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FUNCTIONAL GENOMICS IN PLANTS - AN OVERVIEW

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ABSTRACT
Functional genomics has gained huge prominence in the twenty first century. The availability of the complete genome sequences of commercially important crops has fostered the growth of this field. Functional genomics deals with assigning the gene function to the unknown genes of an organism. It aims to expand our functional knowledge of various macromolecules. ESTs have been used in molecular ecology research for genome-wide studies of gene expression and selection. Microarrays and Serial analysis of Gene Expression are the two widely used techniques for the gene expression profiling. Methodologies like transcriptomics and proteomics play an important role in determining the gene function. However, only one approach cannot serve the purpose. In this review, the focus is laid on the determination of gene function and the role of transcriptomics and proteomics in the studies of functional genomics.

KEYWORDS: genome sequences, crops, macromolecules, ESTs.

INTRODUCTION

Genome sequencing projects have laid the foundation for the advanced biology era. Genome drafts of many crops have been sequenced in the last decade of the last century (Werner, 2010). With the increased focus in the genome sequencing projects, plant research entered an exhilarating period in which genome-wide approaches have become an integral part of plant biology, with potentially highly rewarding but as yet unpredictable biotechnological applications (Bouchez and Hofte, 1998). Genomics is a field of biology which deals with the structure and function of genome. The ultimate goal of genomics is the identification of all the genes of an organism and then determining their function. The emergence of branches of genomics; structural genomics and functional genomics has led to the paradigm shift in solving the problems of biology. Structural genomics involves identifying all of the genes within a single species by the sequencing of large collections of complementary DNAs (cDNAs) and/ or total genome sequencing (Bennetzen, 2001). In the intervening time, the post genome era has emerged by taking the full advantage of the genome sequence data (Holtorf *et al.*, 2002).

The first step of unraveling the challenges of biology is to determine the exact sequence and location of all the genes in any given organism. Emergence of the novel tools using the technology can permit the interrogation of the complete genome all at once and in a single experiment (Holtorf *et al.*, 2002). One of the major efficiencies that has emerged from plant genome research to date is that about 54% of higher plant genes can be assigned some degree of function by comparing them with the sequences of genes of known function (Bevan *et al.*, 1988). The genome sequencing of many commercial crops have been completed which is summarized in table 1. The next step is to understand the expression and function of all the genes in an organism. This review contemplates on how the gene function is determined and on the role of transcriptomics and proteomics in the functional genomics.

TABLE1: List of commercial crops in which genome sequencing is completed

Crop	Scientific name	Year of completion	References
Apple	*Malus domestica*	2010	Velasco *et al.* 2010
Castor	*Ricinus communis*	2010	Chan *et al.* 2010
Chickpea	*Cicer arietinum*	2013	Jain and Mukesh 2013
Chinese cabbage	*Brassica rapa*	2011	Wang *et al.* 2011
Cucumber	*Cucumis sativus*	2009	Huang *et al.* 2009
Grape	*Vitis vinifera*	2007	Jaillon *et al.* 2007
Long grain rice	*Oryza sativa ssp indica*	2002	Yu J *et al.* 2002
Maize	*Zea mays*	2009	Schnable P *et al.* 2009
Musk melon	*Cucumis melo*	2012	Jordi Garcia-Mas *et al.* 2012
Papaya	*Carica papaya*	2008	Ray Ming *et al.* 2008
Pigeonpea	*Cajanus cajan*	2012	Varshney R K *et al.* 2012
Potato	*Solanum tuberosum*	2011	Xu, X *et al.* 2011
Soybean	*Glycine max*	2010	Huang S *et al.* 2010
Sugarbeet	*Beta vulgaris*	2013	Juliane, C.D. *et al.* 2013
Water melon	*Citrullus lanatus*	2012	Shaogui Guo *et al.* 2013

Functional Genomics

Functional genomics refers to a suite of genetic technologies that will contribute tremendously to a comprehensive understanding of gene function, as will concurrent studies in other areas of biology (*e.g.* physiology, biochemistry, ecology, *etc*.) (Bennetzen 2001). The initial idea of the function of an unknown gene may be deduced from its sequence structure using already known functions of similar genes as the basis for comparison (Holtorf *et al.,* 2002). The central theme of functional genomics depends on the way the components of the genome interact (Werner, 2010). The next generation sequencing provides the unlimited data for genomics, epigenetics and transcriptomics.

