ROTHAMSTED RESEARCH

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Biochemistry Department

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Economics of treatments. The 'economic' treatment on winter beans gave a yield increase over 'standard' of 1·6 t ha^{-1} (worth £240 at current prices) but had an increased materials cost of £245. Our results suggest that the extra yield was a result of controlling early chocolate spot by the benomyl + thiram seed treatment (cost £20 ha^{-1}) and *Sitona* larvae by carbofuran granules (£110 ha^{-1}). If ancillary experiments show that, as for spring beans, the much cheaper phorate granules (£18 ha^{-1}) are almost as effective as carbofuran, the combination of seed treatment and granules would be very profitable. The 'full' treatment cost a further £700 ha^{-1}, largely for aldicarb, but despite giving a substantial increase in total dry matter in July, it did not significantly increase grain yield, probably because relatively more was lost from lodging.

The 'economic' treatment on spring beans cost £36 ha^{-1} and gave an increased yield over 'standard' of 0·6 t ha^{-1} (worth £90) on the unirrigated crop and 0·3 t ha^{-1} (worth £45) on the irrigated crop. Because viruses were rare and effects of treatments on root-rots were slight the benefits of 'economic' are attributed to the control of *Sitona* larvae and *Pratylenchus* spp. achieved by the phorate and the improved leaf efficiency from the single spray of benomyl. 'Full' control cost £615 ha^{-1} more than 'economic' but gave no further yield increase. Aldicarb, which hitherto has given excellent pest control, this year failed to control both *Sitona* larvae and *Pratylenchus* spp.—probably because of leaching associated with much early rain. A computer simulation (Briggs, CLU and Nicholls, Insecticides and Fungicides Department) suggested that by the end of March all the phorate would have remained in the top 10 cm of soil whereas none of the aldicarb would have been in this layer, most being below 20 cm. It is therefore surprising that 'full' control gave yields equal to 'economic': possible explanations are control of unrecognised seedling pests by aldicarb before leaching and greater leaf efficiency from the two benomyl sprays included in this treatment. The severity of the attack by rust, irrespective of treatment may have contributed to yields smaller than those in previous experiments.

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Introduction

The Department's research programme has progressed along the same paths as outlined last year. We continue to have a major commitment to studying nitrogen metabolism in cereals and legumes, in particular the primary and secondary assimilation of ammonia, the synthesis of important amino acids and the final deposition of the nitrogen in the storage protein of the developing seed. What has changed over the years is the nature of the techniques that can be brought to bear on this subject; increasingly the selection of mutant plants and the use of recombinant DNA technology are becoming more powerful ways of investigating these topics. We have also continued and increased our commitment to the study of tissue cultures of crop plants. These have the potential of producing desirable variants either by selection of naturally occurring or induced mutants, or by the direct introduction of specific genetic material. Among those attributes most generally cited as desirable is resistance to disease. We are studying one specific system in which resistance to virus infection may be induced in plants.

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Host/plant pathogen relationships

Pathogenesis-related proteins. Extracts made from the leaves of tobacco (Xanthi-nc), in which the spread of virus (TMV) is limited by a hypersensitive reaction, contain a number of proteins which are not present in extracts of uninfected leaves. Although they are produced during flowering as well as in pathogenic conditions, interest continues in their relationship to, and possible role in, restricting pathogen spread.

The aim of this work is to understand the processes that occur in a virus-infected leaf and which determine whether the virus will multiply and spread throughout the plant or remain localised in a small area. The closely related *Nicotiana tabacum* cultivars Xanthi and Xanthi-nc provide an ideal system for investigating this problem since tobacco mosaic virus (TMV) spreads systematically in Xanthi but produces localised necrotic lesions in Xanthi-nc. Furthermore, infection of Xanthi-nc with TMV induces resistance, in that fewer and smaller lesions are formed in all leaves infected subsequently with virus. Analysis of young growing plants has shown that as many as ten novel soluble leaf proteins, termed pathogenesis-related (PR) proteins, accumulate in resistant leaves but are not present in healthy ones. It has been found that injection of healthy leaves with aspirin or polyacrylic acid induces resistance to TMV and causes the appearance of certain PR-proteins. The hypothesis that the PR-proteins are involved in the localisation mechanism is under investigation.

Relationship of PR proteins to resistance. When Xanthi-nc is inoculated with tobacco mosaic virus (TMV) at 20°C, local necrotic lesions appear on the leaves at the sites of infection and the virus is restricted to the cells of the lesion and immediately adjacent tissue. At the same time the leaves become resistant to further infection by TMV and the associated PR-proteins appear. At 32°C, however, the PR-proteins do not accumulate, the resistance breaks down and the virus spreads systemically. We reported previously (*Rothamsted Report for 1980*, Part 1, 180) that at 20°C aspirin-treated leaves also accumulated PR-proteins and became resistant, but at 32°C, in contrast to the response of TMV-infected leaves, PR-proteins accumulate. We have assessed whether the presence of PR-proteins at 32°C affected the course of virus infection of tobacco leaves. The presence of PR-proteins in leaves of Xanthi-nc at 32°C, induced by treatment with aspirin, did not cause systemically infecting TMV to form localised necrotic lesions; however, the spread of virus was greatly reduced. The extent of systemic spread of virus was visualised by transferring plants from 32 to 20°C, when the regions containing virus collapse to form necrotic areas. We found that the areas of virus spread on aspirin-injected half-leaves were much smaller than on water-injected opposite half-leaves.

