Cereal gene mining and manipulating

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Research on cereals in the Gene Expression Unit has focused primarily on barley but also features work on wheat for very different but equally compelling reasons. Barley is of paramount importance to agriculture and the local economy in Scotland and the North of England, where most of the crop is utilised for the production of malt which, when processed to produce whisky, remains the major UK export earner from the food and drink sector. Wheat rivals rice as the leading crop in world-wide production, and is becoming increasingly important in the less-developed world as a staple to meet the food demands of an ever-increasing population. These local and global drivers have led to the two research programmes described in this article. They have different objectives but share common technologies and each has already benefited from the other - the potential for exploitation of this research in the future across the cereals, including rice, is tremendous.

Virus-resistance in Chinese wheat Wheat is a staple food for approaching 40% of the world's population and, in China, one half of its 1.2 billion people rely on the wheat crop for their major food needs. China is thus one of the world's major wheat producers, along with the nations of the former USSR and the USA. The crop is grown on 30 million hectares of arable land in China, and production has trebled over the last 30 years. These impressive gains have been necessary to match population growth, but are continually threatened by disease caused by pests and pathogens, with resulting annual crop losses estimated at about 50%. Reduction in these losses would enhance both food security and the sustainability of this production.

Prominent in the losses incurred in the Chinese winter wheat crop through viral disease is a 10-30% loss in yield of winter wheat resulting from infection by viruses transmitted by soilborne fungi. *Soilborne wheat mosaic virus* (SBWMV) and *Wheat spindle streak mosaic virus* (WSSMV), which cause the bulk of this loss, are both transmitted by the fungus *Polymyxa graminis* in soil. Preventing the infection of roots by the application of fungicide is difficult, expensive, environmentally undesirable and impracticable because the fungus can survive as resting spores in soil for many years. Once a field is infected with viruliferous fungus, there is no way to eradicate the virus. Therefore, the only simple and economical method to control these viruses is by growing virus-resistant wheat cultivars in the infected fields.

Plant breeding for virus resistance involves the identification of resistance genes and their introgression into elite genotypes. There follows an ongoing struggle for supremacy between the breeder and the pathogen, which seeks to evade control. The breeder's resistance

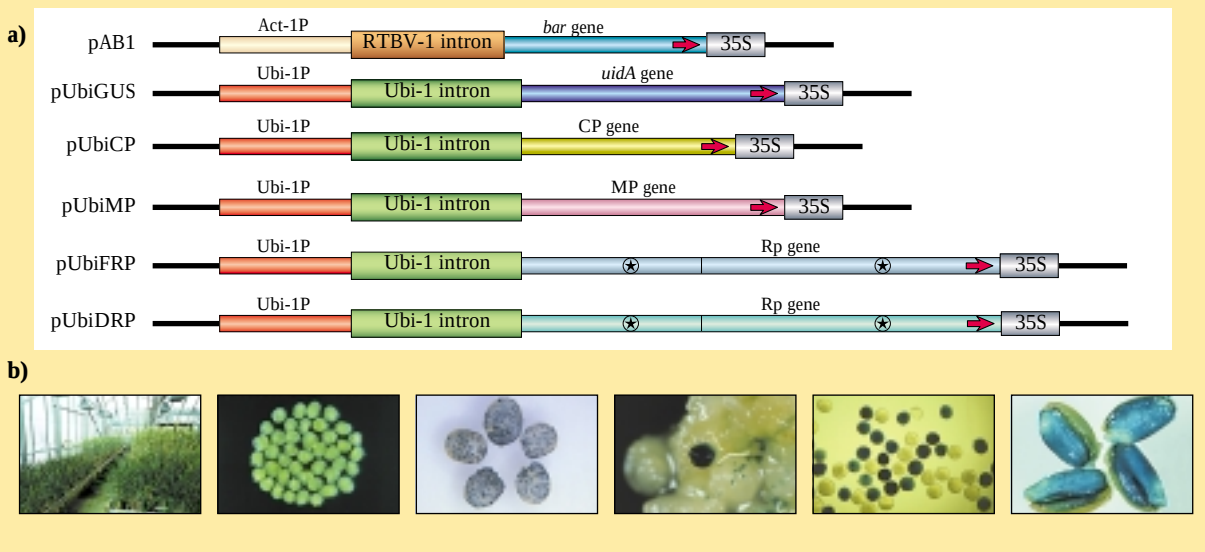

Figure 1 a) Gene constructs for Chinese wheat transformation. b) Stages in the transformation of wheat.

