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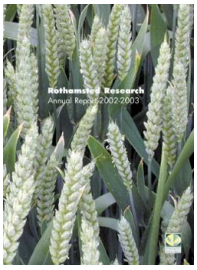
Rothamsted Research Annual Report 2002-2003



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Rothamsted Research (2003) *Genomics and the Classical Experiments ;* Rothamsted Research Annual Report 2002-2003, pp 36 - 39

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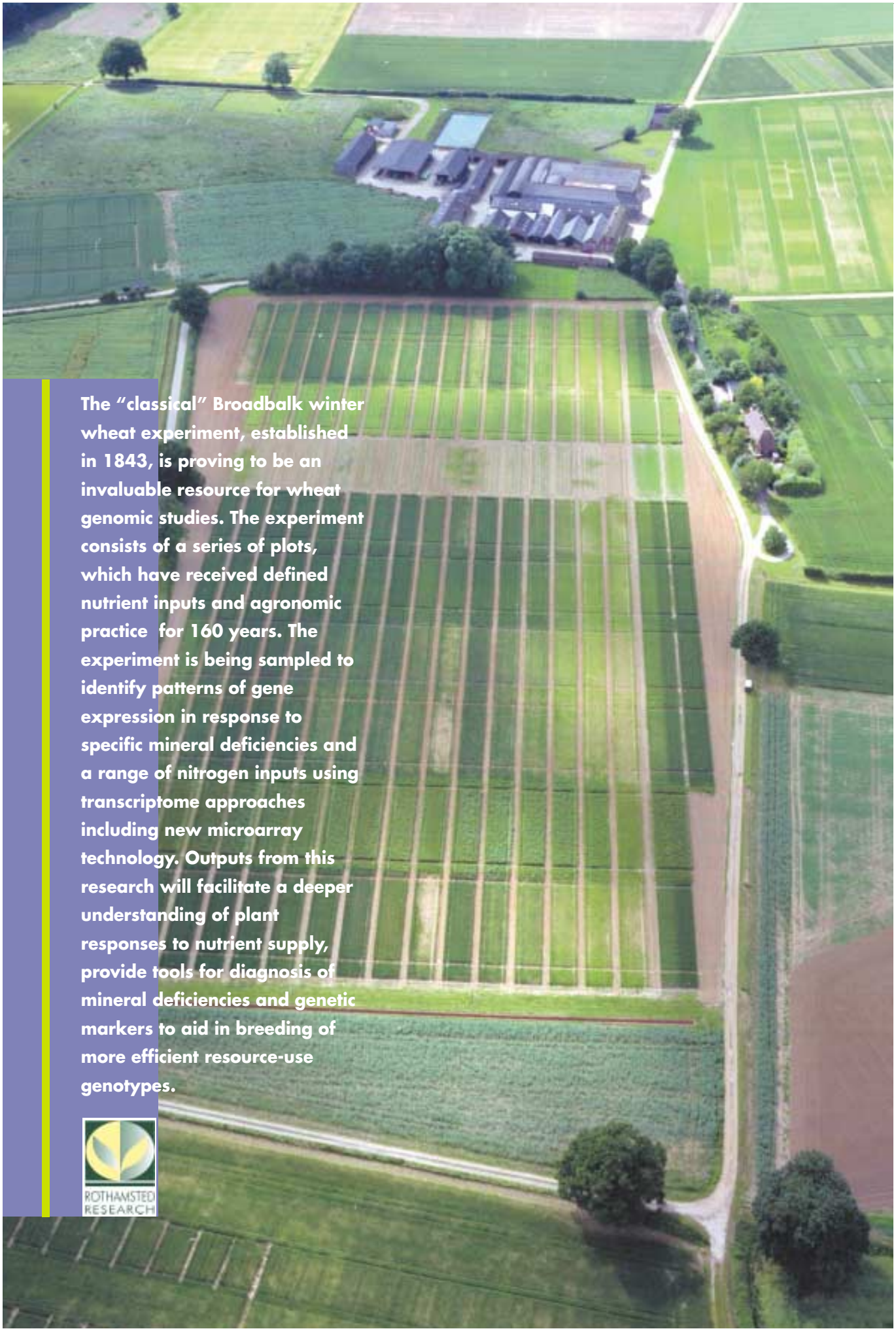

The "classical" Broadbalk winter wheat experiment, established in 1843, is proving to be an invaluable resource for wheat genomic studies. The experiment consists of a series of plots, which have received defined nutrient inputs and agronomic practice for 160 years. The experiment is being sampled to identify patterns of gene expression in response to specific mineral deficiencies and a range of nitrogen inputs using transcriptome approaches including new microarray technology. Outputs from this research will facilitate a deeper understanding of plant responses to nutrient supply, provide tools for diagnosis of mineral deficiencies and genetic markers to aid in breeding of more efficient resource-use genotypes.