Although polyacrylic acid-injected leaves of Xanthi-nc accumulated PR-proteins at 20°C, none was detectable at 32°C but the spread of systemic virus was reduced. This suggested that the reduction of virus spread at 32°C was not related to the presence of PR-proteins. However, we found that, although no PR-proteins were produced at 32°C, on transfer to 20°C greater amounts were accumulated than in control plants treated with polyacrylic acid on the day of transfer to 20°C. This indicated that the production of PR-proteins was primed, but not expressed, at 32°C so that on transfer to 20°C the proteins were produced more quickly. We aim to investigate the possibility that the reduction in virus spread was also primed at 32°C and only expressed after transfer to 20°C.

The amount of PR-proteins formed, and resistance induced, was measured in leaves of Xanthi-nc 7 days after injection with from 5 to 600 μg aspirin ml^{-1}. The amount of PR-Ia was determined from extracts of whole leaves by polyacrylamide gel electrophoresis (PAGE). Resistance to virus infection was measured by injecting each concentration of

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aspirin into at least ten half-leaves; the opposite half-leaves were injected with water. Seven days after inoculation with TMV the lesion areas were measured and the difference in areas between treated and control half-leaves used as a measure of resistance. Below 75 μg aspirin ml^{-1} there was no detectable accumulation of PR-Ia and no significant resistance, but from 75 to 600 μg aspirin ml^{-1} there were non-linear increases in both PR-Ia and resistance. This suggests that there is a threshold concentration of aspirin below which neither PR-Ia synthesis nor virus resistance are induced, and above which there is a correlation between the aspirin concentration injected, the amount of PR-Ia accumulated and the degree of resistance. The similar response of PR-Ia accumulation and resistance to virus injection suggest that they may be related. (Antoniw and Carr, with White and Woods, Plant Pathology Department)

***Synthesis of PR proteins* in vivo.** Besides the four PR-proteins which are obvious in electrophoretic analysis of extracts of TMV-infected leaves of tobacco (Xanthi-nc), and which have been partially characterised (PR-Ia, b, c and II), there are at least six more which are not present in extracts of uninfected leaves. These resemble PR-Ia, b, c and II in that they are relatively resistant to proteolytic enzymes and are more obvious after extracts have been incubated with trypsin and chymotrypsin. However, they are not produced by this digestion, nor are their electrophoretic mobilities significantly modified by it. Any chemical relationship they have to PR-I and II is uncertain but they do not contain the same component polypeptides; when electrophoresed under denaturing conditions, they do not produce the peptides characteristic of these two proteins.

When infected leaves, which are actively synthesising PR-proteins, are briefly exposed to $^{14}CO_2$ and illuminated, PR-Ia, b, c and PR-II become radioactive; PR-Ia retains this label when subsequently electrophoresed in denaturing conditions. Of the other proteins, only one is consistently labelled. The pattern of labelling is not significantly changed when the leaf is exposed to $^{14}CO_2$ before or concomitant with virus inoculation. The labelling of PR-I and II is thus consistent with their metabolic synthesis in these leaves, but the experimental arrangement is unsuited for a pulse-labelling analysis of the process; the large amount of $^{14}CO_2$ necessary to give a convincing labelling of the proteins is extensively incorporated into carbohydrates and subsequently released into synthetic pools over a long period. (Pierpoint and Strowman)

In vitro *synthesis of PR-proteins*. There has been very little study of the changes which occur in the mRNA populations of virus-resistant plants during infection, and the processes which govern the expression of the genes of resistance remain unknown. By investigating the control of synthesis of PR-proteins it is hoped that light can be shed on the mechanisms controlling resistance. Total poly A$^+$ mRNA extracted from healthy and virus-infected Xanthi-nc tobacco seedlings and leaves of mature plants was translated in cell-free systems derived from wheat germ extract and rabbit reticulocyte lysate. The radio-labelled translation products were analysed by PAGE and the gels fluorographed. PR-proteins have been identified among the translation products on the basis of Rf values and by immunoprecipitation. The experiments show that mRNA coding for PR-proteins is present in healthy plants but is not translated. It would seem that virus infection or chemical treatment of tobacco allows the translation of PR-protein mRNA to occur. (Antoniw and Carr, with White, Plant Pathology Department, and Dr T. M. A. Wilson, Liverpool University)