Techniques for large scale expression analysis

Knowing when and where a gene product (RNA and/or protein) is expressed can provide important clues to its biological function. Techniques for gene expression analysis include Northern blotting (detection of a single/few genes by hybridization of labeled probes), Semi-quantitative PCR (visual estimation of the difference in expression level of one or few genes, by PCR, in the cDNA samples reverse transcribed from RNA), Quantitative PCR (estimation of the levels of transcript of one/few genes), Differential display RT PCR (determines the differentially expressed genes between a control and test samples), Serial analysis of gene expression (determine the transcript number of known and novel genes in the tissues tested in terms of short sequences called as tags), microarray, (cRNA hybridized to probes spotted on a chip gives a signal value, which can be used to detect the differentially expressed as well as specific known genes) (Pinky Agarwal *et al.,* 2014). Construction of microarrays and serial analysis of gene expression are the commonly used techniques for large scale expression analysis.

Generation of Expressed Sequence Tags- Prime route for large scale gene discovery

Expressed sequence tag sequencing is generally a prelude to full genome sequencing (Glenn J. Bryan, 2007). Expressed Sequence Tags are obtained by single-pass sequencing of cDNA clones, usually randomly selected from a cDNA library, which represents a tissue of interest (Agnieszka and Jan 2005). ESTs have been collected for many plant species. The most comprehensively surveyed are Arabidopsis (Arabidopsis thaliana; 418,563 in GenBank) and rice (Oryza sativa; 406,624 in GenBank), both of which have also had their entire genome sequenced (Arabidopsis Genome Initiative, 2000; International Rice Genome Sequencing Project, 2005). The first significant potato EST project was reported by Crookshanks *et al.* (2001), who analysed 6077 ESTs, of which 2254 were full length, from a mature tuber cDNA library made from field-grown potatoes (*S. tuberosum* var. Kuras). The list of EST databases are tabulated in table 2.

TABLE 2: List of databases related to EST information

Databases	
Arabidopsis EST's	http://http://www.cbc.med.umn.edu/ResearchProjects/Arabidopsis/
diArk 3.0.	https://www.diark.org/diark
National Center for Biotechnology Information EST database	http://www.ncbi.nlm.nih.gov/dbEST/index.html
The Institute for Genome Research	http://www.tigr.org
Wheat EST Data	https://wheat.pw.usda.gov/NSF/curator/wheat_est.html

Microarrays

Gene expression profiling with microarrays involves mRNA isolation (messenger RNA) and then converting it into fluorescent targets, either DNA or RNA, with or without rounds of amplification (depending on the amounts of starting materials). Targets are then hybridized to the microarrays (Galbraith and Edwards 2010). Once the fluorescent sample is hybridised to a cDNA microarray, unbound material is washed away and the sample hybridized to each element is visualized by fluorescence detection. The use of fluorescent dyes for labelling facilitates the combination of two differently labelled samples in a single hybridization experiment and thus the use of competitive hybridization to reduce experimental error (Aharoni and Vorst, 2001). The core concept of microarrays is that each element provides a unique signal as a result of the hybridization of targets to probes, and, when each signal is measured in parallel, efficient acquisition of information across multiple genes and even across entire genomes becomes possible (Galbraith and Edwards, 2010). The rationale behind this approach is that genes showing similarity in expression pattern may be functionally related and under the same genetic control mechanism (Brown and Botstein, 1999). The use of micoarrays in plants is summarized in table 3.The concept of assigning the gene function using expression profiles was first demonstrated in yeast (Hughes *et al.,* 2000).

