, Miflin, Norbury, Pahlich, Parkin, Rognes, Smith and Wallsgrove)

Storage proteins in cereals

Wheat seed protein. Studies on the gliadin (storage proteins) extracted from whole milled wheat grain have continued, and amino acid analyses of the fractions extracted by different solvents, under different conditions, have been completed. Most nitrogen, and the greatest range of polypeptides, are extracted by 50% (v/v) propan-1-ol containing 1% acetic acid and 1% 2-mercaptoethanol. The amount of lysine (as percentage of protein content) in these fractions increases linearly, from 0·48 to 0·74 μmol%, as the temperature of extraction is raised, both with and without 2-mercaptoethanol in the extractant. The S-amino acid content showed no such relationship. The cyst(e)ine values (determined as cysteic acid) varied from 1·22 to 1·64 μmol% randomly, and the methionine content ranged from 0·91 to 1·21 μmol%, except in the fraction extracted without 2-mercaptoethanol at 4°C which contained only half the average amount of cyst(e)ine. The lysine contents of all fractions extracted by unacidified 50% propan-1-ol were much smaller despite similar polypeptide patterns, the maximum value being around 0·50 μmol%. There was no change in methionine contents but the cyst(e)ine values increased slightly.

Using either 70% (v/v) ethanol or 55% (v/v) propan-2-ol as the extractant similar results were obtained for the amount of nitrogen extracted, the polypeptide pattern and amino acid profile of the extracted protein. The greater cyst(e)ine and smaller lysine contents, in comparison to the values in propan-1-ol extracts, reflects the relative absence of high MW polypeptides, the presence of which is just discernible in extracts made at 60°C. Two-dimensional gels of fractions extracted with 70% ethanol at 4°C with and without, and at 60°C with, 2-mercaptoethanol confirmed the presence of a group of high MW components in the latter. Some preliminary experiments have been made on S-deficient wheat grain, cv. Sappo. Using one-dimensional SDS-PAGE the presence of three high MW bands, one single and a doublet, were detected in grain grown with an excess of nitrogen relative to the S supplied. These bands were not detectable, except as possible traces, in grain grown with an adequate supply of S. Techniques for determining the free sulphate content of grain are being explored.

The storage proteins of wheat endosperm are of prime importance in determining the baking quality of flour; of particular significance is the amount of protein present in an aggregated state. As previously reported, an estimate of this can be obtained by subjecting a urea-detergent solution of gluten proteins to gel-permeation chromatography, when aggregated and monomeric species can easily be distinguished. In order to determine

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whether such aggregates are of physiological origin or rather are a consequence of grain drying and/or dough formation two experiments have been performed. In the first, protein bodies were isolated from developing grain while in the second flour was fractionated in the dry state into protein and starch by differential centrifugation in an organic medium. Subsequent extraction of both protein body and flour protein preparations with urea-detergent yielded solutions, containing both associated and monomeric material, very similar to those obtained from gluten. Disulphide bonds play an important role in the stabilisation of the aggregated state. This is supported by the observation that the aggregates are dissociated in the presence of reducing reagents such as mercaptoethanol, and work is currently in progress to determine the distribution of cysteine residues in several storage proteins. The technique adopted involves specific chemical cleavage at these residues followed by mapping of the resulting peptides according to size by SDS-PAGE, and preliminary results have been obtained for several high molecular weight polypeptides (in the range 85 000–145 000). Although of similar amino acid composition (all contain about 2% cysteine) these proteins exhibit markedly different fragmentation behaviour. Those with molecular weights below 100 000 break down into several large peptides while those of higher molecular weight show no significant decrease in size, a result which strongly suggests that in the latter proteins the cysteine residues are confined to the terminal regions of the polypeptide chain.

Polymorphism of hordein polypeptides. As reported previously the storage proteins of barley (hordeins) can be classified into two groups *B* and *C* which have different chemical properties and which are specified by different complex structural gene loci. Each group consists of several similar polypeptides which probably represent a polymorphic series of proteins coded for by a family of closely related genes. To investigate further the nature of the protein polymorphism both sequence determination and peptide mapping studies have been undertaken. The N-terminal amino acid sequence of a purified *C* hordein fraction from barley cv. Julia was determined using a Beckman 890C Amino Acid Sequencer (in association with Dr J. March, John Innes Institute). Although the *C* hordein fraction was a mixture of polypeptides, a single major sequence was found with secondary residues present at only eight positions out of 30. This indicates the presence of considerable structural homology in component polypeptides. The automatic amino acid sequencer was also used to confirm that the *B* hordein fraction was blocked at the N-terminus.