armoury has been enhanced recently by transgenic methods and, particularly, by the finding that, in dicotyledonous plants (dicots), the expression of a single viral gene as a transgene within the plant can inhibit the spread of virus throughout the plant. Genes for viral coat protein, movement protein and replicase have been used in this strategy, which has been developed at SCRI against potato viruses and has proven effective (see SCRI Annual Report, 1993). We have extended this approach to monocotyledonous cereals by transforming these genes from SBWMV into both model cultivars of wheat and cultivars used in breeding programmes in China.

Genes are introduced into wheat by a biolistic approach, using immature embryos as the target tissue. Example constructs are shown in Figure 1. It features genes encoding SBWMV coat proteins, driven by a constitutive promoter from an ubiquitin gene, including the first intron of the same ubiquitin gene, which stabilises expression in monocots. The construct DNA is coated onto gold particles and introduced into cells of the immature wheat embryo by bombardment. Optimal conditions for this introduction are established by determining the best conditions for transient expression, from a similar construct, of a reporter molecule (GUS) (Fig. 1). Gold particles used for bombardment can be coated with the DNA of both constructs leading, after regeneration and selection, to the generation of stable transgenic wheat plants containing both genes, and which express the GUS reporter as well as the coat protein (expression from pollen and endosperm for example, and transgenic plants, are also shown in Fig. 1). Using this approach, we have been successful, as confirmed by Southern blotting, in transforming the model cv. Bobwhite and three Chinese cvs., Yangmai 93-111, Yangmai 94-141 and Yangmai 2980, used in Chinese breeding programmes. Genes for coat protein, movement protein and replicase, in sense, antisense and defective gene constructs, have all been introduced into these varieties. This EU-funded research includes partners from Europe (IACR, Rothamsted and the Max Planck Institute, Koln) and leading Chinese agricultural research centres who will conduct resistance testing of the transformed wheat to determine if the success achieved with this approach in dicots. has been emulated.

Gene expression in malting barley The focus of our work on barley is related to quality rather than disease resistance. Most of the barley grown in Scotland is processed into malt which supports a £2.4 billion per annum whisky export industry. The production of the optimum barley grain for malting is the result of a great variety of complex biological processes. These range from the deposition of starch in the developing endosperm, and all the plant physiology and biochemistry which support this, to a rapid and even germination of the grain achieving the desired hydrolysis of complex carbohydrate to substrates utilisable by yeast. SCRI's breeders and geneticists have made great inroads into putting these processes into a genetic framework and, aided by molecular marker technology, in applying this knowledge to practical breeding goals. It is clear from their work that properties such as malting quality are governed by multiple genes whose contributions will require dissection and description before any understanding of these complex phenotypes can be reached. Fortunately, a new set of tools which can be applied to these problems is now on stream - these include large-scale gene discovery programmes and high volume parallel gene expression analysis. We have begun the application of both technologies to barley, together with the development of barley transformation which will be essential to the determination of function. When combined with existing strengths in genetic and phenotypic analyses of barley, new strategies for the analysis of complex traits can now be assessed.

The gene discovery element of our malting barley research began with the sequencing of DNA from some 2,000 genes expressed in the grain 3 days after the commencement of the malting process. This was achieved by the construction of a cDNA library from the mRNA population present at this stage and single pass sequencing from individual clones derived from this library to derive expressed sequence tags (ESTs). The process was remarkably informative with little redundancy in the sequence information obtained. The primary analysis of this information (after quality checks) was comparison to existing public databases of all known genes. The results of this analysis can be