Adenine compounds and disease resistance. The resistance induced in *N. tabacum* cv. Xanthi-nc and *N. glutinosa* by infection with TMV has been likened to the interferon system operating in animals (*Journal of General Virology* (1974), **23**, 1–9). Recently Sela (*Trends*

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in Biochemical Sciences (February 1981), 31–33) claimed that pppA$^{2'}$p$^{5'}$A$^{2'}$p$^{5'}$A (2-5A), which is involved in the inducible nuclease mechanism of interferon action in animals, was present in Xanthi-nc. In collaboration with Dr J. Cayley at the Imperial Cancer Research Fund we found that although a radiobinding assay (*Nature, London* (1980), **288,** 189) indicated low levels (< 5 nM) of 2-5A in healthy leaves of Xanthi-nc and *N. glutinosa*, this was not enhanced in infected leaves and was probably an artefact of the assay. Furthermore there was no significant 2-5A synthetase activity or 2-5A-dependent binding protein detectable in the extraction. (Antoniw and Carr, with White, Plant Pathology Department)

Intermediary nitrogen metabolism

Ammonia assimilation. When effective legume root nodules are formed there is a major increase in the amount of glutamine synthetase present in the plant portion of the nodule. Several closely related forms of glutamine synthetase from *Phaseolus* root nodules have been separated and purified to apparent homogeneity. The properties and possible interconversion of these forms are presently being examined in order to establish the number of gene products involved. During nodule formation the activity of glutamine synthetase increases markedly and changes in the activity of the different forms has been followed and related to the induction of nitrogenase and leghaemoglobin. Antibodies raised to nodule-specific glutamine synthetase cross-react with the root form and with the enzyme in nodules of other legume species. The purified anti-glutamine synthetase immunoglobulin has been used to identify the protein obtained by *in vitro* translation of nodule poly A$^+$ RNA. Glutamine synthetase appears to be translated mainly on free polysomes and work is now being initiated to isolate the specific messenger RNA. Complementary cDNA will be made from the enriched fractions as the first step in the isolation of the genes for this important enzyme.

Earlier work in the department established the existence of ferredoxin-dependent glutamate synthase in green leaves, and the key role of this enzyme in ammonia assimilation has been firmly established. Recently work in other laboratories (Matoh *et al.*, *Plant Cell Physiology* (1980), **20,** 1329–1340) has suggested that leaves also contain NAD(P)H-dependent glutamate synthase. We have confirmed the existence of this enzyme in pea and barley leaves, although the NAD(P)H-dependent activity is less than 3% of the total glutamate synthase in light-grown leaf tissue. Total glutamate synthase activity in etiolated leaves is much lower than in green leaves, and NAD(P)H-dependent activity can be up to one-third of the total. Studies on greening leaves have shown that there is a rapid light-induced increase in ferredoxin-dependent enzyme whereas the NAD(P)H-glutamate synthase activity does not alter.

Amino acid biosynthesis. The synthesis of amino acids derived from aspartate, viz. lysine, threonine and methionine, has been further investigated. Characterisation of aspartate kinase from green leaves of barley has shown that three forms of the enzyme can be separated by DEAE-cellulose chromatography. The form first eluted (AK-I) is a minor proportion (10% of the total activity) and is inhibited by threonine. The second and third forms (AK-II and AK-III) are both inhibited by lysine alone and, synergistically, by lysine plus S-adenosylmethionine. Evidence that these are truly separate enzymes coded for by separate genes was obtained by analysing the lysine plus threonine resistant mutants R2501 and R3004. These mutations are the result of two independently inherited genes (see below); extracts of R2501 contain an AK-II which is relatively insensitive to lysine and a normal AK-III whereas those of R3004 have a normal AK-II and a lysine-insensitive AK-III.

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Previously, we reported that isolated intact chloroplasts can synthesise lysine, threonine and methionine from aspartate. Now we have estimated the proportion of the leaf cells' total synthesis of these amino acids that occurs in the chloroplast. Using isolated leaf protoplasts as a source of organelles, it has been established that aspartate kinase, the first enzyme of the pathway, is confined to the chloroplast in both peas and barley. Similarly, homoserine kinase and threonine synthase are wholly localised in the chloroplast. These results together with previous findings suggest that all of the threonine and lysine formed in a leaf cell is synthesised in the chloroplast. Currently we are establishing the localisation of those enzymes responsible for methionine and S-adenosyl methionine synthesis.

Proline has been implicated as an important metabolite in the response plants make to imposed stresses besides its more normal role in amino acid and protein metabolism. However, despite its importance little is known of the biochemical mechanisms of proline synthesis. *In vivo* labelling studies with barley seedlings have confirmed that both glutamate and ornithine may act as precursors of proline. Although [^{14}C]-glutamate is only converted into protein-bound proline, [^{14}C]-label derived from ornithine is found in both protein and soluble proline pools. Barley chloroplasts isolated from gently ruptured protoplasts are able to convert [^{14}C]-glutamate to proline, ornithine and arginine in light-stimulated reactions. These studies are continuing and attempts are being made to find a fully soluble system capable of synthesising the putative intermediates of proline formation.