A detailed study has been made of the polymorphism and structural homology of the *B* hordein polypeptides. Two-dimensional gel mapping of *B* hordein from eight cultivars of widely differing ancestry showed the presence of 47 different polypeptides with between 8 and 16 present in any one variety. The polypeptides differed in their distribution patterns, some being present in a number of varieties while others were restricted to one or two. They also differed in their relative contributions to the total hordein fraction, both within and between varieties. The structural homology of the major polypeptides was compared by cleavage at methionine residues with cyanogen bromide and separation of the peptides on gradient gels. On the basis of this the polypeptides were divided into three classes, which also showed characteristic distributions on the 2-D maps. The structural homology demonstrated for the polypeptides within each group provides support for the hypothesis that the hordein loci are complex multigenic families derived from the duplication and divergence of single ancestral genes.

***In vitro* synthesis of storage proteins.** Poly A^+ RNA, derived from membrane-bound polysomes isolated from developing barley endosperms, supports the synthesis *in vitro* of polypeptides with many of the characteristics of *B* and *C* hordeins. This RNA fraction

https://doi.org/10.23637/ERADOC-1-137

has been further subfractionated by polyacrylamide gel electrophoresis under denaturing conditions (glyoxal, urea or methyl mercury hydroxide) and distinct bands obtained ranging in molecular weight from 200 000 to 700 000. Sections (3 mm in length) were cut from urea gels and the RNA electro-eluted from the gel and used to support protein synthesis the products of which were characterised by SDS-PAGE. Using this technique it has been found that the mRNAs for *B* and *C* hordeins have similar mobilities on urea-agarose gels corresponding to a molecular weight of about 650 000.

One difference between the products of poly A^+ RNA translated using an *in vitro* protein synthesis system, and authentic hordein polypeptides, is that the latter have a greater mobility on SDS-PAGE gels. This indicates that the mRNA transcripts are initially produced as longer polypeptide chains which are subsequently processed to the correct size. If the system in barley is analogous to that in animals it might be expected that this would occur co-translationally as the products are transferred through the endoplasmic reticulum. Experiments with isolated vesicles derived from barley endosperm endoplasmic reticulum have confirmed that this occurs. Further, work using *Xenopus laevis* oocytes (in conjunction with the University of Warwick) has indicated that the 'signals' controlling this processing activity are probably homologous between plants and animals.

Work has also been initiated on the synthesis and deposition of storage proteins from other important seed crops. The membrane-bound polysomes from wheat endosperms have been shown to direct the synthesis of a wide range of polypeptides including some which can be tentatively identified as the high molecular weight storage proteins believed to be important in breadmaking. Attempts to isolate the mRNA specifying these proteins is now under way. Similarly, polysomes and mRNA directing the synthesis of the pea storage proteins have been isolated from developing pea cotyledons. These fractions are being used to study further the various steps involved in the processing and deposition of the cotyledonary storage proteins of peas, particularly in comparison with those steps occurring in the cereal endosperm.

Nucleic acid studies. Work has continued (with Dr R. B. Flavell and Dr R. Thompson at the Plant Breeding Institute) towards the physical isolation of the storage protein genes of barley. Barley endosperm poly A^+ has been used to produce cDNA. This cDNA has been made double stranded and inserted into the plasmid pBR322 and the plasmid cloned in *Escherichia coli*. The *E. coli* colonies produced were hybridised with radioactively labelled poly A^+ RNA preparations from barley endosperms or barley shoots. About 130 colonies were found to hybridise only to the endosperm RNA suggesting that they contain lengths of DNA corresponding to endosperm specific messages. Further analysis of those clones has been undertaken using a variety of techniques.

