ROTHAMSTED RESEARCH

Report for 1982 - Part 1

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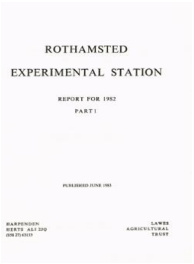

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Introduction

Following the retirement of C. P. Whittingham, the plant metabolism group within the Botany Department merged with the Biochemistry Department on 1 October. This has enlarged the department and as a result we have reorganized our activities by defining a number of research areas. These areas are not seen as *separate* and *discrete* but rather as a number of overlapping spheres of interest best described in terms of a multi-dimensional Venn diagram. Each area has a number of staff associated with it and a group coordinator—these are identified below. The nature of the research carried out in

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the department has changed to some degree, both as a result of the natural development of the original programme and as a result of the merger. Hopefully the result of the new combination will be synergistic rather than just additive. Overall our commitment remains much the same: to define and describe biochemical pathways of importance to the agronomic attributes of plants, to understand how these are regulated and to devise ways of modifying those aspects that appear potentially capable of improvement. The work done contributes to two of the ARC's defined priority areas 'Genetic Manipulation of Crop Plants' and 'Photosynthesis'; our research also has an important bearing on another area currently under much discussion—that of food biopolymers. The general programme of each area is described below and progress reports of selected aspects of the work are presented.

These changes in the organization of the research are accompanied by changes in location. The Biochemistry Department will eventually move out of the Ogg Building where it has been since 1965 and move into the Bawden Building; the Department also will continue to use the new laboratories built in 1980, now named the Emrys Thomas laboratories. A further result of reorganization has been a merging of the Biochemistry and Botany greenhouses into what will become an inter-divisional facility.

At present some work on plant growth regulators is included in the Department's programme and this is described in a final section of this report.

Area 1: Ribulose bisphosphate carboxylase

Work in this area falls within the ARC's priority programme on 'Photosynthesis'. Our role in this programme is the study of the enzymology of the carboxylation and oxygenation of ribulose bisphosphate (RuBP). RuBP carboxylase catalyses both of these reactions; the relative rates of catalysis of these competing processes is thought to determine the relative rates of carbon dioxide fixation and photorespiration. We are therefore trying to understand the mechanism of action of this enzyme and studies involve: improving the specific activity and purity of the isolated enzyme; determining the mechanism of catalysis of the oxygenase and carboxylase reactions using spectroscopic and stopped flow techniques; studying the three-dimensional structure of the enzyme using electron microscopy, crystallographic and computer modelling approaches; attempting to identify plants having RuBP carboxylases with different catalytic properties in order to define the characteristics (particularly affinity for O_2) that are important in determining the differences; and finally separating the subunits of the enzyme and subsequently reconstituting the enzyme. We are also interested in how our work on the isolated enzyme might interact with work at the PBI in which the gene for the large subunit has been isolated and used to direct the synthesis of the protein in *Escherichia coli* minicells. Hopefully, in time it will be possible to devise a system for *in vitro* mutagenesis of the RuBP genes and subsequent testing of the mutated enzyme's characteristics after its production in *E. coli*.

Staff. Area coordinator: A. J. Keys; Research staff: Bird, Boyle, Burton, Cornelius, Gompertz, Gutteridge, Hall, Holbrook, Keys, Parry, Schmidt.

Variation with genotype. Variations in affinity for CO_2 among RuBP carboxylases from different species were established but differences in the relative activities of the enzyme as carboxylase and oxygenase were not so evident (Paper 30). Further measurements of the kinetic properties of RuBP carboxylase from bracken (*Pteris aquilina*) did not confirm an indication that this enzyme might have a relatively lower oxygenase activity than the enzymes from other species studied.

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RuBP carboxylases were partially purified from different species of *Triticum* on each of three occasions and triplicate measurements were made of the velocity of carboxylation (v_c) and velocity of oxygenation (v_0) in the same reaction mixture. Addition of carbonic anhydrase to each reaction mixture was essential because of the large amount of enzyme used and the consequent rapid removal of CO_2 from solution. Significant variations were found in v_c and v_0 between species and occasions. However the mean values of v_c/v_0 for enzymes from *T. urartu*, *T. monococcum*, *T. dicoccum T. dicoccoides*, *T. aestivum* (cv. Maris Dove) and *T. aestivum* (cv. Huntsman) were not significantly different in an analysis of variance.

Effect of temperature and metabolites on v_c and v_o. It has not been clear whether it is the kinetic properties of RuBP carboxylase, or the relative solubilities of O_2 and CO_2, that are the more important in explaining the increase with temperature of photorespiration, relative to photosynthesis, in the leaves of C_3 plants. Rates of carboxylation and oxygenation, catalysed by wheat RuBP carboxylase (*T. aestivum*), were measured at temperatures of 5 to 35°C either in the presence of 14 μM $^{14}CO_2$ and 340 μM O_2 or in reaction mixtures that had been equilibrated at the temperature of measurement, with different gas mixtures (either 300 vpm CO_2 in 21% oxygen or 210 vpm CO_2 in 21% oxygen). With the constant concentrations of CO_2 and oxygen, the ratio v_c/v_0 (4·8) was independent of temperature between 5 and 25°C and fell to only 3·6 at 35°C. Where the solubility of O_2 and CO_2 at the various temperatures determined the substrate concentrations, the ratio fell dramatically with increased temperature. For reaction mixtures equilibrated with 210 vpm CO_2 in 21% oxygen in the gas phase the ratio fell from 4 to 1·8 between 10 and 35°C. This fall is sufficient to explain the relative increase in the photorespiration rate of intact leaves with increasing temperature. It is concluded that the change in the ratio of solubilities of O_2 and CO_2, and not changes in kinetic parameters of RuBP carboxylase, is mainly responsible for the changes, with temperature, in the rate of photorespiration relative to photosynthesis in the leaves of C_3 plants.

No metabolite, from a total of more than 50 (intermediates of photosynthetic, photorespiratory, nitrogen and oxygen metabolism), significantly altered the relative activity of carboxylase and oxygenase. An apparent effect of hydrogen peroxide on the ratio v_c/v_0 resulted from the presence of a trace of catalase-like activity in the purified RuBP carboxylase. The following compounds at 1 mM (in the presence of 6 mM Pi. 16·6 μM $^{14}CO_2$ and 265 μM O_2) inhibited both carboxylase and oxygenase activities; fructose 1,6-bisphosphate, sedoheptulose 1,7-bisphosphate, hydrogen peroxide, 2,3-bisphosphoglyceric acid, inorganic pyrophosphate, phosphoenolpyruvate, oxaloacetate, 2-oxoglutarate and ammonium ion.

Purification, structure and properties. The mode of action of certain effectors of RuBP carboxylase is to stabilize the ternary complex between the protein and the essential cofactors, CO_2 and Mg^{2+}. Additions of such effectors, together with the cofactors, during purification of the wheat enzyme did not consistently increase the specific activity of the product but did change the requirements for activation by CO_2 and Mg^{2+} in a manner that depended on the effector used. Enzyme prepared in the presence of fructose bisphosphate was activated by CO_2 and Mg^{2+} four times faster at 40°C than enzyme prepared without an effector. Furthermore, there was a significant decrease in the subsequent rate of dissociation of the CO_2 and Mg^{2+} from the enzyme. Preparations made in the presence of pyrophosphate were activated, almost instantaneously, at temperatures from 25 to 40°C without any apparent modification of the subsequent rates of dissociation of the cofactors. Especially with pyrophosphate, the purified product is maintained in a conformation, like the native enzyme, that reacts rapidly with CO_2 and Mg^{2+}.

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The effectors mentioned above, and other substances such as inorganic oxyanions that affect the formation of the catalytically active complex of carboxylase with Mg^{2+} and CO_2, are invariably inhibitors of both the carboxylase and oxygenase activities. Phosphate, sulphate and sulphite affect the reaction between the enzyme protein and its cofactors as well as substrate turnover. However, sulphite inhibits catalysis differently from sulphate and phosphate in that its potency increases with enzyme turnover. The time course of carboxylation and oxygenation in the presence of sulphite is biphasic with the second phase being slower and linear. It appears that sulphite modifies an amino acid residue essential for catalytic activity but can do this only during the progress of the reaction. A thiol group on the protein is probably involved but the formation of an inhibitory derivative of RuBP cannot be ruled out completely. Both the oxygenase and carboxylase reactions are similarly affected by sulphite. This provides further support for the view that a single active site is involved in catalysis of both reactions. Further studies on the effects of oxyanions on RuBP carboxylase are in progress. Phosphate, especially, binds at a site close enough to the active site to affect both activation and activity.

Area 2: Photosynthetic carbon and nitrogen metabolism

Previous collaborative work between the two departments had demonstrated essential links between fluxes of carbon and nitrogen in photorespiration and we proposed the operation of a photorespiratory nitrogen cycle (see *Rothamsted Report for 1980*, Part 1, 22). The importance of this cycle *in vivo* was subsequently confirmed by the selection of mutants in *Arabidopsis* which lacked ferredoxin-dependent glutamate synthase, one of the enzymes of the cycle. These mutants died under photorespiratory conditions, probably due to a build up of ammonia (Somerville & Ogren, *Nature* (1980), **286,** 257–259). There are also a number of observations in the literature which suggest that the form of nitrogen nutrition that a plant receives (nitrate or reduced nitrogen) affects the compensation point and thus, by implication, the amount of photorespiration. This research area aims to investigate these links between photosynthesis, carbon and nitrogen metabolism in more depth, particularly by isolating a number of mutants in barley which are unable to grow under normal atmospheric conditions but survive with enhanced CO_2. Such plants have been shown to have biochemical lesions in the photorespiratory pathway and thus offer a means of identifying key steps in photorespiratory metabolism. The Biochemistry Department has long investigated the relationship between photosynthesis and the formation of amino acids in chloroplasts. We have concentrated on two major areas, ammonia assimilation and the synthesis of the aspartate family of amino acids. In both, a major part of the metabolism occurs in the chloroplast at the expense of light energy. The interaction of carbon and nitrogen metabolism has also been probed by developing and using inhibitors of the enzymes involved in the assimilation of ammonia into organic molecules. Some of these compounds, which block glutamine synthetase, also cause ammonia to build up which results in plant death in much the same way as occurs in plants lacking ferredoxin-dependent glutamate synthase.