We have also continued our long-term studies on asparagine metabolism. Although this compound is implicated in the flux of nitrogen through the plant, little is known of its synthesis and metabolism except in seeds. We have attempted to show that enzymes for its synthesis also exist elsewhere in the plant. A very low asparagine synthetase activity has been isolated from pea leaves (0·45 μmol g^{-1} fresh weight h^{-1}). The enzyme is dependent upon glutamine and ATP/Mg^{2+} (*see Rothamsted Report for 1974*, Part 1). The activity is however high enough to account for the known nitrogen flux through asparagine as calculated by ^{15}N-analysis (Bauer *et al.*, *Plant Physiology* (1977), **59**, 915–919).

Photorespiration. In collaboration with Keys, Botany Department, work has continued on examining mutants of barley with altered photorespiratory properties. One mutant has been confirmed as having only 5–10% of the wild type level of catalase. The wild type enzyme exists in two major forms that can be separated either by starch-gel electrophoresis or DEAE-cellulose chromatography. Both forms are missing in the mutant. Back-crossed plants (cv. Golden Promise) have 50% of the normal catalase level (showing both forms) and are able to grow at normal atmospheric CO_2 concentrations. Examination of the F_2 generation shows that the sensitivity to low CO_2 is correlated with low catalase activity and is due to the action of a partially dominant gene. (Bright, Cullimore, Cunliffe, Hill, Joy, Kueh, Lara, Lea, Mazelis, Miflin, Rognes, Pahlich and Wallsgrove)

Cereal endosperm proteins

A major area of research in the Department is the chemistry, genetics and molecular biology of cereal grain proteins. We have been particularly concerned with the alcohol-soluble storage proteins of barley and wheat but this work has extended to include other proteins and other species. The following approaches have been used: (i) chemical analysis of the isolated proteins, (ii) genetic analysis of the loci coding for the proteins, (iii) isolation of mRNA, for these proteins, the synthesis of cDNA and cloning of double-stranded cDNA, (iv) studies of the deposition of these proteins *in vivo* and *in vitro*. The results obtained have allowed us to classify the prolamin storage proteins in wheat,

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barley and rye into three groups: the high molecular weight, the sulphur-rich and the sulphur poor prolamins. This classification has considerably helped our understanding of these proteins and their contribution to the technological properties of the grain. The results are reported under the headings of the different cereals.

Barley

Analyses of normal and mutant cultivars. The high molecular weight prolamin fraction, which we have termed 'D' hordein, has been characterised. Small amounts of this fraction are extracted in aqueous-alcohol but extraction is greatly enhanced by the addition of 2-mercaptoethanol and acetic acid. The 'D' hordein gives a single band on SDS-PAGE with an apparent molecular weight of 105 000 but equilibrium centrifugation suggests that the protein is considerably smaller (about 54 700 d). Amino acid analysis showed that, like other prolamins, it contained much glutamate plus glutamine (30–34 mol%) and little lysine and other basic amino acids (a total of about 5 mol%). However, in common with the HMW prolamins of wheat and rye, it was rich in glycine (about 15 mol%) and was, for a prolamin, relatively poor in proline (about 12 mol%). Unfortunately the fraction appears to be blocked to Edman degradation and so no N-terminal amino acid sequences could be obtained. Although the gene(s) coding for 'D' hordein is thought to be on chromosome 5 our detailed genetic analysis has not been completed.

Little work has been done on 'C' hordein this year but our studies on the isolation of the 'B' hordein genes have continued. Poly A^+ RNA, derived from membrane-bound polysomes of developing endosperms, support the synthesis of hordeins in an *in vitro* protein synthesis system. A cDNA library, derived from such RNA, was constructed in pBR322 (see last year's report). Over 20 clones from this library have been characterised with respect to their hybridisation to various size classes of RNA and their ability to select mRNA coding for polypeptides of a range of molecular weights. One group of 54 cDNA clones was tentatively identified as carrying cDNA related to 'B' hordeins on the basis of cross-hybridisation analysis and *in vitro* translation of plasmid-selected mRNA. One of these 54 clones, pHvE-c16, which itself hybridises to 39 other clones, has been sequenced and found, by comparison with published C-terminal and peptide sequences, to be related to the B1 hordein polypeptides. The mRNA from which pHvE-c16 was derived has a 3′ untranslated region which is at least 100 nucleotides long or about 8% of the length of the mRNA (1300 nucleotides).