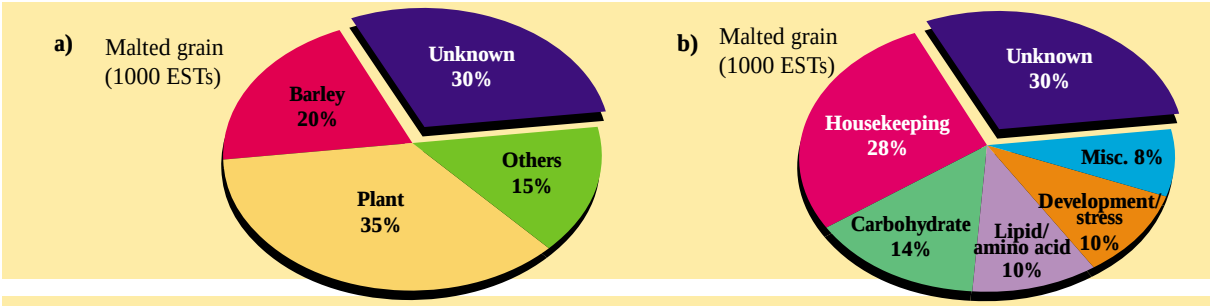

Figure 2 EST composition. a) 70% of the malted barley ESTs show significant homology to existing database sequences, primarily plant sequences, while 30% show no significant homologies. b) The ESTs to which a putative function can be assigned by homology have been classified according to that function: most deal with cellular housekeeping and organ level responses to the malting, but a significant proportion is involved in determining the intensive carbohydrate, lipid and amino acid metabolism activated during this process.

presented in several ways, as illustrated in Figure 2. Full interpretation of this data presents a daunting task in bioinformatics. Individual sequences have founded novel investigations in our own and collaborators' labs. These have goals relating to a much wider area than malting quality. They include (as examples): a determination of the prevalence of microsatellites and single nucleotide polymorphisms in the expressed portion of the barley genome and their exploitation in diversity studies and association genetics; the cloning of disease resistance gene 'islands'; the characterisation and activity of barley retrotransposable elements; and the development of novel selectable markers for barley transformation. The assembled sequence information also offers a preliminary insight into various metabolic processes contributing to malting quality in the germinating grain during malting. We can begin to assess these at the level of a metabolic pathway rather than, as previously, as single enzymatic conversions. This approach will be greatly enhanced by further EST sequencing in malting tissues and extended by the collection of EST information from the developing grain - the sequencing of 40,000 ESTs will be undertaken in the Genomics Unit over the next 3 years.

The above data represent a snapshot of gene expression in the barley grain at one stage of malting. A further way to add value to this information is to put it in a temporal and spatial context. This can be achieved by depositing the sequences on microarrays and interrogating these with complex probes encompassing the range of genes expressed at specific times in the malting process or from specific tissues of the malting grain. A good versus poor malting quality comparison can also be included. These parallel gene expression studies are now in progress, while the pace of technological advance in this area is breathtaking. Data derived from them, combined with genetic, phenotypic and bioinformatic analyses, will bear on the selection of genes, or gene sets, for manipulation towards the improvement of malting quality and other complex traits. Accelerated breeding or transgenic routes can be used as appropriate to achieve the desired improvements. For many genes, stable transformation will offer the most definitive determination of gene function. Progress in barley transformation has been greatly increased by our experience with wheat but priorities remain in increasing efficiency and minimising genotypic dependency. Just as the development of transformation technology in wheat has benefited barley, much of what we learn about gene expression in barley will complement research on wheat and other cereals.

Prospectives The new millennium heralds a new era in biological research in which we can take a more integrated view of molecular, cellular and whole plant processes. The wealth of new information from genomics will impose significant challenges in bioinformatics if the maximum value is to be derived from it, in combination with existing genetic and phenotypic information. If this enterprise is successful, we can expect much increased definition in the selection of targets to effect desired phenotypic changes. These will be facilitated by the precision engineering of genomes involving the introduction of highly targeted changes to specific (sets of) genes with no collateral modification. In addition, candidate genes emerging from such studies will provide new opportunities to connect sequence diversity to phenotype and provide more informative ways of describing plant genomes. Coupling the power of an integrated science approach to tackle complex biological problems is an exciting endeavour that will require connections between discovery and hypothesis driven research.