Staff. Area coordinator: P. J. Lea; Research staff: Festenstein, Hall, Hill, Kendall, Keys, Lea, Mazelis, Turner, Walker, Wallsgrove.

Effect of environment and nitrate supply on photosynthesis and growth of wheat. This work was carried out in the Botany Department in conjunction with Driscoll, Lawlor, Mitchell and Young (now all in Physiology and Environmental Physics Section).

The study of the effects of nitrate nutrition and temperature on photosynthetic metabolism of wheat (var. Kolibri) leaves (*Rothamsted Report for 1981*, Part 1, 56–58)

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was extended to include two light intensities (350 and 650 μmol quanta of 400–700 nm m^{-2} s^{-1}). Nitrate concentration was greatest in leaves of plants grown with added nitrate and in warm conditions; it decreased as leaves aged, particularly in the absence of added nitrate and in the cold. Nitrate reductase activity was greatest in leaves from plants grown in the cold with added nitrogen but also decreased with leaf age. Total nitrogen as a fraction of dry matter was greatest in cold conditions with added nitrate and smallest in the cold without nitrate. Thus nitrate content is not directly related to nitrate reductase activity or to total nitrogen. Total dry matter and leaf area per plant were greater in warm conditions than in cold and with added nitrate. Nitrate particularly stimulated growth of tillers. Soluble protein per unit area of leaf was increased by cold and nitrate but decreased more rapidly with age in nitrate deficient plants.

RuBP carboxylase protein and activity were increased by high light and nitrogen. Specific activity of the extracted carboxylase protein was not affected by the environment in which the plants were grown. Net photosynthesis per unit area of detached leaves was greater in leaves with more carboxylase but not in proportion to amount of carboxylase present. Net photosynthetic rates of attached leaves were greater in the plants grown at 350 than at 650 μmol quanta m^{-2} s^{-1}. Stomatal resistance was greatest in leaves grown in the cold. Preliminary analyses suggest that net photosynthesis was similar in all treatments at equivalent internal CO_2 concentration and light intensities. This is consistent with previous observations (*Rothamsted Report for 1981*, Part 1, 57) that quantum yield and photorespiration rates were unaffected by growth conditions. Increased chlorophyll and decreased chlorophyll a/b ratio suggested that light harvesting would be superior in the cold grown plants with added nitrogen but this did not result in greater CO_2 assimilation. Photosystem I (PSI) activity was greater in chloroplasts from cold grown plants than warm, and slightly greater for plants grown at the lower light intensity. PSI+II was not altered by the conditions of growth. Oxygen evolution by leaf pieces, measured polarographically, was faster for leaves grown in cold than in warm conditions and faster for leaves from bright than from dim light. Fluorescence emission from PSII at 685 nm, measured by Drs M. Bradbury and N. R. Baker (University of Essex), was unaffected by treatments although variability was large.

Thus, although differences in nitrate supply, temperature and light conditions during growth altered the amounts of chlorophyll and possibly thylakoid proteins, they did not change the intrinsic photoresponses or efficiency of light harvesting. Neither did conditions of growth greatly affect the processes of CO_2 assimilation and photorespiration. Nitrate supply increased chlorophyll and protein but without large effects on the physiology of the intact leaf. The main effect of temperature was on the expansion of leaves and the main effect of nitrate was to stimulate the growth of tillers.

Photorespiratory mutants of barley. Mutants of barley have been selected on the basis of their inability to grow satisfactorily under photorespiratory conditions (i.e. normal air) but which recover on transfer to a CO_2-enriched atmosphere (see *Rothamsted Report for 1980*, Part 1, 53). One of these mutants, R(othamsted) Pr 79/4, has been found to be deficient in catalase. This is now thought to contain a recessive mutation which is probably at the structural locus for microbody-located catalase. The effect of this lesion on the fixation and further metabolism of $^{14}CO_2$ was examined. The mutant accumulates ^{14}C in 6-phosphogluconate. The results are consistent with an observation of Brennan and Anderson (*Plant Physiology* (1980) **66,** 815–817) that glucose-6-phosphate dehydrogenase can be activated *in vitro* by H_2O_2 and that the dark activation of this enzyme is inhibited by catalase. It has been suggested (Grodzinski & Butt, *Planta* (1977) **133,** 261–266) that a significant proportion of the CO_2 released in photorespiration originates from the chemical oxidation of glyoxylate by H_2O_2 produced as a result of the action

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of glycollate oxidase. If this were so then the effect should be enhanced in RPr 79/4 which lacks the ability to break down H_2O_2. The metabolism of [^{14}C]-glycollate by leaves of the mutant and of Maris Mink, the wild-type parental cultivar, therefore was investigated. The results showed that there were no significant differences between the two and suggest that the chemical action of H_2O_2 on glyoxylate does not make a significant contribution to CO_2 release during photorespiration.

Inhibition of ammonia assimilation. Glutamine synthetase and glutamate dehydrogenase are the major enzymes responsible for the assimilation of ammonia; the former in higher plants, the latter in fungi and both, depending on genotype and environment, in bacteria. We have been seeking inhibitors of these enzymes for further investigations into ammonia assimilation. A number of derivatives of 5-aminoisophthalic acid have been synthesized that inhibit glutamate dehydrogenase. 5-N-Allylaminoisophthalic acid was the most potent, with a K_I of 0·52 mM, although it had little effect on plant growth. However, inhibitors of glutamine synthetase, such as methionine sulphoximine or phosphinothricin rapidly cause the death of a wide range of plants. Ammonia concentrations in the treated plants increase to a maximum of 16 μmoles g^{-1} fresh weight in *Pisum sativum* whilst the amount of glutamine decreases almost to zero. When supplied at micromolar concentrations, to either nitrate-reducing or nitrogen-fixing blue-green bacteria, they also inhibit glutamine synthetase and ammonia is excreted into the medium. The potential of this system for the biological production of ammonia is being examined by Dr M. G. Guerrero of the University of Seville, Spain.

Localization of the enzymes of the aspartate pathway. In previous annual reports, we reported that many of the key enzymes of lysine and threonine synthesis are located solely in the chloroplast and that this is likely to be the only place in the leaf cell where these two amino acids are made. We have now examined the localization of the enzymes of methionine and S-adenosylmethionine (AdoMet) biosynthesis in the leaves of *Pisum sativum* and *Hordeum vulgare*. Cystathionine synthase is totally located in the chloroplast but only 60 % of cystathionine lyase activity is present in the chloroplast with the remainder of the activity being in the cytoplasm and mitochondria. Methionine synthase and methionine adenosyltransferase are not located in the chloroplasts and the latter enzyme is definitely not associated with any organelle. Thus it appears that homocysteine is synthesized in the chloroplast, but the synthesis of methionine and S-adenosylmethionine occurs in the cytoplasm.

Area 3: Metabolic regulation

Even before the genetic manipulation programme began the Department was interested in selecting for mutants of barley altered in their ability to regulate their synthesis of amino acids. Such mutants, which are usually dominant, are potentially useful in gene transfer studies as well as in providing an understanding of how plant metabolism is regulated. Subsequently other recessive mutations, which would also be specifically useful for monitoring gene transfer, have been selected. Work in this area will aim to determine mechanisms by which the regulation of fluxes is achieved in metabolic pathways with important agronomic attributes, to attempt to modify such controls and also to provide material for studies in cell biology (Area 4). Currently this involves the selection of mutants of barley and *Solanum* species that (a) accumulate threonine and/or lysine and/or methionine; (b) accumulate proline; (c) lack nitrate reductase; (d) lack alcohol dehydrogenase; (e) are resistant to disease toxins or herbicides. Work is also in progress on the expression of the mutant phenotypes in different stages of plant growth or different

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organs as well as in tissue culture and on determining the biochemical and genetical nature of the selected mutants. Certain of the well characterized mutants are being investigated to determine if there is any change in their agronomic attributes such as nutritional quality or disease resistance.

Staff. Area coordinator S. W. J. Bright; Research staff: Arruda, Bright, Creissen, Dunlop, Franklin, Hill, Karp, Kueh, Mayne, Mazelis, Nelson, Norbury, Risiott, Steven, Roberts, K. Smith, Wallsgrove.

Aspartate pathway. The three previously isolated barley mutants resistant to lysine plus threonine (R2501, R3004 and R3202) have continued to provide fundamental information on the regulation of carbon flux through the aspartate pathway. The first enzyme in the pathway by which all the aspartate enters, aspartate kinase, exists in three isoenzymic forms (AKI, AKII, AKIII). The mutants each contain either AKII or AKIII enzymes which are either completely insensitive to lysine or lysine plus AdoMet or retain different degrees of sensitivity. The proportions of the AKII and AKIII isoenzymes also vary. Analysis of isoenzymes and growth in the presence of lysine (8 mM), threonine (8 mM) and arginine (1 mM) allowed the identification of a double mutant line homozygous for the *Lt1b* and *Lt2* mutant genes from R3202 and R3004 respectively. This line is being used to examine the pattern of amino acid accumulation in the presence of two feedback deregulated isoenzymes.