The cDNA clones are being used to study the organisation of the hordein genes in normal barley cultivars and the two high-lysine mutant lines Risø 1508 and Risø 56. Mutant 56 is greatly deficient in 'B' hordeins and in mRNA for these proteins whereas 1508 is deficient in both 'B' and 'C' hordeins and also the corresponding mRNA. The synthesis of the 'D' hordeins both *in vivo* and *in vitro* seems little affected by the mutations. Using the characterised cDNA clones we have shown that almost no mRNA for the 'B' hordeins are present on mutant polysomes. From these results we would suggest that the mutation acts prior to translation of the mRNA on membrane-bound polysomes. Preliminary evidence on the genomic organisation of the *Hor-2* ('B' hordein) locus using 'genomic blots' confirms the conclusions of others, based on conventional genetic analysis, that Risø 56 contains a mutation in the structural genes for 'B' hordeins whereas Risø 1508 does not.

Analyses of wild and old barley grain. The six-rowed, brittle-rachised wild barley *H. agriocrithon* has been proposed as the ancestor of cultivated *H. vulgare*. However grain of *H. agriocrithon* has been collected rarely and it has been shown that similar plants can be produced by hybridisation between *H. vulgare* and *H. spontaneum*, the

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two-rowed, brittle-rachised, wild barley which is widespread in the middle east. We have analysed (with Mr P. Murphy, University College of North Wales, Bangor, Dr J. Witcombe, ICARDA, Syria) grain from plants grown from material collected in the field in North India and identified, on the basis of morphological characters, as *H. vulgare* (cultivated), *H. spontaneum* (weedy) and *H. agriocrithon* (probably also weedy). Comparison of the storage protein (hordein) patterns showed that the *H. agriocrithon* was a hybrid between *H. spontaneum*, which was genetically homogeneous for this character, and one genotype of *H. vulgare*, which showed great genetic variability in hordein pattern. Experimental crosses between *H. vulgare* and *H. spontaneum* grown in the glasshouse gave similar material to that collected in the field and identified as *H. agriocrithon*, thus confirming the hybrid nature of this material.

We have also made a comparison of the amino acid and protein compositions of barley grain from Egyptian archaeological sites dated between approximately 1000 and 3000 years BC and grain grown at Rothamsted between 1852 and 1977. The relative amino acid composition of the archaeological samples was remarkably similar to that of the recent grain, the main differences being decreased relative amounts of lysine and methionine. We are unable, however, to demonstrate the presence of salt-soluble proteins by electrophoresis, or of hordein by electrophoresis or immunoassay. Salt-soluble protein and hordein fractions extracted from the oldest Rothamsted samples gave less clearly resolved patterns on electrophoresis and isoelectric focusing than fractions from recent grain, indicating some protein degradation. The rate of degradation was apparently faster for 'C' hordein than 'B' hordein polypeptides. Scanning electron microscopy showed little difference in the relative amounts of large and small starch grains in the different samples. Transmission electron microscopy showed that although the cells of the starchy endosperm of the 1000 BC grain were partially disorganised, they did contain structures which resembled the protein bodies present in similar sections of recent grain.

Wheat. Work on conditions for extracting wheat prolamins has been completed and the fractions analysed for their amino acid and polypeptide compositions. The results have shown that the most efficient solvent is 50% v/v propan-1-ol, containing 1% acetic acid and 1% 2-mercaptoethanol, although this solvent may also extract relatively more (but essentially only a small total amount) non-prolamin proteins than other solvents. These techniques are currently being used to analyse wheat grown under different regimes of nitrogen and sulphur nutrition.

Previous work has shown the importance of a group of high molecular weight (HMW) prolamins in relation to breadmaking properties. These proteins are homologous to the 'D' hordeins and have similar amino acid compositions; they also have anomalously large molecular weights on SDS-PAGE relative to equilibrium centrifugation. Several of these components have been purified from different varieties using gel filtration, ion exchange chromatography and preparative isoelectric focusing. The N-terminal amino acid sequence of one of these was determined (with Mr J. F. March, John Innes Institute); it showed that two cysteines, out of a total of five or six, were located in the first 20 residues. This confirms previous indications, from the cleavage of the polypeptides at cysteine residues (*see* last year's *Report*), that the cysteines are located close to or at the end(s) of the polypeptide chain.

We are also attempting to isolate an RNA fraction enriched for the mRNA for these HMW prolamins. A comparison of the *in vitro* translation products of free and membrane-bound polysomes from developing wheat endosperms showed that the membrane-bound polysomes are markedly enriched for the desired mRNA. The *in vitro* translation products were identified as HMW prolamin on the basis of their extraction in a solvent containing 50% propan-1-ol, 2% 2-mercaptoethanol and 1% acetic acid, their mobility

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on SDS-polyacrylamide gels and their characteristic high ratio of incorporation of ^{3}H-glycine to ^{3}H-serine. In addition, like the *in vivo* proteins, they showed a pattern on SDS-PAGE which was cultivar-dependent. When poly A$^+$ RNA was extracted from the membrane-bound polysomes, improved synthesis of the HMW proteins was obtained when a reticulocyte lysate was used instead of the wheat germ system for *in vitro* protein synthesis. Further purification of this RNA was attempted by several methods. The most promising procedure involves fractionation of poly A$^+$ RNA on denaturing agarose gels. The mRNA coding for the HMW proteins appears to migrate more slowly than the 26S ribosomal RNA giving it a molecular weight of 2×10^6, large enough to code for polypeptides twice the size of those of the HMW prolamins.