The advantages of microarrays in expression profiling are: requirement of a small sample can be completed in limited time as all the genes can be measured once in a single experiment and the accuracy of result. There are limitations like multiple di erent genes (members of the same gene family) will often cross-hybridize, thereby leading to a single spot that hybridizes to more than one gene product. To overcome this limitation, oligonucleotide chips that are unique to individual genes are being used (Bennetzen, 2001). These chips are based on a method to synthesize large amounts of different oligonucleotides in situ on a glass support using light-directed, solid-phase, combinatorial chemistry developed by Affymetrix (Bouchez and Hofte 1998).

TABLE 3:. Reports on the use of DNA microarrays in plants (Source: Aharoni and Vorst 2001)

Biological Context	Plant species	Microarray Type and Scale[a]	Reference
Expression in roots and leaves	*Arabidopsis*	cDNA; 48 clones	Schena *et al.*, 1995
Expression in major plant organs	*Arabidopsis*	cDNA; 1443 clones	Ruan *et al.*, 1998
Strawberry ripening and flavour, flower development	Strawberry, Petunia	cDNA; 1701 strawberry and 480 petunia clones	Lemieux *et al.*, 1998; Aharoni *et al.*, 2000
Expression in rosette leaves of two accessions	*Arabidopsis*	cDNA; 673 clones	Kehoe *et al.*, 1999
Mapping the trait for defense response to fungal pathogen	*Arabidopsis*	Oligo; 412 polymorphisms	Cho *et al.*, 1999
Response to mechanical wounding and insect feeding	*Arabidopsis*	cDNA; 150 clones	Reymond *et al.*, 2000
Response to nitrate treatments	*Arabidopsis*	cDNA; 5524 clones	Wang *et al.*, 2000
Response to treatments with defense related signaling molecules & fungal pathogen	*Arabidopsis*	cDNA; 2375 clones	Schenk *et al.*, 2000
Expression regulated by the circadian clock	*Arabidopsis*	Oligo; 8200 genes represented	Harmer *et al.*, 2000
Expression associated with systematic acquired resistance (SAR)	*Arabidopsis*	cDNA; 10,000 clones	Maleck *et al.*, 2000
Phytochrome A mediated response	*Arabidopsis*	Oligo; 412 polymorphisms	Spiegelman *et al.*, 2000
Expression in developing seeds	*Arabidopsis*	cDNA; 2715 clones	Girke *et al.*, 2000
Expression analysis of the glutathione- *S*-transferase gene family	Maize	cDNA; 42 clones	McGonigle *et al.*, 2000
Identification of downstream genes in MAP kinase 4 signaling pathway	*Arabidopsis*	cDNA; 9861 clones	Petersen *et al.*, 2000
Response to herbivory and herbivore-induced volatiles	Lima bean	cDNA; 2032 clones	Arimura *et al.*, 2000
Expression in different tissues	Rice	cDNA; 1265 clones	Yaza- ki *et al.*, 2000
Diurnal and circadian-regulated genes	*Arabidopsis*	cDNA; 11,521 clones	Schaffer *et al.*, 2001
Expression under drought and cold stresses	*Arabidopsis*	cDNA; 1300 clones	Seki *et al.*, 2001
Identification of repetitive genomic elements in 17 *Vicia* species; phylogenetic resconstruction	*Vicia* spp.	Repetitive genomic fragments; 1152 clones	Nouzova´ *et al.*, 2001
Response to high light	*Synechocystis* sp PCC6803	1.0 kb PCR fragments; 3079 clones	Hihara *et al.*, 2001
Salt stress induced gene expression	Rice	cDNA; 1728 clones	Kawasaki *et al.*, 2001
Gene expression folowing exposure to high salinity	Ice plant, *Arabidopsis*	cDNA; 2600 ice plant and 9212 *Arabidopsis* clones	Bohnert *et al.*, 2001

[a] cDNA, cDNA microarray; oligo, oligonucleotide array.