R3004 and R2501 plants accumulate threonine in the soluble fraction of the seed. In both cases the soluble threonine is increased from less than 1% to more than 10% of the total seed threonine without major changes in other components of the soluble pool. R3004 plants grown in the field maintain this characteristic. It seems therefore that all the excess aspartic acid entering the pathway is channelled into threonine rather than the two other end products lysine and methionine. A plausible rationale for this observation comes from our knowledge of the regulatory properties of other enzymes in the pathway in barley. The first branch point in the pathway regulates the partitioning of aspartic acid semialdehyde between the synthesis of lysine and that of methionine and threonine. The first enzyme unique to the lysine pathway, dihydrodipicolinic acid synthase is stringently inhibited by lysine with an $[I_{0.5}]$ value for the barley leaf enzyme of 10–20 μM. In contrast there is a significant fraction of the other branch enzyme, homoserine dehydrogenase, insensitive to feedback inhibition by threonine. Threonine synthase is activated by AdoMet (Aarnes, *Planta* (1978) **140,** 185–192) thus regulating the portioning at the second branchpoint of homoserine phosphate between methionine and threonine synthesis. These mutants have thus allowed the integration of information from studies of isolated enzymes and the whole plant. If our explanation is correct then lysine accumulation should only be possible in a plant with deregulated mutant forms of both aspartate kinase and dihydrodipicolinate synthase. We are looking for such mutants by screening M_2 embryos derived from remutagenized R3004 for resistance to the inhibitory effects of the lysine analogue aminoethylcysteine. From 20 000 embryos screened, a number of mutant lines with heritable resistance have been identified.

Proline accumulation. Further genetical analysis of three hydroxyproline-resistant barley mutants (R5201, R6102, R6902) has showed them all to be allelic. A fourth mutant R6901 has a different phenotype with particularly good root growth in the presence of hydroxyproline. R5201 has increased free proline in both leaf and root tissue whereas R6901 has an increase only in the roots. Externally fed proline allowed a significant improvement of the growth of young aseptically grown barley plants in the presence of 150 mM NaCl. The increased pools of free proline in the mutants were, however, too

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small to make more than a marginal improvement in their growth in the same conditions. Plants of R5201 and Maris Mink were grown to maturity in the glasshouse under a trickle irrigation system with 0, 75, 125, 250 mM NaCl added to the basic medium. The higher leaf content of free proline in the mutant was apparent in all treatments at all times. However, the increased proline was, once again, too small to make any significant difference to growth or yield in the presence of salt. Proline biosynthesis was examined by feeding radioactively labelled glutamic acid to young leaves of R5201 and its parent cv. Maris Mink. In spite of the increased free pool of proline in the mutant, labelled proline accumulated at least as fast in the mutant as the parent. Externally fed proline decreased the incorporation of radioactive label into proline in both mutant and parent leaves, but to a much greater extent in the parent. These results suggest that there is, in the mutant, a regulatory enzyme in proline biosynthesis with decreased sensitivity to feedback inhibition by proline. Unfortunately, the most likely enzyme candidate for regulation, glutamate kinase, is extremely difficult to assay.

Nitrate reductase. Chlorate kills plants because it is reduced to toxic chlorite by the enzyme nitrate reductase. In order to select plants lacking nitrate reductase we have screened mutated M_2 barley seed for chlorate resistance and recovered three mutants from which F_1 seeds have been obtained after crossing with wild type plants. The enzyme nitrate reductase consists of an apoprotein and a low molecular weight molybdenum-containing cofactor. The cofactor is also required for the activity of the enzyme xanthine dehydrogenase. One of these mutants, R9401, has been characterized (with Dr Wray, St Andrews University) as containing a monogenic, recessive mutation affecting synthesis of the molybdenum containing cofactor (*cnx*-type). The mutant seedlings still contain the apoprotein as measured by nitrate-inducible cytochrome c reductase, a cofactor-independent partial activity of nitrate reductase. These seedlings have very low (<1%) of zero nitrate reductase activity and will not grow with nitrate as sole nitrogen source.

Thirty-six mutants of *Physcomitrella patens* have been isolated by their inability to grow on nitrate (with Professor Cove, Leeds University). After further characterization, 19 strains were shown to be totally unable to utilize nitrate as a nitrogen source. Fifteen strains were identified as putative *cnx* mutants and in at least two of the strains growth could be restored by high levels of molybdenum. Four strains had normal levels of nitrate and nitrite reductase, but two of these were identified as possible nitrate uptake mutants.

Cultured cereal caryopses. One of the problems in studying the metabolism of the cereal endosperm has been that, hitherto, it would only develop when attached to the plant either intact or in detached tiller culture. Culture of isolated barley caryopses has now been achieved and the technique should allow grain development to be studied uninfluenced by the parent plant. Single caryopses (fresh wt 20–30 mg; dry wt 4–7 mg) separated from glasshouse-grown plants 8–12 d after anthesis, were cultured for periods of up to 20 d in 0·8 ml of defined sterile liquid medium. Increases in fresh weight, dry weight, storage protein and starch were comparable to that *in vivo*. The effects of sucrose concentration, pH and sources of nitrogen and sulphur were examined in the standard medium. The relative proportions of the B and C storage hordeins were different to that synthesized *in vivo*, nevertheless this system should provide a valuable tool to examine regulation of hordein synthesis in the developing cereal seed.

Area 4: Plant cell biology

Many of the potential techniques for transferring genetic information between plants, other than by conventional sexual crossing, involve the use of plant protoplasts. Similarly,

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introduction of novel genetic material may also be via protoplasts. For the practical application of such techniques it is necessary to be able to regenerate whole plants from protoplasts for our major crop species. This is one of the major aims of the programme. A second aim is to develop appropriate mechanisms for gene transfer at various levels of complexity. In particular the work is concerned with the isolation and culture of protoplasts of wheat, barley, potato and rape; with morphogenesis and plant regeneration from protoplast-derived colonies and cultured plant parts; with describing the variation present in the regenerated plants (so-called 'somaclonal' variation) and attempting to understand and control it; with the transfer of single and polygenic traits via protoplast fusion; with gene transfer using *Agrobacterium* as natural vector; and with studies on the integration of transferred genes.

Staff. Area coordinator: M. G. K. Jones; Research staff: Boccon-Gibod, Bright, Creissen, Foulger, Jarrett, Jones, Karp, Lancaster, Maddock, Nelson, Ooms, Risiott, Roberts, Wilcox.

Wheat and barley. The cereals in general, and wheat and barley in particular, do not respond well in tissue culture. It has previously been reported that wheat plants can be regenerated consistently from embryogenic and shoot-forming cultures of immature embryos and inflorescences (see *Rothamsted Report for 1981*, Part 1, 45). This has been repeated routinely in experiments throughout the past year, and genotypic differences in morphogenetic capacity have been confirmed.

Embryogenic and shoot-forming tissues, as well as individual immature embryos, have been used to initiate a large number of suspension cultures. The aim is to establish morphogenetic suspensions capable of regenerating whole plants, and to use this material as a source of protoplasts. A number of distinct lines have been obtained, including one finely-divided and rapidly-growing line (from cv. Maris Butler) which shows some morphogenetic capacity. Cell aggregates $< 500\ \mu m$ in diameter will grow readily on a wide range of agar media. Localized areas of chlorophyll production in the resulting callus and, more rarely, leafy structures have been formed. A single plantlet with both leaves and roots has been obtained, but this did not survive to be transplanted into soil.

Phenotypic variation has been observed amongst wheat plants regenerated through tissue culture (*Rothamsted Report for 1980*, Part 1, 42). To attempt to understand and possibly control this 'somaclonal' variation, cytogenetic studies of regenerated plants and phenotypic assessments of their progeny (in collaboration with the Plant Breeding Institute, Cambridge and Nickerson R.P.B. Ltd, Rothwell) have been undertaken.

The cytological analysis has been made of regenerants in two wheat cultivars—Highbury and Copain. There is evidence of changes in chromosome number and structure in both cultivars. In Highbury, 20 out of 60 plants studied were aneuploids (wheat is normally a hexaploid with 42 chromosomes). Meiotic analysis showed that 8% of the regenerants had interchanges and 12% showed chromosome breakage. In Copain, 19 out of 48 plants were aneuploids with chromosome numbers in a slightly wider range than for Highbury. Meiotic data are not yet available but a structural chromosome change (probably an interchange) has been identified in root-tip preparations.

The preliminary phenotypic assessment indicates that morphologically normal lines are obtained from the majority of regenerants, but also that morphological differences (height, awn length, fertility) are transmitted to the progeny of a proportion of wheat regenerants. In contrast, no variation was observed amongst the progeny of a much smaller number of diploid barley regenerants. The significance of this variability may depend on whether it can be manipulated, either to generate desired variation, or to reduce it when uniformity through the tissue culture stage is required.

