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Review Article

Application of Gene Profiling in Agriculture

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A B S T R A C T

In an effort to remedy in agricultural crisis, finding the genes that are able to defend against biotic stress would be of great value. It is an obvious interest to determine which genes would be involved in the regulation and with what gene expression level in response to plant infection. Gene Profiling can be used to determine the gene expression level of the messenger RNA (mRNA) in a given cell population. Gene Profiling is the study of the transcriptome, which is the set of all mRNA or transcripts produced in a population of cells. The transcriptome varies under stress conditions because all mRNA transcripts in a cell are a reflection of the genes that are being actively expressed under any stress conditions. Two technologies can be useful tools for transcriptomics. One of the technologies is serial analysis of gene expression (SAGE) can be used, which has been developed by Velculescu *et al.,* (1995). SAGE technology is also a powerful tool that allows the analysis of overall gene patterns. As another approach to transcriptomics, microarray analysis, which utilizes labeled cDNAs hybridized to an array of DNA elements as probes affixed to a solid support. Using microarray analysis, high densities are achievable and enable the measurement of over 10,000 genes. Microarray is one of the popular technologies that contain information derived from hundreds or even thousands Microarray is one of the popular technologies that contain information derived from hundreds or even thousands of samples. The level of gene expression of an organism in a variety of normal, developmental, and disease states can be quantified by SAGE technology in the same way as microarray technology.

Keywords

Gene Profiling, Transcriptomics, Microarray, SAGE, cDNA-AFLP

Introduction

Approaches for gene identification and functional characterization involve in protein characterization, peptide sequence determination, and identification of the corresponding DNA sequence. More recently, expressed sequence tags (ESTs), microarrays, large-scale gene expression (transcriptome) profiling, and associated informatics technologies are rapidly becoming commonplace in the plant sciences. These 'genomic' approaches typically take advantage of technologies for characterizing large numbers of nucleic acid sequences, bioinformatics, and the expanding collection of nucleic acid sequence data from diverse taxa. These techniques attempts to combine large-scale DNA sequence, gene expression, protein, metabolite, genotype, and/or phenotype data to develop a comprehensive understanding

of biological process. Combination of these approaches also makes it possible to extract more meaningful functional information as new DNA sequence data are generated (Adams *et al.,* 1991).

Expressed sequence tags: A tools for expression analysis

Expressed sequence tags are created by sequencing the 5'and/or 3' ends of randomly isolated gene transcripts that have been converted into cDNA. Despite the fact that a typical EST represents only a portion (approximately 200–900 nucleotides) of a coding sequence, this partial sequence data has substantial utility. For example, EST collections are a relatively quick and inexpensive route for discovering new genes confirm coding regions in genomic sequence facilitate the construction of genome maps can sometimes be interpreted directly for transcriptome activity and provide the basis for development of expression arrays (DNA chips). In addition, high-throughput technology and EST sequencing projects can result in identification of significant portions of an organism's gene content and thus can serve as a foundation for initiating genome sequencing projects (Bourdon *et al.,* 2002).

Gene expression profiling

A variety of methods have been developed for quantifying mRNA abundance in plant tissues. Although the established and reliable method of RNA gel-blot analysis can be quite sensitive and allows for the accurate quantification of specific transcripts, this method is adapted for genome-scale analysis. Differential display uses low stringency PCR, a combinatorial primer set, and gel electrophoresis to amplify and visualize larger populations of cDNAs representing mRNA populations of interest. Differential display has important advantages when compared with scale-limited approaches such as RNA-blot analysis (e.g. minimal mRNA is required, parallel profiling of mRNA populations is feasible), yet this technique suffers from output that is not quantitative and positives are often difficult to clone and confirm (Debouck, 1995).

cDNA-AFLP

More recently the principles of AFLP have been applied to cDNA templates (i.e. cDNA-AFLP) and this approach has been used to identify differentially expressed genes involved in a variety of plant processes. This technique offers several advantages over more traditional approaches. In this poorly characterized genome scan be investigated in a high-throughput manner. Because the stringency of cDNA-AFLP PCR reactions is quite high (which is not the case with differential display) the fidelity of the cDNA-AFLP system allows much greater confidence in acquired data and differences in the intensities of amplified products can be informative. In addition, this technique allows a wide variety of tissue types, developmental stages, or time points to be compared concurrently (Bachem *et al.,* 2001).