The 'B'-hordein related cDNA clones (see above) were used to look for the presence of homologous mRNA sequences in the poly A$^+$ RNA fraction derived from the membrane-bound polysomes of developing endosperms. Analysis, by hybridisation of radioactive cDNA to poly A$^+$ RNA that had been fractionated by electrophoresis, suggested the presence of mRNA related to several of the clones. Translation *in vitro* of mRNA selected by hybridisation to certain clones gave products with the mobility of the sulphur-rich wheat prolamins on SDS-PAGE but these have not been identified further. These results are consistent with the suggested homologies between the cereal prolamins.

Rye. Two groups of polypeptides, which together represent over 90% of the prolamin storage protein (secalin) fraction of rye, were purified by ion exchange chromatography and gel filtration (in collaboration with Dr D. D. Kasarda, USDA, Albany). These fractions had molecular weights of 40 000 and 75 000 by SDS-PAGE and 33 000 and 54 000 by sedimentation equilibrium ultracentrifugation respectively. Although each gave a single wide band on SDS-PAGE, isoelectric focusing showed that each contained a number of polypeptides. Amino acid analysis showed that these were sulphur-rich prolamins; both groups had similar compositions with large amounts of glutamate plus glutamine and proline and little lysine. The N-terminal amino acid sequences of the two groups were identical at 17 of the first 20 positions and were similar to those reported for γ_2 and γ_3 gliadins (S-rich prolamins) of wheat. Both groups had C-terminal histidine.

Poly A$^+$ RNA isolated from developing endosperms supports the synthesis, in an *in vitro* wheat germ system, of both the 40 000 and 75 000 γ-secalins as well as the ω-secalins. This latter group are the sulphur-poor prolamins. The poly A$^+$ RNA has been further fractionated by agarose gel electrophoresis under denaturing conditions into three separate fractions which are enriched for one of the above groups of secalins. These fractions are being used to produce cDNA for subsequent cloning and the production of a rye prolamin cDNA library. (Bahramian, Blanco, Burgess, Byers, Faulks, Festenstein, Field, Forde, Forde, Hill, Kreis, Matlashewski, Miflin, Parmar, Pywell, Rahman, Shewry, Smith, K., Smith, S. and Wallace)

Genetic manipulation of plants

The aim of this programme is to extend the range of useful variation available to plant breeders. A number of approaches are being tried including: assessment of the variation induced by tissue culture techniques, selection of mutants containing potentially useful genes, selection of mutants which may act as recipients in transformation experiments and definition of conditions for obtaining transformation with foreign genetic material. The progress made in each of these areas is reported below.

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Protoplast and tissue culture studies

Wheat. Work on wheat has been concerned with the isolation and culture of protoplasts, and plant regeneration from cultured immature embryos and inflorescences. Various plant tissues have been examined as sources of protoplasts and a range of pretreatments and culture conditions investigated. The plant material used has included young leaves and shoot cultures, cell suspension cultures and immature embryos, both directly after excision and after different periods of growth in culture. So far suspension cultures have yielded protoplasts which are mostly vacuolate; limited first divisions have been observed in a few cases, but these have not been sustained. However, these suspensions can only be induced to form roots, and not shoots, suggesting that they are unlikely to be a source of cells fully competent to divide and ultimately give rise to intact plants. Protoplasts have been obtained directly from immature embryos. These are highly cytoplasmic and they remain alive for extended periods, showing apparent synthesis of cytoplasm. Thus they appear promising as a source of morphogenetically competent protoplasts although as yet they have not been induced to divide.

Standard procedures have been developed for regeneration of wheat plants via culture of immature embryos and inflorescences on a semi-defined medium. Shoots initiated on morphogenetic and embryogenic cultures, in which production of leafy shoots is clearly preceded by embryoid-like structures, can be cultured separately. These shoots develop into plantlets which can be transferred to pots and grown to maturity in the glasshouse.

Using glasshouse-grown and field-grown material, a range of 25 winter and spring wheat varieties has been screened for their suitability for tissue culture procedures. Over 7000 embryos and 700 inflorescences have been tested. This has revealed clear differences between genotypes in the incidence and extent of the morphogenetic response, and apparent variation amongst regenerated plants in characters such as plant height and fertility. The progeny of these plants is being examined in collaboration with Rothwell Plant Breeders Ltd to determine whether this is genetically stable.