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Potato. Plants have been regenerated from isolated mesophyll protoplasts of nine UK potato cultivars and from the diploid wild species *Solanum brevidens*. Refinements to the isolation and culture techniques have led to improvements in protoplast yield and in the efficiency of plant regeneration. Plants have also been regenerated from cultured explants of leaf, rachis, petiole and tuber of 11 cultivars. Protoplast-derived plants of cv. Maris Bard and plants regenerated from cultured explants of cv. Désirée were grown for one or two tuber generations in pots before being planted in the field in N. Ireland (in cooperative experiments with Dr N. Evans, DANI). Thirty-three vegetative or tuber characteristics were measured or calculated and the data from 23 of these were subjected to statistical analysis. Variation in the tissue culture-derived plants was apparent in numerous characters and exceeded that observed in the control plots of the parent cultivars.

The protoplast-derived plants of the tetraploid (2n=4x=48) cultivars Maris Bard and Fortyfold were also analysed cytologically and extensive chromosome variation was found. The majority of Maris Bard regenerants were aneuploid with chromosome numbers ranging from 46–93 and most of the plants had very high chromosome numbers (88–93). In Fortyfold, 30% of the regenerants were normal and the variants were in a more limited aneuploid range (46–49). Structural chromosome changes were also found. The chromosomal variation correlated with the morphological variation observed in regenerated plants. Chromosomes have also been studied in plants regenerated from cultured excised leaf pieces. In the two tetraploid cultivars studied so far the majority of plants were normal (19/20 plants 2n=4x=48).

Plants regenerated from protoplasts of the diploid wild species *S. brevidens* (2n=2x=24) have also been examined cytologically. Approximately 50% of the plants were normal and, of the remainder, most were tetraploid (2n=4x=48) although a few aneuploids were found (2n=25, 46, 47). We have preliminary evidence that the nature of the chromosome variation is influenced by factors such as the culture procedure and the ploidy of the source material.

Experiments to fuse various protoplast combinations from crop plants, including potatoes, have been started. Culture procedures now enable plants to be regenerated from protoplasts of most potato cultivars, but the efficiency needs to be improved for full use of the system for genetic manipulation and fusion studies. There are strong indications that further improvement can be made.

Transformation by *Agrobacterium*. Various methods have been explored to transform plants by the introduction of a limited number of foreign genes. Of the crop plants, potato appears to be particularly amenable to infection by various *Agrobacterium tumefaciens* strains, although prolonged incubation periods of up to 6 months are required to obtain transformed shoots. All the transformed shoots recovered so far lack roots, but grafting the shoots on to stems of normal young potato plants allows the transformed shoots to continue their growth. Some of these grafted shoots spontaneously develop aerial stolons, and under 12 h day length these form tubers. Very little morphological or cytogenetical variability has been observed among untransformed plants recovered from induced crown gall on stems of tetraploid potato cultivars. Crown galls induced on young rape plants form shoot-like structures, but so far no obvious meristems have been observed. Crown galls can also be induced on young bean and pea plants from excised cultured embryos, but no differentiation has been observed from such galls irrespective of the *Agrobacterium tumefaciens* strain used to induce the galls. *Agrobacterium rhizogenes* efficiently induces root formation on most dicotyledonous crop plants tested. Attempts to regenerate plants from such root tissues are in progress. Preliminary studies on the growth characteristics of potato crown gall tissue indicate that an improvement in

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culture technique for potato protoplasts is required for routine experiments aimed at recovering transformed plants by co-cultivation with *Agrobacterium* or by incubation with DNA.

Area 5: Disease resistance

One of the objectives of crop improvement, either by conventional or novel means, is the incorporation of disease resistance traits into crop cultivars. However, little is known at the molecular level of how plants resist disease and there is scarcely any information on the way in which disease resistance genes work. Together with workers in the Plant Pathology department, we have had a long-term interest in the phenomenon of induced resistance to virus infection in tobacco (*Nicotiana tabacum*) and the relationship of the pathogenesis-related (PR) proteins to this process. We have obtained considerable biochemical information on these proteins and have now extended this to isolate the mRNA for them; the aim now is to produce complementary DNA which will be inserted into a plasmid and this cloned in *Escherichia coli*. By this means we should eventually be able to isolate the genes for these proteins. Our studies of disease resistance have now been extended to encompass another project in which the aim is to attempt to identify the molecular nature of the genes for resistance to potato virus Y (and perhaps potato virus X) that are present in certain potato cultivars. Currently we are testing the feasibility of different approaches of recognizing the resistance-gene product at the RNA and protein level. This research area is one that extends across departmental boundaries to include members of the Plant Pathology Department and part of the relevant work is reported in the Plant Pathology Department report (see pp. 190–191).

Staff. Area coordinator: B. J. Miflin; Research staff: Antoniw, Burrell, Carr, Ooms, Pierpoint, Strowman, and Carpenter and White (Plant Pathology Department).

PR-proteins in transformed tobacco. *Nicotiana tabacum* cv. White Burley transformed by infection with *Agrobacterium tumefaciens* produces a callus tissue that grows without the addition of plant growth substances. Polyacrylamide gel electrophoresis (PAGE) of extracts of the callus showed a pattern of protein-bands characteristic of the PR-proteins produced in White Burley on infection with the Ni118 strain of TMV or treatment with aspirin (*Rothamsted Report for 1979*, Part 1, 29). A similar pattern of protein bands was found on PAGE of extracts of transformed Petit Havana tobacco. In contrast, no PR-proteins were detected in shoots derived from the seed of transformed Petit Havana tobacco, which had been grafted on to normal plants (after transformation these plants do not develop a normal root system (Wullems, Molendijk, Ooms & Schilperoort *Cell* (1981) **24,** 719–727)). The results show that PR-proteins are formed in crown gall callus not as a direct consequence of the insertion of T-DNA into the plant genome, but as a result of the physiological state of the tissue, which is associated with high levels of certain plant growth substances which are already known to induce PR-proteins in tobacco (*Rothamsted Report for 1980*, Part 1, 180).

Other PR-proteins in virus-infected leaves. Besides the four PR-proteins which are obvious in electrophoretic extracts of TMV-infected leaves of tobacco (Xanthi-nc) and which have been partially characterized (PR-1a, b, c and 2), there are at least five more (A–E; R_m 0·55, 0·46, 0·41, 0·36 and 0·25) which are not present in extracts of uninfected leaves. These resemble PR-1a, b, c and 2 in that they are relatively resistant to proteolytic enzymes and are more obvious after extracts have been incubated with trypsin and chymotrypsin. However, their chemical unrelatedness has been emphasized by their behaviour during electrophoresis in a variety of conditions, and during chromatofocusing.

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Suggestions that they include a cellulose or chitinase have not been confirmed. Cellulases and exochitinases do not increase upon virus-infection, and although an endochitinase does increase 3–7 fold in these circumstances, its electrophoretic mobility is unlike any of the PR proteins. Although not likely to be chitinases, three of the proteins are absorbed from solution on to chitin, suggesting that they may be N-acetylglucosamine-specific lectins. It is not yet clear if A–E include any of the protease-inhibitors that are induced by damaging leaves, but some of the components of cell walls that are thought to be responsible for the induction of the protease-inhibitors, induce the formation of PR-proteins in detached leaves.

Area 6: Cereal seed proteins

The Department has had a long term interest in the chemical and physical characterization of cereal seed storage proteins, in the biology of their deposition and in their genetic and evolutionary relationships. This work has continued and has been extended by using recombinant DNA technology. There is thus considerable overlap between this area and the subsequent one on gene isolation. The current aims of our programme are to compare, in all of the above aspects, the prolamin storage proteins of wheat, barley and rye and to investigate the nature of other storage proteins such as the globulins of oats. We are also studying the effect of the relative supplies of nitrogen and sulphur on the amounts of the different storage proteins synthesized. Part of this concern is related to the effect of S on baking quality and we are continuing to investigate the relationship between the chemical and physical properties of the seed proteins, particularly of wheat, and their use in food technology. The storage proteins are deposited in protein bodies within the seed and we are seeking to understand the mechanisms involved for both prolamin and globulin storage proteins.

Staff. Area coordinator: P. R. Shewry; Research staff: Bunce, Byers, Burgess, Faulks, Festenstein, Franklin, Karp, Miflin, Parmar, Shewry, S. J. Smith, Tatham; some of the research reported below was also done in conjunction with staff in area 7.

The effect of applied N and S on the yield and composition of cereal grain protein. It has been shown with many cereals that sufficient S must be available to the plant if it is to obtain maximum benefit from applied N. If there is insufficient S relative to the N supply, yields of grain and of grain protein fall and the S-amino acid content of the grain protein decreases. With wheat, this last observation related to protein quality is particularly important because it is the only cereal grain (with the exception of a little rye) used for making bread. No single property determines the bread-making quality of wheat, but disulphide bonding capacity is regarded as an essential requirement for dough formation. Hence the importance of having grain protein with an adequate cysteine content.

Grain N and S status of British wheat. The interaction of available N and S on the quality of wheat grain is of practical importance in wheat-growing countries where the soil can be S-deficient, e.g. Australia and part of mid-West USA. S-deficiency is not a characteristic of British soils, but, because of the increasing use of S-free fertilizers, it was thought necessary to carry out a survey on commercially produced wheat grain in order to ascertain the current range of variability of N and S contents. One hundred and seventy two grain samples of known N content from the 1981 Home Grown Cereal Authority (HGCA) wheat collection were analysed for S and their N:S ratios determined. Only six samples had a S content of $< 0{\cdot}12$ (as percentage of dry matter), all the others falling within the range 0·12–0·18%. A low S content alone is not sufficient evidence of S deficiency,

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N:S ratios are considered to be a more reliable guide. In the vegetative tissues of wheat it is considered that enough S is being supplied for protein synthesis if the total N:total S and protein N:protein S ratios are $\leqslant$17:1 and 15:1 respectively. The corresponding values for wheat grain are less certain although a total N:total S ratio of 17:1 has been proposed (Randall, Spencer & Freney *Australian Journal of Agricultural Research* (1981) **32,** 203–212). On this basis none of the HGCA samples can be considered as S-deficient. However, the average N:S ratio of 20 samples from one area was 14:1 (range 12·3–16·1:1) which was considerably higher than from the other sites. These samples also had the highest average protein content (12·0, ranging from 9·5–15·9 as percentage of DM), indicating heavy N fertilization. While there is sufficient S currently available to balance the N supplied, it is suggested that this area should be monitored in future. It is proposed to analyse the 1982 HGCA wheat grain samples similarly.