Colony hybridisation techniques, using DNA purified from eight of the cloned plasmids as probes, has indicated that a considerable amount of cross hybridisation occurs between the clones consistent with them corresponding to a family of related mRNAs. Barley endosperm poly A^+ RNA has been fractionated by electrophoresis in agarose gels containing methyl mercury hydroxide. The RNA has then been transferred to diazotised paper, to which it is covalently bound, and hybridised with ^{32}P labelled DNA isolated and purified from several of the cloned plasmids. In this way the size classes of the poly A^+ RNA hybridising with the different clones of cDNAs can be determined. The 20 clones characterised so far can be divided into seven groups which hybridise to different RNAs ranging in size from about 710 to 2350 nucleotides long. Again there is evidence for the presence of clones containing closely related nucleic acid sequences. In separate experiments, using two different approaches, the cloned DNA, fixed to nitrocellulase or diazotised paper, has been hybridised with endosperm poly A^+ RNA

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and the RNA fraction specifically hybridising with the DNA has been subsequently eluted and incorporated into an *in vitro* protein synthesising system to test its messenger properties. Such fractions have been found to have mRNA activity. Preliminary analyses of the products suggests that they are hordein-like proteins. (Byers, Bahramian, Burgess, Cunliffe, Faulks, Festenstein, Forde, Forde, Field, Gayler, Kreis, Hill, Matthews, Miflin, Parmar, Pierce, Rahman, Shewry, Smith and Wallace)

Regeneration of plants from protoplasts

An essential prerequisite for the genetic manipulation of crop plants is the ability to regenerate reproducibly plants from protoplasts. Currently, this problem presents a major barrier to progress and one which we are trying to overcome. This year we have concentrated on the crops potato, rape, wheat and barley.

Potato. Great interest has been attached to the observation of Shepard *et al.*, 1980 (*Science* **208**, 17–24) that a considerable variation in agronomic characters occurs amongst plants regenerated from single protoplasts derived from the same leaf of plants, of the American *tetraploid* variety Russet Burbank. Some of this variation may have significance for plant breeders. However, for genetic manipulation studies one would ideally require a regenerative system in which, in control populations no variation existed. It is therefore of interest that Wenzel, 1979 (*Advances in Protoplast Research* 327–340) working with protoplasts isolated from *in vitro* propagated shoot cultures of *dihaploid* potato clones reports uniformity of protoplast-derived clones. It is unclear at present whether the differences observed by the two groups are due to the differing genetic complexity of the source materials, to the different techniques employed or to both. We have consequently attempted to apply the shoot culture technique to British commercial tetraploid varieties. Numerous plants have been regenerated from protoplasts of Maris Bard and these are now forming tubers. They will be placed under field trials in collaboration with potato breeders (Northern Ireland Department of Agriculture) to determine their uniformity. Numerous calluses have also been obtained from Maris Piper protoplasts and their ability to form plants is currently being tested. We are also using growth chamber-grown plants as a source of protoplasts but, in contrast to the US group, will attempt regeneration by methods used for our shoot-culture derived protoplasts. The protoplast systems developed will be incorporated into the Department's programme on the isolation of biochemical mutants.

Rape. There are two main reasons for our interest in regenerating rape plants from protoplasts: (1) workers at the John Innes Institute are investigating the use of cauliflower mosaic virus as a possible vector for the transformation of plant cells, consequently a reproducible system for protoplast culture in the *Brassica* genus is required. (2) Rape breeders have a strong interest in F_1 hybrid seed production and for this purpose a source of male sterility is highly desirable. In conjunction with Dr G. Pelletier (INRA, France) we hope to develop reproducible protoplast regeneration systems as the first step in attempts to produce cytoplasmic hybrids between rape and male-sterile cytoplasms derived from radish.

Using shoot cultures of rape cv. Victor as the protoplast source we have obtained numerous callus colonies but so far only one plant has been produced. Dividing colonies have also been obtained from protoplasts of the types of interest in the male-sterile production programme. Any plants produced will also be tested for uniformity in parallel with the potato programme.

https://doi.org/10.23637/ERADOC-1-137

Wheat and barley. Despite extensive experimentation in several laboratories efforts to induce division in mesophyll protoplast populations have failed. Consequently, workers in this area are seeking alternative sources of dividing, morphogenetically competent, cells. Possible sources are tissue cultures derived from immature embryos and immature influorescences. Indeed using this approach with pearl millet, *Pennisetum americanum*, Vasil and Vasil, 1980 (*Theoretical and Applied Genetics* **56**, 97–99) have reported regeneration of plants from protoplasts. In barley, shoot-forming tissue cultures can be reproducibly obtained from immature embryos cv. Golden Promise but not from other varieties tested. These mutants are being crossed with Golden Promise to determine the tissue culture response of recombinants. Plant-forming cultures have also been obtained from wheat immature embryos and influorescences. All plants obtained from tissue cultures will be tested for variation in agronomic characters. Suspension cultures derived from the morphogenetic cultures yield protoplasts capable of division (3–5% division in wheat) but this division is not sustained.

