High-throughput gene expression analysis at SCRI

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Exploitation of genomics resources Major investments have been made in the last few years at SCRI to establish extensive catalogues of genes, as expressed sequence tags in crop plants and through whole genome sequencing in plant microbial pathogens. In order for these resources to be fully exploited in crop improvement and research programmes, high-throughput monitoring of gene expression is required. Determination of coordinated changes in gene expression over time, throughout development, or in response to manipulation, stress or pathogens, will allow novel insights into how they respond to environmental challenges.

Utilisation of current technology Parallel gene expression technologies currently being developed and utilised at SCRI include microarrays and serial analysis of gene expression (SAGE). These tools enable the simultaneous analysis of the activities of many thousands of genes, providing targets for detailed downstream studies. There are two main microarray platforms being optimised at SCRI. Spotted arrays, which consist of DNA probes (as PCR products or oligonucleotides) deposited onto modified glass slides, and Affymetrix GeneChip technology, where each gene is represented by a set of short oligonucleotide probes. RNA is isolated from target tissues and labelled as cDNA, prior to hybridisation against the arrayed gene probes. SCRI has acquired a Genetix QArray Mini spotting robot for microarray fabrication, along with an Applied Precision ArrayWoRx scanner for data acquisition. In addition, for GeneChip studies, a hybridisation and analysis node has recently been purchased. Following data extraction, exploitation of data is subsequently performed using GeneSpring (Silicon Genetics) software, which allows identification of differentially expressed genes, comparison of expression profiles, clustering analysis and links to metabolic pathways. The Computational Biology programme and BioSS are providing support for analysis and data storage.

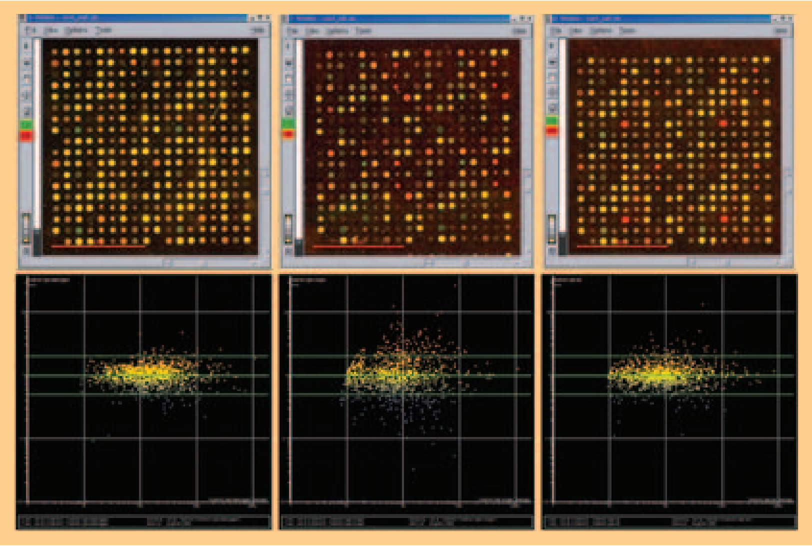

Figure 1 Differential gene expression in stressed barley root tissue.

Collaborative projects Affymetrix GeneChips are now available for barley from a collaborative effort between SCRI and a USDA-funded initiative. These arrays contain approximately 23,000 probe sets representing high-quality barley gene sequences. It is envisaged that several collaborative projects will utilise these arrays, including dissection of malting characters and grain development, and plant response to abiotic stress. GeneChips representing 22,000 *Arabidopsis* genes will also be utilised wherever appropriate. The genome sequence of the potato pathogen *Erwinia carotovora* ssp. *atroseptica* has recently been completed at SCRI and each gene (approx. 5000) will be represented on Agilent oligonucleotide arrays, along with a set of potato genes (approx. 1000) implicated in defence responses. These arrays will be used to identify novel pathogenicity genes *in vitro* and *in planta*, and to study host-pathogen interactions. In addition, projects are also underway to investigate regulation of alternative splicing in *Arabidopsis*, global control of carotenogenesis in potato, dormancy in raspberry, the role of transcription factors in biotic responses of potato and potato-nematode interactions. Linear amplification technologies are also being developed to allow expression analysis from small numbers of target cells.