Effects of S supply on the grain proteins of barley. A study was made of the effects of sulphur starvation on the grain of two cultivars of barley (Athos and Sundance). The low S grain were smaller than the normal grain and had individual dry weights of about 23–25 mg compared with 44 mg. They contained a similar amount of N g^{-1} dry wt (about 26–27 mg) but less S (0·8 to 1 mg compared with 1·5 to 1·8 mg), and a smaller proportion of cysteine and methionine. The proportion of the total grain-N present in the hordein (storage protein) fraction was reduced from about 50 to 27%. This fraction also contained less cysteine and methionine and electrophoretic analysis showed decreases in the amounts of the S-rich 'B' and 'D' hordein polypeptides. The salt-soluble protein fraction also contained less cysteine and methionine and electrophoresis showed that some components were either reduced in amount or absent. There was little effect on the amino acid or polypeptide compositions of the glutelin fraction, but non-protein nitrogenous components were greatly increased (from about 7 to 30% of the total N).

A more detailed study was made of the accumulation and synthesis of hordein in the developing grain of cv. Sundance. Electrophoretic analyses of hordein fractions from early (15 days after anthesis), late (30 days) and mature developmental stages showed that 'C' hordein accounted for over 90% of the total fraction in the early S-deficient grain compared to 40% in the normal grain. This proportion then declined to about 80% in the late and mature S-deficient grain and 25–30% in the normal sample. *In vitro* translations of polysomes and polysomal poly A^+ RNA from the early and late developmental stages, and of total RNA from the early stage only showed increased proportions of 'C' hordein in the translation products of the RNA fractions from the S-deficient grain. This is consistent with the differences in the pattern of hordein accumulation.

The relative amounts of mRNAs for 'B' and 'C' hordein polypeptides in the S-deficient and normal grain were compared by immobilizing the RNA on nitrocellulose filters and then hybridizing it to ^{32}P-labelled cDNA sequences known to be related to 'B' and 'C' hordein mRNAs (see area 7). The results indicated that the total concentrations of mRNAs for 'C' hordein were higher in the polysomal RNA and polysomal poly A^+ RNA fractions from the S-starved grain than in the same fractions from the normal grain. It was concluded that there was a close relationship between the proportions of hordein polypeptides accumulated and the populations of mRNAs coding for them.

Immunological relationships of cereal prolamins. It has been shown that there are common antigenic determinants for 'A' and 'B' hordeins and also for 'B' and 'C' hordeins. The reactions of different hordeins with antisera to 'A' hordein and to 'C' hordein have been quantified in nephelometric tests and the study extended to other prolamins and prolamin fractions. Antiserum to 'C' hordein did not react with total prolamins from maize, millet, sorghum or rice, but reacted with prolamins of wheat (gliadins) and rye

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(secalins). Wheat ω-gliadins reacted strongly, as expected from their homology in amino acid sequence with 'C' hordeins. There was also reaction with γ_3-gliadin, 75K γ-secalin and, to a lesser extent, 40K γ-secalin of rye. These fractions are themselves related in their N-terminal amino acid sequences and the reaction suggests some structural relationship with 'C' hordein; α(A)-gliadin and β-gliadin were much less reactive.

An antiserum to high molecular weight (HMW) subunit 2 of wheat (cv. Highbury) also reacted strongly with ω-gliadins and to a lesser extent with α-, β- and γ_3-gliadins. The antiserum also reacted with HMW subunits from cultivars Cheyenne and Maris Butler, but all the HMW subunits gave less reaction than most of the other gliadins. There was no reaction with 'C' hordein. These studies are of value in indicating structural relationships between different prolamin fractions.

Chromosomal location of the structural genes for 'D' hordein. Lawrence and Shepherd (*Theoretical and Applied Genetics* (1980) **59,** 25–31) showed that the HMW prolamins are coded for by structural genes on the long arms of the homologous chromosomes of group 1 in wheat, 1R in rye and 5 in barley. We have analysed F_2 and F_3 grain of crosses between barley cultivars with marker genes on the long and short arms of chromosome 5. These showed that 'D' hordein is controlled by a single locus (called *Hor 3*) located about 9 cM from the centromere on the long arm of chromosome 5. It is closely linked to *nec 1*, a locus which determines necrotic spotting on the young leaves (8·0 ± 1·5 cM), and more loosely to *wst 5* (white leaf stripes) (37·4 ± 4·7 cM), *Hor 1* ('C' hordein) (64·8 ± 12·7 cM) and 'B' (black lemma) (65·9 ± 10·0 cM). This location is analogous to that of the loci (designated *Glu 1*) coding for HMW subunits on the long arms of chromosomes 1A, 1B and 1D of wheat. These are about 9 cM from the centromere and 66 cM from *Gli 1*, which codes for ω-gliadins (homologous to 'C' hordein) and γ-gliadins (unknown hordein relationship) (Payne, Holt, Worland & Law *Theoretical and Applied Genetics* (1982) **63,** 120–138).

Purification, characterization and synthesis of the globulin storage proteins of oats. In oats the major storage proteins are globulins. These have been purified by column chromatography and sucrose density gradient ultracentrifugation. Three globulins have been identified, with sedimentation coefficients of approximately 3, 7 and 12. The major globulin component is the 12S fraction, and this has been purified by gel filtration chromatography on Sepharose S300 and zonal isoelectric precipitation. Results from SDS-PAGE separations suggest that the 12S globulin consists of pairs of one small and one large subunit, with apparent molecular weights (M_r) between 19 000, 25 000 and 35 000 and 42 000 respectively, and stabilized by disulphide bonds. These correspond to the subunits of the 12·1S globulin described by Peterson (*Plant Physiology* (1978) **62,** 506–9) and have been purified by a combination of preparative polyacrylamide gel electrophoresis and gel filtration chromatography. Isoelectric focusing showed that the large subunit preparation contained about 20 major bands with isoelectric points (pIs) of 5–7 and the small subunit preparation 4 or 5 major bands with pIs of 8–9. The large subunits were richer in glutamate+glutamine and the small subunits in aspartate+asparagine and in lysine. An enriched fraction of the 3S and 7S globulins was obtained by precipitation of the 12S globulin at pH 4·8. SDS-PAGE of the 7S globulin showed that it contained mainly 55 000 polypeptides, with some minor components. The migrations of the major components of the 3S and 7S globulins on SDS-PAGE were similar under reducing and non-reducing conditions. Antisera against the 12S oat globulin have been raised in rabbits, and a purified anti-globulin IgG fraction tested for cross-reactivity against oat prolamin, albumin and globulin. The only positive reaction was against the oat globulin fraction. Polysomes have also been isolated from the membrane

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fraction of developing oat endosperms and fractionated by sucrose density gradient centrifugation. The translation products of these polysomes were analysed by SDS-PAGE under reducing conditions and a range of products were observed with apparent M_rs up to 70 000, the most intense staining bands were around 60 000. When precipitated with anti-globulin IgG, the major band of M_r 58 000 to 60 000 was precipitated, indicating that the oat globulin precursor molecule is approximately the same size as the unreduced dimer. The properties of the 12S oat globulins are very similar to those of the 11S (legumin) proteins found in legume seeds; there are also some common features between the 7S oat globulin and 7S (vicilin) legume seed globulins.

Area 7: Gene isolation and expression

This area is part of the ARC's priority programme on genetic manipulation. Our primary interest was to isolate the genes for the storage proteins of cereals. To this end we have constructed a number of 'libraries' of cloned complementary DNA (cDNA), derived by reverse transcription of mRNA from endosperms, in the plasmids pBR322 and pUC8 grown in *Escherichia coli*. We are currently identifying which of these cDNA clones contain sequences related to various storage proteins and attempting to obtain clones containing inserted sequences equivalent to the full length of the mRNA. Such clones can be used in a number of studies as outlined below. The DNA can also be sequenced and this sequence used to predict the primary amino acid sequence of the corresponding protein; this approach will be particularly valuable in our study of cereal protein biopolymers in relation to food technology (see area 6). The cDNA clones will also be used in identifying the various structural genes present in a genomic library of barley nuclear DNA currently being constructed using phage λ. Besides the prolamin storage proteins we are also interested in identifying cDNA clones related to other important grain proteins such as chymotrypsin inhibitors and β-amylase. A further project in this area is the isolation of the genes for glutamine synthetase. In each instance, besides obtaining information on the structural properties of the proteins, the factors affecting the expression of the genes will also be studied. The isolated full length cDNA and genomic DNA clones of certain of the proteins will also be used for studies on gene transformation in higher plants (see area 4).

Staff. Area coordinator: B. J. Miflin; Research staff: Blanco, Burgess, Cullimore, B. G. Forde, J. Forde, Fry, Kreis, Lara, Miflin, Pywell, Rahman, Saarelainen; some of the research reported was done in conjunction with staff in area 6.