Morphogenetic tissue cultures and individual immature embryos are also being used to initiate suspension cultures. The cell-lines established are being tested for their ability to give rise to plants on appropriate media. We intend to isolate and culture protoplasts from such suspensions that remain morphogenetic.

Potato. Plants have been regenerated from leaf mesophyll protoplasts of three UK cultivars. They have also been regenerated from callus formed on excised pieces of leaf, rachis, petiole and tuber. The regenerated plants from both sources vary morphologically from the original plants. This variation is greatest in plants derived from protoplasts. Tubers have been derived from many of these plants and will be tested in the field to see if this variation persists and if any of it is of agronomic value (with Northern Ireland Department of Agriculture). However, whilst the observed variation may be useful in cultivar improvement programmes it is a problem if specific genes are to be introduced into a constant background. We are therefore attempting to identify and study the causes and the control of induction and expression of this variation. A part of this, cytological analysis of the regenerated plants has been started.

Plants have also been regenerated from protoplasts of the diploid wild species *Solanum brevidens*, which may be useful for genetic studies and in the production of biochemical mutants.

Selection of mutants

Alcohol dehydrogenase. When barley plants are subject to flooding there is a five- to six-fold increase in the level of alcohol dehydrogenase (dependent on protein synthesis)

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in both leaves and roots. Three distinct sets of isoenzymes (set I, set II, set III) can be separated by electrophoresis or DEAE-Sephacel chromatography. In a single gene mutant of barley (Adhl-M9, Harberd & Edwards, *Barley Genetics Newsletter* (1980)) only set III is present. The three forms have similar substrate affinities, pH optima and molecular weights (82 000); the only major difference is that the K_m for ethanol in the reverse direction is higher for set I. Set I enzyme from leaves has been purified 1200-fold. Mutants lacking the major (set I and II) isoenzyme groups may be useful recipient systems for transformation with purified nuclear genes for alcohol dehydrogenase from plant or other sources. This depends on the ability to select efficiently for the transformants. Cell suspensions from immature embryos of the mutant Adhl-M9 and wild type have been established. These will be used to test whether anaerobic conditions will efficiently discriminate between these genotypes and hence provide a useable selection system for transformants.

Nitrate reductase. Mutants exist in tobacco which lack nitrate reductase and do not grow on nitrate (Mendel & Muller, *Molecular and General Genetics* (1979), **177,** 145–153). Previously reported mutants of barley (Warner *et al. Nature, London* (1977), **269,** 406–407) have low nitrate reductase but grow normally on nitrate. Such mutants are of little use for transformation work. We have therefore attempted to isolate non-leaky nitrate reductase-minus mutants of barley which do not grow on nitrate. We have done this by selecting plants capable of growing in the presence of chlorate. So far we have identified and grown on eight plants which are chlorate-resistant; seven of these appear to have less than 10% of the normal level of nitrate reductase. One of these plants has so far set seed when crossed to normal barley; a proportion of the progeny also lack nitrate reductase. These nitrate reductase-minus plants grow very poorly on nitrate and thus appear to be suitable recipients into which a functional nitrate reductase gene might be transferred.

Seventeen mutants of the moss *Physocomitrella patens* have been isolated which are also unable to grow on nitrate as a sole nitrogen source (with Professor D. Cove, Leeds University). Thirteen mutants have no measurable nitrate reductase activity when transferred from a urea to a nitrate medium, which induces maximum activity in the wild type strain. Preliminary tests suggest that the mutants fall into three complementation groups.

Amino acid metabolism. Analysis of two further barley mutants resistant to lysine plus threonine (R3004, R3202) showed that both contained altered aspartate kinase. Using normal and mutant types we have shown that there are at least three isoenzymes of aspartate kinase in barley, one sensitive to feedback-inhibition by threonine and two to lysine or lysine plus S-adenosylmethionine. Of the two lysine-sensitive enzymes one (AK-II) is insensitive in R3202 and R2501 and the other (AK-III) in R3004. The resistance genes in R3202 and R2501 do not appear to be linked to that in R3004. R3004 and R2501 (but not R3202) accumulate soluble threonine in the seed. Cell suspension cultures from immature embryos of Bomi, R3202 and R2501 have been established. Three hydroxyproline-resistant mutants have been analysed. Two are allelic mutations (R5201, R6102) but unlinked to a third (R6902). All three lead to increases of three- to six-fold in soluble proline pools within the leaf. Increased leaf proline content does not affect susceptibility to attack by pests and diseases.