Transformation studies. Attempts are being made to obtain rapidly growing suspension cultures of barley mutants, including the lysine plus threonine resistant mutant mentioned above, to see if these traits are expressed in culture. If they are the resistance genes can be used as markers in cell fusion experiments. At the request of the GM programme committee we are also selecting mutants lacking either alcohol dehydrogenase or nitrate reductase in barley and potato as a prelude to transformation studies in these crops. (Bright, Creissen, Franklin, Freeling, Jarrett, Lancaster, Maddock, Norbury, Roberts, Risiott, Rogerson and Thomas)

Staff and visitors

Outside support. The Department gratefully acknowledges the financial support for personnel and materials that have been provided by various organisations including the Home-Grown Cereals Authority, Ciba-Geigy Ltd., Shell Research Ltd, the Potato Marketing Board and the EEC.

Visitors. The Department was pleased to welcome Dr M. Freeling from the University of California and Dr K. Gayler from the University of Melbourne both for 9 months, Dr S. E. Rognes from the University of Oslo for 7 months, Dr D. Boothby from the North London Polytechnic and Dr N. Rogerson from the Northern Ireland Department of Agriculture both for 6 months, and Dr E. Pahlich from the University of Giessen for 4 weeks.

Visits abroad. Members of the Department attended and gave talks at many conferences abroad chiefly by invitation. P. J. Lea attended the US Department of Energy conference on 'The Energetics of Nitrogen Fixation', Michigan State University, USA 13th FEBS Meeting, Jerusalem, Israel, the 5th International Congress on Photosynthesis, Halkidiki, Greece, and also lectured on UNEP training courses in Nairobi, Kenya, and Belgrade, Yugoslavia, and at various university campuses in the USA. M. Byers, J. M. Field and B. J. Miflin attended the INRA 'Workshop on the Physico-Chemical Properties of Wheat Gluten Proteins' Nantes, France. B. J. Miflin attended a Societé Physiologie Végétale Français Symposium on 'Photorespiration' in Toulouse, France, the Joint Canadian and International Association of Plant Physiologists Meeting in Calgary, Canada, on 'Nitrogen and Crop Yield', and the Sixth EMBO Symposium in Heidelberg, Germany, with M. Kreis. E. Thomas presented papers at a CNRS/NSF conference on plant tissue culture, Orsay, France, and at an EEC Symposium on proteins in oilseed

https://doi.org/10.23637/ERADOC-1-137

crops held at Braunschweig, West Germany. S. W. J. Bright gave seminars at the University of California, Davis, USA; INRA, Versailles, France; the Risø National Laboratory Denmark, and University of Groningen, Netherlands. W. S. Pierpoint attended the NATO Advanced Study Institute in Sounion, Greece. G. N. Festenstein gave a poster demonstration at the 13th FEBS Meeting in Jerusalem. P. R. Shewry and R. M. Wallsgrove returned from the USA both after a year's leave of absence.

Staff. Margaret Holden retired in October after 36 years at Rothamsted. She joined the newly formed Biochemistry Department in 1944 to work with N. W. Pirie on metabolic changes occurring in leaf extracts. An interest in such changes, catalysed by pectin esterases, ribonucleases, chlorophyllases, lipoxidases, etc., remained for much of her research life, but she also contributed to the biochemistry of pathogen-infected plants and was seconded to the Cocoa Research Institute in Ghana between 1954 and 1958 to work on the swollen-shoot disease. She was Acting Head of the Biochemistry Department in 1973. Apart from her research she has contributed to many aspects of life in Rothamsted ranging from secretary of the Staff Union to a very vigorous member of the Overseas Housing Association. Her knowledge of the fungi, the macroscopic ones especially, is recognised both locally and nationally. She has been active in the British Mycological Society for many years, and currently helps to produce and edit the *Bulletin*.

During the year Jayne Matthews successfully completed her Ph.D. and left to do post-doctoral work at the University of Wisconsin, Madison, USA, and Jane Ray resigned; Julie Cullimore from the University of East Anglia, Janice Forde from the University of Edinburgh, Valerie Jarrett from Wye College, Sheila Maddock from the University of Cambridge, Pamula Everitt, Ruth Risiott, Jan Roberts, and Jacqueline Wallace joined the Department.

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