Serial analysis of gene expression (SAGE)

In SAGE polyadenylated mRNA is isolated from cells with oligodT coupled to biotin. The polyadenylated message is precipitated out with magnetic beads coupled to avadin that binds to biotin. The mRNA is then reversed transcribed and double stranded cDNA is created. Thus, a cDNA library is created. A four base cutter, usually Nla III cuts the cDNA, leaving the 3' portion of the cDNA attached to the bead. The cDNA attached to the bead is ligated to a linker

containing a type IIS restriction site and digested with a 'tagging' enzyme, usually BsmFI. This gives rise to 15bp fragments or tags. The fragments are ligated and amplified with PCR. Only those fragments that ligated tail-to-tail are amplified by PCR because the PCR primers used are derived from the sequence of the linker. These tail-to-tail fragments are called ditags. These ditags are concatermerized and cloned. The clones are sequenced. By sequencing the clones the identity and the abundance of a transcript is established. The number of tags for a given sequence by the total tags gives the absolute abundance of the tag (Velculescu *et al.,* 1995).

Microarrays

The most commonly used technology to profile the expression of thousands of transcripts simultaneously is microarrays. cDNA and Oligonucleotide arrays are two types of platforms commonly used.

In cDNA arrays cDNAs from a clone collection orcDNA library are spotted on nylon membrane or glass slide. As many as 30,000 cDNA can be spotted on a microscope slide with each spot corresponding to a unique cDNA. The second type of microarray uses oligonucleotides.

These are either etched on a silicon chip by photolithography. The oligonucleotide or cDNA spotted array is hybridized to cDNAs synthesized from the mRNA or total RNA extracted from the cell or tissue of interest. The cDNA from two different samples are labeled with fluorescent dyes such as Cy3 (green) and Cy5 (red). These samples can be different cell populations or treatment conditions. The cDNA labeled with Cy3 and Cy5 are mixed together and hybridized against the same array. The two populations compete for the same targets or probe spots on the array. The array is scanned with two different wavelengths following hybridization and washing. The spot intensity at the two wavelengths is determined.

A ratio or log ratio between the two fluorescent intensities is calculated. Alternatively, radioactivity can be used to increase the sensitivity of the assay but at the cost of decreased density of the array. Microarray is a high throughput method for profiling gene expression but many variables influence the outcome of the experiment (Paul *et al.,* 2004).

Use of gene expression profiling

Gene expression profiling technology is currently being used to investigate a variety of different physiological and developmental processes in plant species, via a variety of different profiling techniques. Some examples include responses to different stresses, environmental conditions, pathogens and symbionts (Puthoff *et al.,* 2003) and various developmental processes.

Advantages and disadvantages of gene expression profiling

Advantages

Great scope for miniaturization, for high-throughput applications and for development of integrated, automated systems.

Simultaneous detection and quantification of thousands of hybridization events

Disadvantages

DNA array instruments, DNA chip production, probes, and bioinformatics are fairly expensive.

References

Adams, M., Kelley, J., Gocayne, J. *et al.*, 1991. Complementary DNA sequencing: expressed sequence tags and human genome project. Science, 252, 1651–1656.

Bachem, C., Horvath, B., Trindade, L., Claassens, M., Davelaar, E., Jordi, W., Visser, R. 2001. A potato tuber-expressed mRNA with homology to steroid dehydrogenases affects gibberellin levels and plant development. Plant J., 25, 595– 604.

Bourdon, V., Naef, F., Rao, P., Reuter, V., Mok, S., Bosl, G., Koul, S., Murty, V., Kucherlapati, R., Chaganti, R. 2002. Genomic and expression analysis of the 12p11-p12 amplicon using EST arrays identifies two novel amplified and over expressed genes. Cancer Res., 62, 6218–6223.

Debouck, C. 1995. Differential display or differential dismay. Curr. Opin. Biotech., 6, 597–599.

Paul, A.-L., Schuerger, A., Popp, M., Richards, J., Manak, M., Ferl, R. 2004. Hypobaric biology: Arabidopsis gene expression at low atmospheric pressure. Plant Physiol., 134, 215–223.

Puthoff, D., Nettleton, D., Rodermel, S., Baum, T. 2003. Arabidopsis gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J., 33, 911–921.

Velculescu, V. E., Zhang, L., Vogelstein, B., Kinzler, K. W. 1995. Serial analysis of gene expression. Science, 270:484-487.