Characterization of cDNA clones for cereal storage proteins. Previous work identified one group of cDNA clones (including clones pHvE-c16 and pHvE-c179), derived from barley endosperm mRNA, as being related to 'B' hordein sequences (see *Rothamsted Report for 1981*, Part 1, 42). This was based on evidence from hybrid-selection translation and sequence information. Further studies have identified clones, including pHvE-c251, which select mRNAs that, upon translation in an *in vitro* wheat germ protein synthesis system, direct the synthesis of 'C' hordeins, and also clones (amongst them pHvE-c155) that select mRNA related to 'D' hordeins. The identity of the 'D'-hordein clone has been further confirmed by using it to identify cDNA clones, derived from wheat endosperm mRNA, which select mRNAs that direct the synthesis of wheat high molecular weight prolamins (these prolamins are homologous in composition to 'D' hordeins). The DNA inserted in the wheat clones pTaE-c256 and pTaE-c237 has been partially sequenced and found to contain sequences that predict a carboxyl terminal sequence for

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the protein that is identical to that determined by carboxypeptidase cleavage of a purified high molecular weight polypeptide.

Hordein gene organization in normal and mutant barley lines. We have used the above clones to investigate further the nature and expression of the hordein loci *Hor 1* (C-hordein), *Hor-2* (B-hordein) and *Hor 3* (D-hordein). Using B-hordein related clones and mRNA from endosperms of cultivars having different alleles at the *Hor 2* locus, we have obtained evidence that the protein polymorphism is due to the presence of a family of mRNAs and not to post-translational modifications. Furthermore, hybrid-selection translation suggests that the B-hordein clones fall into two distinct categories, one hybridizing to mRNAs that direct the synthesis of class I and II polypeptides and the other to mRNAs specifying class III polypeptides; this division held true irrespective of the *Hor-2* allele present in the cultivar from which the mRNA population was obtained. The basis for distinguishing the polypeptides with class I, II and III was cyanogen bromide cleavage mapping and has been reported previously (*Rothamsted Report for 1980*, Part 1, 40). These results are consistent with the *Hor-2* locus being a cluster of related genes comprising a multigene family which can be divided into two sub-families. Further resolution of the locus will depend on more sequence information and the isolation of clones of genomic DNA derived from the locus.

Certain mutant lines of barley accumulate smaller amounts of hordein in the grain which thus have a higher lysine content. We have used our characterized clones to attempt to identify the nature of the mutation. In Risø 56, a γ-ray induced mutant of Carlsberg II, the amount of 'B' hordein is reduced and this is due to the absence of the major class II and III hordein polypeptides present in Carlsberg II (there are no known class I polypeptides in this cultivar). Correlated with the lack of polypeptides, we have been unable to find any evidence for mRNAs for these polypeptides despite using a number of different RNA fractions and techniques. Analysis of the genomic DNA of Carlsberg II and Risø 56, shows that about 100 kilobases of DNA, which in Carlsberg II hybridizes with DNA from 'B'-hordein related clones, is missing from the mutant. We therefore conclude that the high-lysine phenotype of Risø 56 is due to a major structural mutation in the *Hor-2* locus.

Risø 1508 is due to a recessive mutation *lys 3a* located on a separate chromosome from the *Hor* loci. The major effect is the absence of 'C' hordeins and of 'B' hordein belonging to class I; there is less effect on class III 'B' hordeins and no effect on 'D' hordein. Again, analysis of the mRNA populations in the mutant, using *in vitro* translation and different nucleic acid hybridization techniques with our characterized cDNA clones, suggests that the changes in the amounts of polypeptides reflect changes in the populations of endosperm mRNA. However, we have not found any differences in the genomic organization of the *Hor-2* locus. Our results therefore suggest that the *lys 3a* mutation probably acts by differentially affecting the transcription of the *Hor* loci or the subsequent processing of the RNA transcripts. There is no information as to how this may occur.

The expression of the hordein genes during endosperm development. We have studied the accumulation of the different hordein polypeptides of cv. Sundance during endosperm development in the field. As a group the hordeins accumulate relatively late in comparison to other proteins and continue to accumulate until the grain ceases to grow. Within the hordeins the 'C'-hordeins form a greater proportion of the total hordein early (22 days after anthesis) than they do at maturity. Furthermore the 'B' hordeins do not all accumulate at the same rate; those polypeptides belonging to class I make up a larger fraction of 'B' hordein as development proceeds. Because hordeins do not appear to turn over we have equated rate of synthesis with rate of accumulation and suggest that the expres-

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sion of the hordein loci is modulated both within and between loci during grain development. To gain further information we have again used the characterized cDNA clones and have also translated various RNA fractions *in vitro*. All of the approaches suggest that changes in the relative amount of the hordein proteins are due primarily to changes in the relative amounts of the different mRNAs. These results suggest that the controls on the absolute and relative rates of hordein synthesis operate at the level of *Hor* gene transcription, although we cannot yet rule out the possibility of alterations in turnover rate of the different mRNAs.

Plant growth regulators

Gibberellins in developing wheat grains. The capacity of developing wheat grains to synthesize gibberellins was investigated by culturing detached wheat ears in a defined medium without added growth substances (*Rothamsted Report for 1980*, Part 1, 55–56). Wheat ears (var. Maris Huntsman) were harvested from the field 2 weeks post anthesis and cultured for 1 week. Grains of similar age and size were also harvested from intact plants. Gibberellins (GA's) were purified by ion exchange column chromatography and reverse-phase partition high performance liquid chromatography (HPLC) and collected fractions were assayed using the barley endosperm and dwarf rice assays. The GA's of combined HPLC fractions were examined by combined gas chromatography-mass spectrometry (GC-MS) using fused silica capillary columns. The very low amounts of GA's present in grains at week 2 were insufficient for characterization by combined GC-MS. However, after 1 week in culture the following compounds were conclusively identified in respect to relative retention time and reference MS; GA_{55}, GA_{60}, GA_{20}, GA_{61}, GA_{62}, GA_{19}, GA_{54}, GA_{44}. In addition, the 3-*epi* isomer of GA_{54}, 2β-OH GA_{54} and its 3-*epi* isomer were tentatively identified. This spectrum of GA's was similar qualitatively to those from grains of intact plants. Thus detached wheat ears have the capacity to synthesize endogenous GA's. Preliminary experiments showed that feeding the plant growth retardant, ancymidol, to detached wheat ears reduced the content of biologically-active GA's in developing grains. (Appleford, Lenton, Millard and Radley, with Professor J. MacMillan, University of Bristol)

Staff and visitors

Outside support and collaboration. The Department gratefully acknowledges the financial support for personnel and materials that have been provided by the Home-Grown Cereals Authority, Shell Research Ltd, the Potato Marketing Board, Directorates DG VI (Agriculture) and DG XII (Education and Science) of the EEC, Sigma Chemical Company and NATO. We have also shared SERC/CASE research students with the Universities of Leeds, St Andrews, Liverpool, Newcastle and Bath. As well as numerous collaborations within the ARS we also recognize the value of the collaboration that we have received with members of Birkbeck and Queen Elizabeth Colleges, and the Middlesex Hospital Medical School, University of London, the University College of North Wales; the Universities of Nottingham, Cambridge, Kent, Essex, Bristol; the University of Seville, Spain; the University of Ottawa, Canada; the University of Oslo, Norway; the Swedish University of Agricultural Sciences, Svalov, Sweden; the Vrije Universiteit, Brussels, Belgium; the Department of Agriculture, Northern Ireland; the Imperial Cancer Research Fund Laboratories; Rothwell Plant Breeders; Risø National Laboratory, Denmark; the Department of Physiology, Carlsberg Laboratory, Copenhagen, Denmark; and the USDA Western Regional Research Centre, Albany, USA.

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Visitors. During the year the Department was pleased to welcome for extended visits: Dr P. Arruda, Universidade Estadual de Campinas, Unicamp, Brazil; Dr T. Bryngelsson, The Swedish University of Agricultural Sciences, Svalov, Sweden; Dr J. Boccon-Gibod, ENITAH, Angers, France; Dr I. Jonassen, Carlsberg Laboratory, Copenhagen, Denmark; Prof. M. Mazelis, University of California, Davis, USA; Mr I. Pervez, UCNW, Bangor; Dr S. Rognes, University of Oslo, Norway; Ms Ingrid Verbruggen, Vrije Universiteit, Brussels, Belgium.

Visits abroad. Members of the Department attended the following conferences or visited overseas universities as indicated; 5th International Congress of Plant Tissue and Cell Culture, Tokyo, Japan (S. W. J. Bright); 1st International Symposium on the Molecular Biology of the Plant Bacteria Interaction, Bielefeld, Germany (Julie Cullimore); Meeting of Coordinators of the OECD Photosynthesis Programme, Paris, France (A. J. Keys); Phytochemical Society North America Meeting on 'Mobilization in Germination', Ottawa, Canada (P. J. Lea); Engineering of Plants Conference, University of California, Davis, USA (B. J. Miflin); Norwegian Biochemical Society Meeting, Norway (B. J. Miflin); ASPP Meeting, Urbana, USA (B. J. Miflin); NATO/FEBS Meeting on Genome Organisation, Portese, Italy (B. G. Forde, B. J. Miflin); NATO Conference on Genetic Engineering in Eukaryotes, Pullman, USA (G. Ooms); SEB Meeting, Leiden, the Netherlands (Julie Cullimore, P. J. Lea, B. J. Miflin, G. Ooms); Universities of Washington, Seattle, USA, and of Leiden and Wageningen, the Netherlands (G. Ooms), Florida and Minnesota, USA (Sheila Maddock), Leiden, the Netherlands (Angela Karp); Louvain-la-Neuve, Belgium (M. Kreis), Toronto and Ottawa, Canada and Milan, Italy (B. J. Miflin), Carleton, Ottawa, Canada and Khartoum, Sudan (P. J. Lea); Risø National Laboratory, Roskilde, Denmark and Carlsberg Research Laboratory, Copenhagen, Denmark (Angela Karp, M. Kreis); Swedish University of Agricultural Sciences, Svalov, Sweden (Angela Karp); Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben and Institut für Biochemie der Pflanzen, Halle, DDR (A. J. Keys); ARCO Plant Cell Research Institute, Dublin, USA (G. Ooms); Istituto Sperimentale per la Cerealicoltura, Bergamo, Italy (B. J. Miflin); A. N. Bach Institute of Biochemistry and K. A. Timiriazev Institute of Plant Physiology, Moscow, Institute of Plant Breeding and Genetics, Odessa, USSR (P. R. Shewry); IAEA Research coordination meeting, Vienna, Austria (P. R. Shewry).

