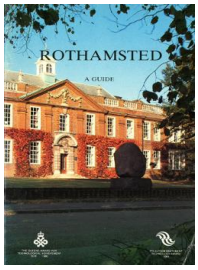
This work is licensed under a Creative Commons Attribution 4.0 International License.

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

ROTHAMSTED RESEARCH

Guide to the Work of the Departments 1984

Full Table of Content



Molecular Sciences Division

Rothamsted Research

Rothamsted Research (1985) *Molecular Sciences Division ;* Guide To The Work Of The Departments 1984, pp 28 - 33

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

Potato diseases

With the expansion of mechanical handling and longer term storage of the potato crop, fungal and bacterial diseases attacking the tubers, especially through wounds, have become more important. Some can be soil-borne but air-borne inoculum may be an important source of re-infection of healthier, nuclear stocks in the earliest stages of multiplication. However, later and in the production of ware crops, the most important source of inoculum is the seed tuber. These diseases have several effects; they may prevent or delay emergence, decrease yield, alter tuber-size distribution, rot tubers in store, or cause unsightly blemishes, which are especially important in potatoes sold ready-washed in transparent packs. Nuclear stocks are produced by rooting stem cuttings, a technique devised at Rothamsted, or now by micropropagation. Such stocks rapidly become as heavily contaminated and infected as any others unless they are protected during multiplication by fungicides; with no effective bactericide, bacterial disease has increased in importance. Control by fungicides, pioneered in this Department, is now widely practised to protect the ware crop in store. The aim is to improve the effectiveness of fungicides by finding better ways of applying them, not only for the ware crop but also through disease control in seed crops.

Moulding of stored grain and hay

Spore-trapping methods developed at Rothamsted are used worldwide to trap airborne pollen and fungus spores, to identify causes of hay fever and asthma and to provide advice for sufferers. Farmer's lung was shown to be an allergic reaction in the alveoli to inhaled spores of thermophilic actinomycetes from heated straw, hay and grain. Other diseases of man and farm animals have been shown to have similar causes. Requirements for preventing moulding of hay using chemical preservatives have been defined and the effects of fungicides on the microflora, yield and quality of grain and on grain deterioration in storage are being investigated. The ecology of fungi which may produce mycotoxins during storage of hay, grain and rape seed is being studied.

MOLECULAR SCIENCES DIVISION

The Molecular Sciences Division was formed with the expectation that it would lead to the application of a spectrum of physical, chemical, biomolecular and cellular techniques to problems in plant science of agricultural importance; it was implicit in this hope that the total effect would be synergistic. Unfortunately serious budgetary reductions mean that the Station will not be able to continue research in Molecular Structures. Nevertheless we still believe in the validity of the approach which will be prosecuted with the resources remaining.

BIOCHEMISTRY DEPARTMENT

Following the retirement of C. P. Whittingham, the plant metabolism group within the Botany Department merged with the Biochemistry Department on 1st October 1982. This has enlarged the Department and as a result we have reorganized our activities by defining a number of research areas. The nature of

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

the research carried out in 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 AFRC'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 is described below.

Ribulose bisphosphate carboxylase

Work in this area falls within the AFRC'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 Plant Breeding Institute, Cambridge, 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.*

Photosynthetic carbon and nitrogen metabolism

Previous collaborative work had demonstrated essential links between fluxes of carbon and nitrogen in photorespiration and we proposed the operation of a photorespiratory nitrogen cycle.

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. 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 bio-

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

chemical 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.

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. 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 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.

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, 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.

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

Disease resistance

One of the objectives of crop improvement, either by conventional or novel means, is the incorporation of disease resistant 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 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 genes for these proteins. Our studies of disease resistance have 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 for recognizing the resistance-gene product at the RNA and protein levels.

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.

Gene isolation and expression

This area is part of the AFRC'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 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 Cereal seed proteins). The

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

A potato plant derived from the variety Maris Bard that was produced using novel genetic manipulation techniques. This is the first example in the world of the application of these methods to a commercial variety of a major food crop.

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 be studied. The isolated full length cDNA and genomic DNA

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

clones of certain of the proteins will be used for studies on gene transformation in higher plants (see Plant cell biology).

SOILS DIVISION

The Division includes the Soils and Plant Nutrition Department (which incorporates the Soil Physics section of the previous Physics Department) and the Soil Microbiology Department. Within this Division we aim to combine the study of the processes of soil behaviour and development, with their practical use for the growth of crops. Thus leaching affects profile development – and the loss of fertilizer nitrogen; clay behaviour affects structure formation – and the best method of cultivation; organic matter turnover is an essential part of soil microbiology – and also vital for the nitrogen nutrition of crops. An important aspect of our work is on soil-root relationships and plant nutrition. The dynamic relation between the root system and the soil requires that they be treated as a single system, and the activities of microorganisms are essential components of this. Our aim, on the basis of scientific principles, is to control plant growth and composition accurately, rather to rely on empirical tests. The broad scope of the Division's work is exemplified by the fact that it is heavily involved in computer simulation and advisory systems, and also in biotechnology, with the work on genetic engineering of symbiotic bacteria and fungi. With these interests, the Division must necessarily collaborate closely with others, expecially Agronomy and Crop Physiology and Molecular Sciences, and with the Soil Survey of England and Wales.

SOIL MICROBIOLOGY DEPARTMENT

General soil microbiology

Soil contains very many different kinds of microorganisms in vast numbers, including bacteria, viruses, actinomycetes, fungi, protozoa and algae. They break down and transform organic and inorganic materials of the soil, so making nutrients available to crop plants. Some are beneficial, as those promoting good soil structures and fixing nitrogen from the air, and others are harmful, such as those causing disease. Methods of identifying and counting soil microorganisms still require much improvement, and work on this is in progress. It is particularly important for the study of the population in the 'rhizosphere' next to the root.

Mycorrhizas

Mycorrhizas are non-pathogenic fungal infections of plant roots. Those of the vesicular-arbuscular (VA mycorrhizal fungi) type are widespread in many crop plants. We are studying the biology of these fungi, and the effects of soil type, fertilizer and pesticide treatments and seasonal factors on them. Their influence on other soil microbes, including those fixing nitrogen or causing disease, may be considerable.

Their main importance is that, in soils containing little available phosphorus, mycorrhizal infection increases phosphate uptake and thereby improves plant growth, and the mechanisms whereby this occurs are being studied. Methods of exploiting mycorrhizas to improve productivity are being actively tested in upland grassland, vegetable production and horticulture.