Transformation studies. We have started a programme on the transfer of genetic material into plants using the tumour-inducing (Ti) plasmid of the crown gall organism *Agrobacterium tumefaciens*. The aim is to be able to introduce new genetic information, e.g. single genes, into host plants probably at the protoplast level. So far potato protoplasts have been treated with *A. tumefaciens* and calluses have been obtained. Some of

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these grow in the absence of phytohormones, a characteristic of crown gall cells. These shoots will be assayed for the presence of bacterial DNA and the ability to produce opines. Attempts will be made to regenerate normal plants containing inserted plasmid DNA. (Ahmed, Boccon-Gibod, Bright, Creissen, Dunlop, Franklin, Freeling, Jarrett, Karp, Kueh, Lancaster, Lea, Maddock, Mayne, Nelson, Norbury, Ooms, Risiott, Roberts, Rognes and Thomas)

Glasshouse facilities

The Ninnings glasshouse finished in July has been taken over by the Department and by early October all available bench space (86 m^2) had been occupied. This development represents an increase of 300% in available growing space and provides much needed header house space. The glasshouse of 'Cambridge' design is divided into three bays (each 65 m^2), independently controlled for temperature and lighting, attached to a service corridor (98 m^2). The lighting is provided by 41 400 W SONT lamps in each bay which give an even 180 μeinsteins m^2 s^{-1} as measured 1–8 m below the lamps. The house has been designed so that 66% of the bench area has automatic watering facilities, it has temperature control to within 1°C of set temperature or 1°C of ambient if higher than set temperature and limited aphid proofing has been provided for. The house will satisfy a long-standing need for glasshouse facilities for all areas of our programme. (Franklin)

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, Sigma Chemical Co. Ltd and the EEC.

Visitors. During the year the Department was pleased to welcome for extended visits: Mr S. Ahmed, Jute Research Institute, Bangladesh; Dr K. W. Joy from Carleton University, Canada; Dr M. Mazelis from University of California, Davis, USA, Dr E. Pahlich from Justus-Liebig University, Giessen, Germany; Dr S. E. Rognes from University of Oslo, Norway; Mr M. Lara and Ms Lourdes Blanco from Centro de investigacion sobre Fijacion de Nitrogeno, Cuernavaca, Mexico; Dr J. Boccon-Gibod from ENITAH, Angers, France; Dr M. Freeling from University of California, Berkeley, USA, and Mr G. Matlashewski from University of Ottawa, Canada.

Visits abroad. Members of the Department attended the following conferences or visited overseas universities as indicated; almost without exception, one or more invited talks or seminars as well as contributed papers were given: International Botanical Congress, Sydney (P. J. Lea and B. J. Miflin); Phytochemical Society of Europe Seed Protein Conference, Versailles, France (Marjorie Byers, J. M. Field, Janice Forde, B. G. Forde, M. Kreis, B. J. Miflin, and P. R. Shewry); International Conference on 'New Frontiers and Future Perspectives in Plant Biochemistry', Nagoya, Japan (B. J. Miflin); Inauguration Symposium, Institute for Nitrogen Fixation, Cuernavaca, Mexico (B. J. Miflin); EEC Workshop on Cereal Seed Proteins, Versailles, France (B. G. Forde, M. Kreis, B. J. Miflin, and P. R. Shewry); American Society of Plant Physiology (Julie V. Cullimore); Bat-Sheva Seminar on N_2-Fixation, Weizmann Institute of Science, Rehovot, Israel (P. J. Lea); Gordon Conference on Plant Tissue Culture, USA (Sheila E. Maddock); Gordon Conference on Plant Molecular Biology, USA (B. G. Forde); Swedish Biochemical Society, Umea, Sweden (P. J. Lea); Ciba-Geigy Ltd, Basle, Switzerland (P. J. Lea

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and D. Parkin); UNEP Training Course, Saltillo, Mexico (P. J. Lea); NATO Exchange Fellowship, University of Oslo, Norway (S. W. J. Bright); and 2nd International Youth Symposium on Regulation of Metabolism in Plants, Varna, Bulgaria (R. M. Wallsgrove).

Staff. Emrys Thomas died on Saturday 23 May after a short illness. He came to Rothamsted in October 1979 to lead a team on the regeneration of crop plants from isolated protoplasts as part of the ARC's programme on genetic manipulation. He gained his Ph.D. for studies with plant tissue cultures in the laboratories of Professor H. E. Street, and subsequently worked at the Max-Planck-Institut in Ladenburg and at the Friedrich Miescher Institute in Basle before coming back to Britain. During this time he built up a reputation as an expert in plant protoplast technology and morphogenesis. He quickly established himself at Rothamsted and he was the first in Britain to regenerate plants from protoplasts derived from a commercial variety of potato. He built up an active team and was starting to attack the immensely difficult problem of regenerating plants from cereal protoplasts. He was an absolutely dedicated scientist and his love of, and his enthusiasm for his subject was infectious; during his short time here he initiated many collaborative projects. His tragic and premature death robs his field of a major international inspiration and influence, and his colleagues of a generous co-worker and a warm-hearted friend.

During the year J. M. Field, M. B. Bahramian, D. Parkin and Jacqueline Wallace left and G. Ooms (from the University of Leiden), Angela Karp (from University College of Wales, Aberystwyth) Jacqueline Pywell, and Eileen Ward joined the staff.

Publications

Thesis

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