Staff. I. F. Bird was appointed as full-time Station Safety Officer from 1 December. During the year Audrey Faulks, Valerie Jarrett and A. Strowman left, and N. Bunce (from Queen Elizabeth College, London), D. Foulger (from University College, London), R. Fry (from the University of Essex), M. G. K. Jones (from the WPBS) and A. Tatham (from University of Essex) joined the Biochemistry Department. M. Kreis transferred to the permanent staff from an EEC-supported post. M. Burrell transferred to the Department from Insecticides and Fungicides. Myra Dunlop, R. Mayne, S. Rahman and G. Schmidt completed their Ph.D's.

Publications

Book

1 (Loewus, F. A., Nozzolillo, C.) & Lea, P. J., Eds. (1983) *Recent advances in phytochemistry, Vol. 17. Mobilization of reserves in germination.* New York, Plenum.

https://doi.org/10.23637/ERADOC-1-129

BIOCHEMISTRY DEPARTMENT

Theses

2 Dunlop, M. K. (1982) *Biochemical and genetical studies of inorganic nitrogen metabolism in the moss* Physcomitrella patens. Ph.D. Thesis, University of Leeds.

3 Mayne, R. G. (1983) *Dehydrogenases in* Hordeum vulgare. Ph.D. Thesis, University of London.

4 Rahman, S. (1982) *The synthesis of seed storage proteins in barley*. Ph.D. Thesis, University of London.

5 Schmidt, C. N. G. (1982) *Comparative enzymology of reductive amination reactions in* Escherichia coli *and thermophilic* Bacillus. Ph.D. Thesis, Paisley College of Technology.

General Papers

6 Bright, S. W. J., Jarrett, V. A., Nelson, R., Creissen, G. P., Karp, A., Franklin, J., Norbury, P. B., Kueh, J. S. H., Rognes, S. E. & Miflin, B. J. (1983) Modification of agronomic traits using *in vitro* technology. In: *SEB seminar symposium series Plant Biotechnology*. Ed. H. Smith & S. Mantell. Cambridge: Cambridge University Press, 251–265.

7 Bright, S. W. J., Maddock, S. E., Forde, B. G., Kreis, M. & Miflin, B. J. (1982) Towards the genetic manipulation of barley. *British Plant Growth Regulator Group Monograph 7*, 36–43.

8 Bright, S. W. J., Rognes, S. E., Kueh, J. S. H. & Miflin, B. J. (1982) Lysine-sensitive aspartate kinase isoenzymes in barley are the products of separate unlinked genes. *Plant Physiology* **69,** S126.

9 Bright, S. W. J., Nelson, R. S., Karp, A., Jarrett, V. A., Creissen, G. P., Ooms, G., Miflin, B. J. & Thomas, E. (1983) Variation in culture-derived potato plants. *Proceedings of 5th International Conference on Plant Tissue and Cell Culture, Tokyo, 1982*, pp. 413–414.

10 Bright, S. W. J., Kueh, J. S. H., Franklin, J. & Miflin, B. J. (1981) Proline-accumulating barley mutants. *Barley Genetics. IV. Proceedings of the Fourth International Barley Genetics Symposium, Edinburgh 1981*, pp. 858–863.

11 (Dale, P. J.) & Jones, M. G. K. (1983) Studies on callus and plant regeneration from tissues and protoplasts of the forage grass *holium multiflorum. Proceedings of the 5th International Conference on Plant Tissue and Cell Culture, Tokyo, 1982*, pp. 579–580.

12 (Doll, H., Jensen, J., Køie, B.), Kreis, M. (& Torp, J.) (1982) Studies of barley high-lysine mutants and seed proteins at Risø. In: *Induced mutants for cereal grain protein improvement*. Vienna: International Atomic Energy Agency, pp. 165–170.

13 Fowden, L. (1982) Agriculture and food. *Chemistry and Industry*, No. 16, pp. 582–589.

14 (Hooykaas, P. J. J.), Ooms, G. & (Schilperoort, R. A.) (1982) Tumors induced by different strains of *Agrobacterium tumefaciens*. In: *Molecular biology of plant tumors*. Ed. G. Kahl & J. Schell. New York: Academic Press Inc., pp. 373–391.

15 Lea, P. J., Wallsgrove, R. M. & Miflin, B. J. (1983) The biosynthesis of amino acids in plants. In: *The chemistry and biochemistry of the amino acids*. Ed. G. C. Barrett. London: Chapman and Hall, pp. 201–232.

16 Lea, P. J., Awonaike, K. O., Cullimore, J. V. & Miflin, B. J. (1982) The role of ammonia assimilating enzymes during nitrogen fixation in nodules. *Israel Journal of Botany* **31,** 140–154.

https://doi.org/10.23637/ERADOC-1-129

17 LEA, P. J. & JOY, K. W. (1983) Amino acid interconversion in germinating seeds. In: *Recent advances in phytochemistry*. Ed. F. A. Loewus, C. Nozzolillo & P. J. Lea, Vol. 17, pp. 77–109.

18 (LEAVER, C. J.), FORDE, B. G., (DIXON, L. K. & FOX, T. D.) (1982) Mitochondrial genes and cytoplasmically inherited variation in higher plants. In: *Mitochondrial Genes*. Ed. P. P. Slonimski. Cold Spring Harbor Laboratory, pp. 457–470.

19 MIFLIN, B. J., BRIGHT, S. W. J. & THOMAS, E. (1981) Towards the genetic manipulation of barley. In: *Barley Genetics IV. Proceedings of the Fourth International Barley Genetics Symposium, Edinburgh 1981*, pp. 919–926.

20 MIFLIN, B. J., SHEWRY, P. R., FAULKS, A. J., FORDE, B. G., KREIS, M., MATTHEWS, J., BAHRAMIAN, M. B., THOMPSON, R., BARTELS, D. & FLAVELL, R. (1981) The analysis of the *Hor 2* locus of barley. In: *Barley Genetics IV. Proceedings of the Fourth International Barley Genetics Symposium, Edinburgh 1981*, pp. 604–611.

21 OOMS, G. (1982) Tumoren bij planten ontstaan door manipulaties van bacterium. *Kanker* **7**, 10–12.

22 PIERPOINT, W. S. (1983) Is there a Phyto-interferon? *Trends in Biochemical Sciences* **8**, pp. 5–7.

23 RADLEY, M. E. (1982) Some factors affecting grain set in wheat. *British Plant Regulator Group Meeting Monograph 7*, 140–149.

24 SHEWRY, P. R. & MIFLIN, B. J. (1982) Genes for the storage proteins of barley. *Qualitas Plantarum Plant Foods for Human Nutrition* **31**, 251–267.

25 SHEWRY, P. R. & MIFLIN, B. J. (1982) Gluten: a sticky phenomenon to understand. *Milling Feed and Fertiliser*, 18–19.

26 SHEWRY, P. R., MIFLIN, B. J., FORDE, B. G. & BRIGHT, S. W. J. (1981) Conventional and novel approaches to the improvement of the nutritional quality of cereal and legume seeds. *Science Progress* **67**, 575–600.

27 SHEWRY, P. R., WOLFE, M. S., SLATER, S. E., PARMAR, S., FAULKS, A. J. & MIFLIN, B. J. (1981) Barley storage proteins in relation to varietal identification, malting quality and mildew resistance. In: *Barley Genetics IV. Proceedings of the Fourth International Barley Genetics Symposium, Edinburgh 1981*, pp. 596–603.

28 THOMAS, E., MIFLIN, B. J., BRIGHT, S. W. J. & LANCASTER, V. A. (1982) The regeneration of plants from protoplasts of agriculturally important species. In: *Variability in plants regenerated from tissue culture*. Ed. E. D. Earle & Y. Demarly. New York: Praeger Publishers, pp. 56–68.

29 WHITE, R. F. & ANTONIW, J. F. (1982) Current research and future prospects for direct control of virus diseases. *Proceedings 1981 Crop Protection Conference on Pests and Diseases*, 759–768.

RESEARCH PAPERS

30 BIRD, I. F., CORNELIUS, M. J. & KEYS, A. J. (1982) Affinity of RuBP carboxylase for carbon dioxide and inhibition of the enzymes by oxygen. *Journal of Experimental Botany* **33**, 1004–1013.

31 BOYLE, F. A. & KEYS, A. J. (1982) Regulation of RuBP carboxylase activity associated with photoinhibition of wheat. *Photosynthesis Research* **3**, 105–111.