ROTHAMSTED RESEARCH

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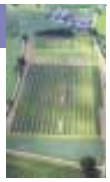

Genomics and the Rothamsted Classical Experiments

Malcolm J Hawkesford, Peter Barraclough, Jonathan R Howarth, Chungui Lu and Keith Edwards

Aerial view of The Broadbalk Classical Experiment. (left)

Background

The Rothamsted Classical Experiments were established in the 19th century by Sir John Lawes and Sir Henry Gilbert to investigate crop production and the influence of various combinations of inorganic and organic fertilisers on crop yields. One objective was to determine if the new inorganic fertilizers were as effective as farmyard manure in elevating crop yield. In addition, plots were established to express specific mineral deficiencies and these have been maintained with only minor modification ever since. The Broadbalk experiment represents a single field in which experimental plots have become established with distinct properties in relation to mineral availability and soil quality. It is possible to sample a wide range of treatments at this single site. Grain yields for the various plots have been recorded continuously and sample information is shown in Table 1. A clear response to applied nitrogen is evident with traditional farmyard manure comparing favorably to inorganic N applications. The specific mineral deficiencies have substantial influences on. Since 2000, one plot has received no sulphur (S) fertilizers. In 2002 yields on this plot were reduced for the first time by c 1 t ha^{-1} compared to the controls.

In 2002, the first extensive samplings for molecular analyses were undertaken. Tissue samples were harvested both during the vegetative growth phase and at defined time points during early grain development. Messenger RNA (mRNA) was extracted from these tissues and is being subjected to transcriptome analyses using microarray and cDNA AFLP (amplified fragment length polymorphism) techniques.

Microarrays for whole transcriptome analysis

The BBSRC Investigating Gene Function (IGF) initiative has sponsored the development of a 10,000 unigene microarray chip. This has been produced in a collaboration between Rothamsted Research and Professor Keith Edwards (University of Bristol). The 10,000 genes represent a substantial proportion of the whole wheat genome and are drawn from a range of cDNA libraries from different tissues, grown under a variety of conditions. A collaborative project is underway between Rothamsted Research and the University of Bristol to

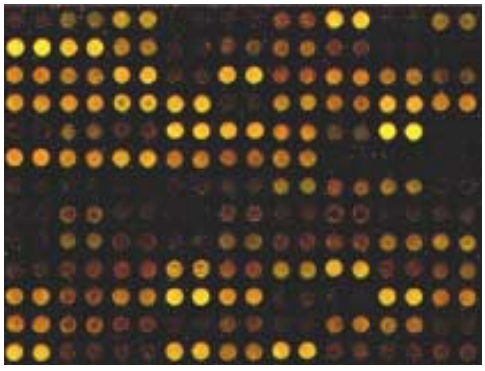

Figure 1. Hybridization of 10,000 duplicated cDNAs on a single glass slide and close-up

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analyze gene expression in wheat grain in relation to nitrogen inputs using material sampled from Broadbalk. Figure 1 shows an example microarray, with the 10,000 genes (amine-modified DNA) spotted in duplicate. This array has been hybridized with cDNAs made from two mRNA populations from wheat grain supplied with high and low rates of N. The cDNA populations are labeled with either a red or green fluorescent dye. The fluorescence intensity is directly related to the level of gene expression and the relative fluorescence of the two dyes, which may be accurately and independently quantified, compares the effect of the treatment on expression for each individual spot or clone. The fluorescence intensities for each spot may be plotted graphically (Figure 2). Most genes are expressed at similar levels and are plotted within the green delimiting lines, which represent experimental error. Points above the upper line or below the lower line represent preferential expression in the high and low nitrogen treatments, respectively. Each of these points represents one gene of the unigene set, whose sequence is known, and in many cases, whose identity has also been ascertained. The differentially expressed genes may be clustered into functional groups, and responses of whole biochemical pathways, at the level of gene expression, may be monitored in a single experiment. The output from this project is providing insights into the coordination of gene expression and biochemical pathways during grain development in response to nitrogen nutrition.

Screening for nutrient-regulated genes

Genes responding to specific nutrient deficiencies are also being identified. The approach is to sample leaf tissues from the nutrient deficient plots on Broadbalk at a time of rapid vegetative growth and high nutrient demand. The mRNA is extracted and a cDNA AFLP analysis is conducted. In this approach sets of oligonucleotide primers are used to generate cDNA fragments representing every gene expressed under each treatment. These fragments are then separated by gel electrophoresis allowing gene expression profiles to be compared (Figure 3). Using appropriate primer combinations, several thousand fragments can be resolved. An advantage of this approach is that gene analysis is not restricted to clones represented on the microarrays, where stress-induced genes may be under-represented. The differentially expressed fragments are excised directly from the gels, amplified, cloned and sequenced. The identity of the cDNA is determined by interrogation of gene sequence databases and the corresponding gene may then be isolated from a genomic library. Particular emphasis is placed on the isolation of the genomic region adjacent to the coding sequence as this contains the relevant control regions. Fusion of these control regions (the promoter) with a reporter gene allows the easy monitoring of expression when this construct is re-introduced into a plant. Methods for both transient and stable expression are being used to characterize the control regions. To date a number of candidate genes whose expression is controlled by specific nutrient limitations have been identified.