Serial Analysis of Gene Expression (SAGE)

SAGE allows simultaneous, comparative and quantitative analysis of gene specific, 9- to 10-bp sequence tags (Velculescu *et al.*, 1995). The schematic representation of the SAGE procedure is depicted in figure1. SAGE allows the quantitative and qualitative evaluation of transcripts by identifying the gene corresponding to each tag and by determining the abundance of the individual tags. The comparison of gene expression patterns in different physiological states by SAGE can also provide unbiased and quantitative analysis of the genes that are differentially expressed in a variety of processes (Ji-Yeon Lee and Dong-Hee Lee, 2003). Differentially expressed genes have been identified by changes in tag abundance. In maize, Quantitative reverse transcription-PCR for selected transcripts indicated high correlation with tag frequency (Poroyko *et al.*, 2005). In rice, transcriptome of three major tissues has been surveyed, and the research indicated that most of the tag-identified and up-regulated genes were found related to enhancing carbon- and nitrogen-assimilation, including photosynthesis in leaves, nitrogen uptake in roots, and rapid growth in both roots and panicles (JingYue Bao *et al.*, 2005). In sugarcane, from 480 sequenced clones, 9,482 valid tags were extracted, with 5,227 unique sequences, from which 3,659 (70%) matched at least a sugarcane assembled sequence (SAS) with putative function; while 872 tags (16.7%) matched SAS with unknown function; 523 (10%) matched SAS without a putative annotation; and only 173 (3.3%) did not match any sugarcane ESTs when SAGE is used to characterise the leaf transcriptome (Tercilio and Antonio, 2007).

Comparison of DNA microarrays to SAGE

The ultimate use of DNA microarrays and SAGE in functional genomics is expression profiling. In microarrays, the concept is mRNA hybridization which differentiates it from SAGE. For SAGE analysis prior information of mRNA sequences is not needed. But, in terms of reliabity and accuracy, SAGE is of paramount importance in the expression studies.