32 BRIGHT, S. W. J., KUEH, J. S. H., FRANKLIN, J., ROGNES, S. E. & MIFLIN, B. J. (1982) Two genes for threonine accumulation in barley seeds. *Nature* **299**, 278–279.

https://doi.org/10.23637/ERADOC-1-129

33 (Bray, R. C.) & Gutteridge, S. (1982) Numbers and exchangeability with water of oxygen-17 atoms coupled to molybdenum (V) in different reduced forms of xanthine oxidase. *Biochemistry* **21,** 5992–5999.

34 Carr, J. P., Antoniw, J. F., White, R. F. & Wilson, T. M. A. (1982) Latent mRNA in tobacco (*Nicotiana tabacum*). *Biochemical Society Transactions* **10,** 353–354.

35 (Cayley, P. J.), White, R. F., Antoniw, J. F., (Walesby, N. J. & Kerr, I. M.) (1982) Distribution of the ppp(A2′p)nA-binding protein and interferon-related enzymes in animals, plants and lower organisms. *Biochemical and Biophysical Research Communications* **108,** 1243–1250.

36 Cullimore, J. V., Lea, P. J. & Miflin, B. J. (1982) Multiple forms of glutamine synthetase in the plant fraction of *Phaseolus* root nodules. *Israel Journal of Botany* **31,** 155–162.

37 Cunliffe, D., Leason, M., Parkin, D. & Lea, P. J. (1983) The inhibition of glutamate dehydrogenase by derivatives of isophthalic acid. *Phytochemistry* **22,** 1357–1360.

38 Fentem, P. A., Lea, P. J. & (Stewart, G. R.) (1983) The action of inhibitors of ammonia assimilation on amino acid metabolism in *Hordeum vulgare* L. (cv. Golden Promise). *Plant Physiology* **71,** 502–506.

39 Fentem, P. A., Lea, P. J. & (Stewart, G. R.) (1983) Ammonia assimilation in the roots of nitrate and ammonium grown *Hordeum vulgare* (cv. Golden Promise). *Plant Physiology* **71,** 496–501.

40 Festenstein, G. N. & Hay, F. C. (1982) Immunochemical studies on barley seed storage proteins. *Plant Science Letters* **26,** 199–209.

41 Field, J. M., Shewry, P. R., Miflin, B. J. & (March, J. F.) (1982) The purification and characterization of homologous high molecular weight storage proteins from grain of wheat, rye and barley. *Theoretical and Applied Genetics* **62,** 329–336.

42 Field, J. M., Shewry, P. R., Burgess, S. R., Forde, J., Parmar, S. & Miflin, B. J. (1983) The presence of high molecular weight aggregates in the protein bodies of developing endosperms of wheat and other cereals. *Journal of Cereal Science* **1,** 33–41.

43 Gutteridge, S., Parry, M. A. & Schmidt, C. N. G. (1982) The reactions between active and inactive forms of wheat ribulose bisphosphate carboxylase and effectors. *European Journal of Biochemistry* **126,** 597–602.

44 Jones, M. G. K. & (Dale, P. J.) (1982) Reproducible regeneration of callus from suspension culture protoplasts of the grass *Lolium multiflorum*. *Zeitschrift für Pflanzenphysiologie* **105,** 267–274.

45 Karp, A., (Rees, H. & Jewell, A. W.) (1982) The effects of nucleotype and genotype upon pollen grain development in Hyacinth and Scilla. *Heredity* **48,** 251–261.

46 Karp, A. & (Jones, R. N.) (1982) Cytogenetics of *Lolium perenne*, Part 1: Chiasma frequency in inbred lines. *Theoretical and Applied Genetics* **62,** 177–183.

47 Karp, A., Nelson, R. S., Thomas, E. & Bright, S. W. J. (1982) Chromosome variation in protoplast-derived potato plants. *Theoretical and Applied Genetics* **63,** 265–272.

48 Karp, A. & (Jones, R. N.) (1983) Cytogenetics of *Lolium perenne*, Part 2: Chiasma distribution in inbred lines. *Theoretical and Applied Genetics*, **64,** 137–145.

49 Kirkman, M. A., Shewry, P. R. & Miflin, B. J. (1982) The effect of nitrogen nutrition on the lysine content and protein composition of barley seeds. *Journal of the Science of Food and Agriculture* **33,** 115–127.

https://doi.org/10.23637/ERADOC-1-129

50 KUEH, J. S. H. & BRIGHT, S. W. J. (1982) Biochemical and genetical analysis of three proline-accumulating barley mutants. *Plant Science Letters* **27,** 233–241.

51 MATLASHEWSKI, G. J., (ADELI, K., ALTOSAAR, I.), SHEWRY, P. R. & MIFLIN, B. J. (1982) *In vitro* synthesis of oat globulin. *FEBS Letters* **145,** 208–212.

52 MAYNE, R. M., LEA, P. J. & MIFLIN, B. J. (1982) Expression and properties of alcohol dehydrogenase in *Hordeum vulgare* cv. Sundance. *Plant Physiology* **69,** 126S.

53 MIFLIN, B. J. & BURGESS, S. R. (1982) Protein bodies from developing seeds of barley, maize, wheat and peas: The effects of protease treatment. *Journal of Experimental Botany* **33,** 251–260.

54 (MURPHY, P. J., WITCOMBE, J. R.), SHEWRY, P. R. & MIFLIN, B. J. (1982) The origin of six-rowed 'wild' barley from the Western Himalaya. *Euphytica* **31,** 183–192.

55 OOMS, G., (BAKKER, A., MOLENDYK, L., WULLEMS, G. J., GORDON, M. P., NESTER, E. W. & SCHILPEROORT, R. A.) (1982) T-DNA organization in homogeneous and heterogeneous octopine-type crown gall tissues of *Nicotiana tabacum*. *Cell* **30,** 589–597.

56 OOMS, G., (HOOYKAAS, P. J. J., VAN VEEN, R. J. M., VAN BEELEN, P., REGENSBURG-TUINK, A. J. G. & SCHILPEROORT, R. A.) (1982) Octopine Ti-plasmid deletion mutants of *Agrobacterium tumefaciens* with emphasis on the right side of the T-region. *Plasmid* **7,** 15–29.

57 OOMS, G., (MOLENDIJK, L. & SCHILPEROORT, R. A.) (1982) Double infection of tobacco plants by two complementing octopine T-region mutants of *Agrobacterium tumefaciens*. *Plant Molecular Biology* **1,** 217–226.

58 OOMS, G., (REGENSBURG-TUINK, T. J. G., HOFKER, M. H., HOEKEMA, A., HOOYKAAS, P. J. J. & SCHILPEROORT, R. A.) (1982) Studies on the structure of cointegrates between octopine and nopaline Ti-plasmids and their tumour-inducing properties. *Plant Molecular Biology*, **1,** 265–276.

59 RAHMAN, S., SHEWRY, P. R. & MIFLIN, B. J. (1982) Differential protein accumulation during barley grain development. *Journal of Experimental Botany* **33,** 717–728.

60 (RODEN, L. T.), MIFLIN, B. J. (& FREEDMAN, R. B.) (1982) Protein disulphide-isomerase is located in the endoplasmic reticulum of developing wheat endosperm. *FEBS Letters* **138,** 121–124.

61 SCHMIDT, C. N. G. & (JERVIS, L.) (1982) Partial purification and characterization of glutamate synthase from a thermophilic Bacillus. *Journal of General Microbiology* **128,** 1713–1718.

62 SHEWRY, P. R., FIELD, J. M., (LEW, E. J-L. & KASARDA, D. D.) (1982) The purification and characterization of two groups of storage proteins (secalins) from rye (*Secale cereale* L.). *Journal of Experimental Botany* **33,** 261–268.

63 SHEWRY, P. R., FIELD, J. M., PARMAR, S. & MIFLIN, B. J. (1982) 'D' hordein, a new group of prolamin storage proteins in barley. *Barley Genetics Newsletter*, 28–29.

64 SHEWRY, P. R., KIRKMAN, M. A., BURGESS, S. R., FESTENSTEIN, G. & MIFLIN, B. J. (1982) A comparison of the protein and amino acid composition of old and recent barley grain. *New Phytologist* **90,** 455–466.

65 SHEWRY, P. R., PARMAR, S. & MIFLIN, B. J. (1983) Extraction, separation and polymorphism of the prolamin storage proteins (secalins) for rye. *Cereal Chemistry*, **60,** 1–6.

66 SHEWRY, P. R., FRANKLIN, J., PARMAR, S., SMITH, S. J. & MIFLIN, B. J. (1983) The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *Journal of Cereal Science* **1,** 21–31.

https://doi.org/10.23637/ERADOC-1-129

67 THOMAS, E., BRIGHT, S. W. J., FRANKLIN, J., LANCASTER, V., MIFLIN, B. J. & GIBSON, R. (1982) Variation amongst protoplast-derived potato plants (*Solanum tuberosum* cv. Maris Bard). *Theoretical and Applied Genetics* **62,** 65–68.

68 WALLSGROVE, R. M., LEA, P. J. & MIFLIN, B. J. (1982) The development of NAD(P)H-dependent and ferredoxin-dependent glutamate synthase in greening barley and pea leaves. *Planta* **154,** 473–476.

69 WALLSGROVE, R. M., LEA, P. J. & MIFLIN, B. J. (1982) Intracellular localisation of aspartate pathway enzymes in the leaves of higher plants. *Plant Physiology* **69,** 111S.

https://doi.org/10.23637/ERADOC-1-129