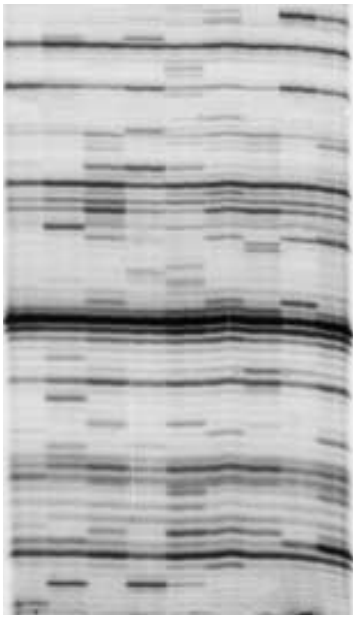

Figure 3. Section of a cDNA AFLP gel showing transcripts from nutrient deficient plants grown on Broadbalk

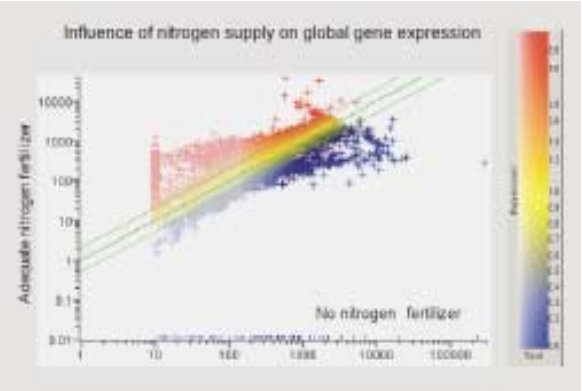

Figure 2. Scatter graph for visualization of relative expression between two treatments (high and low nitrogen-supply on Broadbalk) from a microarray experiment

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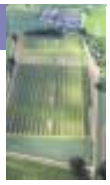

Table 1. Grain yields of winter wheat (cv. Hereward) from a Broadbalk section of plots which have had wheat grown continuously (Section 1). Treatments are: farmyard manure (FYM), various levels of N in multiples of 48 kg/ha (N0 to N6) and K, Mg and P-deficient plots.

Treatment	Mean grain yield (1996-2000) ± std dev. (t/ha @ 85% dry matter)
FYM + N2	8.20 ± 0.50
FYM	5.99 ± 1.03
N0	1.28 ± 0.35
N1	3.18 ± 0.28
N2	5.28 ± 0.69
N3	6.10 ± 1.09
N4 (control)	7.16 ± 0.53
N5	7.62 ± 0.88
N6	8.00 ± 0.62
K-deficient (+N4)	3.61 ± 0.77
Mg-deficient (+N4)	4.77 ± 0.61
P-deficient (+N4)	1.91 ± 0.41

Exploitation

Differential gene expression, which correlates with nutrient use efficiency, may be directly responsible for the variation in this important trait, or may be a consequence of nutrient availability. If a direct causal relationship can be demonstrated then allelic variation of specific genes may become targets for selection in plant breeding programmes or for modification by genetic engineering to achieve improved nutrient use efficiency. Alternatively, if consequentially related, such genes may also provide useful indicative markers for breeding programmes or in the case of nutrient-deficiency induced changes in gene expression useful diagnostic markers. Laboratory-based analysis of marker-gene expression using, for example, PCR techniques, could be routinely supplied to the agricultural community (see Figure 4). However, it is highly desirable to achieve real-time analysis in the field, such that nutrient requirements of a standing crop may be directly fulfilled by appropriately precise fertilizer application. To supply this need, the concept of 'smart plants' has been proposed. The smart plants, which are transgenic lines containing the nutrient regulated promoter coupled to the reporter gene, would signal incipient deficiency in a sensitive and specific manner. The ideal reporter gene would give an easily measurable visible signal dependent upon nutritional status. Suitable sensors, perhaps located on the tractor supplying the fertilizer would respond to signals from either the crop plant or reporter plants scattered throughout the field ('sentinel plants'). This would enable the precise delivery of fertilizer.

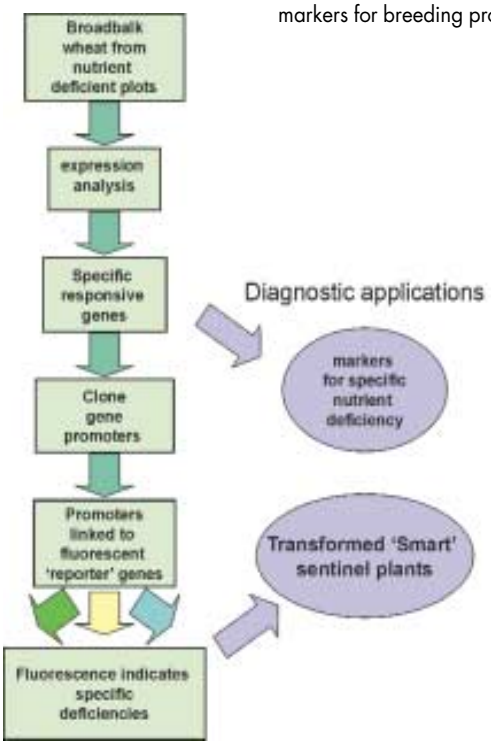

Figure 4. Development of diagnostic applications from expression profiling experiments