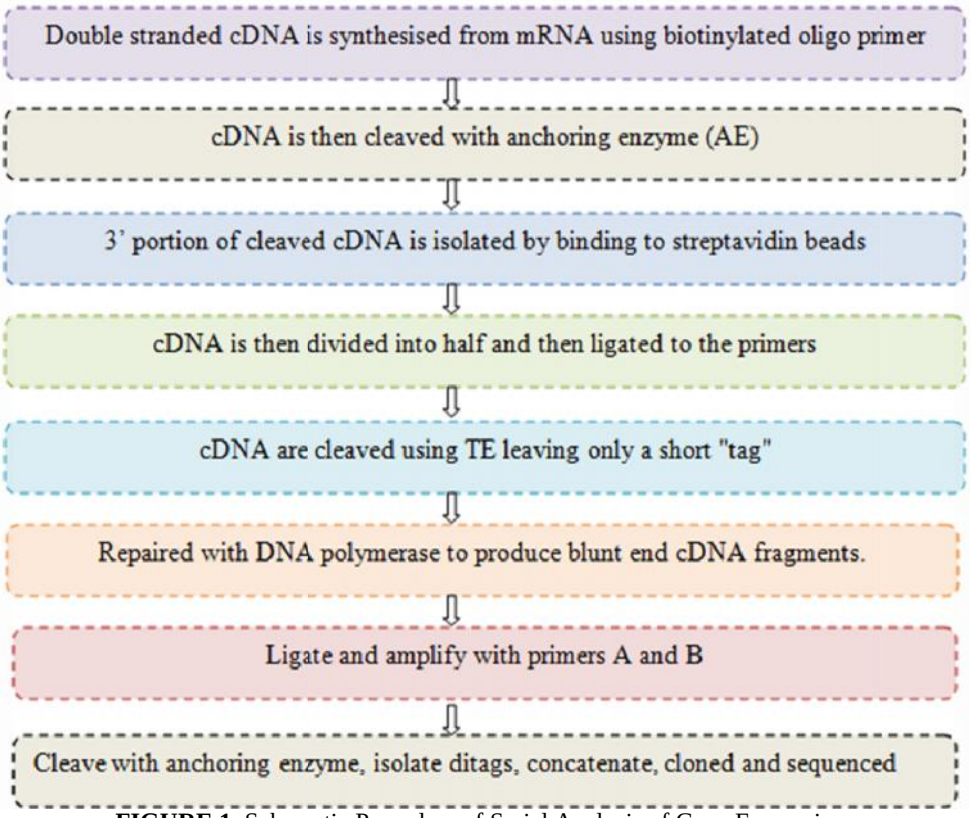

FIGURE 1: Schematic Procedure of Serial Analysis of Gene Expression

Reverse Genetics in Plants

Traditionally, breeders focused on the phenotype of interest, then mutated it to find out which genes are involved in the expression of genotype. However, this strategy was time taking. To overcome this limitation, breeders started using reverse genetic approaches to identify mutations in the gene of interest which are believed to involve some particular process. Mutational approaches have been extremely successful in recent years for the study of the genetic and molecular bases for any trait in plant biology (Bouchez and Hofte, 1998). The marginal frequency of occurrence of direct gene knock out in flowering plants by homologous recombination emphasizes the importance of insertional mutagenesis (Puchta and Hohn, 1996; Reski, 1998; Mengiste and Paszkowski, 1999). In some cases gene knock out may not show an informative phenotype due to the redundancy of genes. In such cases the information of gene expression comes into picture (Holtorf *et al.*, 2002).

Role of transcriptomics

Transcriptomics is the branch of genomics which deals with study of complete set of transcripts encoded in a genome. Transcripts are nothing but the mRNA sequences. Comparison of transcript profiles between healthy and disease plants, or under different external conditions, or as a function of time, reveal the changes in gene expression patterns. Both microarrays and whole transcriptome shot gun sequencing are used in the transcription profiling. To understand the gene function, it is necessary to know not only when, where, how the gene is expressed but also about the other genes that co regulate the gene function (Holtorf *et al.*, 2002). By monitoring the transcriptome function gene function of many unknown genes can be determined. Role of transcriptional modifications and post transcriptional changes in the alteration of gene function can be determined using transcriptomics. The advantage of transcriptome profiling in the functional genomics is the simultaneous analysis of large number of genes while its demerit is it being time-taking process (Holtorf *et al.* 2002).

Role of Proteomics

For the determination of gene function, the information of protein expression is as important as that of mRNA (Bouchez and Hofte, 1998). Proteomics is the branch of genomics which deals with the study of all proteins in the genome. To study the abundance and posttranslational modifications of several hundred proteins in parallel generally two-dimensional PAGE is followed (HumpherySmith & Blackstock, 1997). Mass spectrometry is used for the rapid identification of the components of a complex mixture of proteins; sequencing of proteins and nucleic acids and analysis of post-translational modifications or substitutions relative to an expected sequence. In yeast proteome approach is used to study the gene function through the generation of knockout or over expression mutants and for the analysis of changes in protein profiles on two dimensional gels (Bouchez and Hofte (1988). Santoni *et al.*, 1994 concluded that generalized proteomes can constitute a powerful resource, with future completion of Arabidopsis genome sequencing, for genome-wide exploration of plant function. Besides its applications, there is much scope for the development of the branch of proteomics.

CONCLUSION

The availability of the genome drafts in various crop species has shaped the goal of functional genomics. A variety of techniques are available to determine the gene function. However, there are merits and demerits for all the approaches. Depending upon the facilities available and the objectives of the research, the approach can be selected. To utilise the benefit of the available genomic information on plant genes, only the multidisciplinary integrated approach will allow the functional characterization of plant genes (Holtorf *et al*., 2002). Knowledge of the gene function helps in developing the field of genetic engineering (Chris and Shauna 1999). Steady step up of the transcriptomic technology will offer even more perspectives for the fast and comprehensive analysis of plant gene function.

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