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# A proteomic road to acquire improvement in crop productivity.

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## INTRODUCTION

Genomics is the field of genetics that attempts to understand the content, organisation, function and evolution of genetic information contained in whole genome. Large-scale genome projects have greatly changed the face of biology. Genomics has often been referred to as a new field that has led to a paradigm shift in the way science is performed. By taking full advantage of the vast amount of sequencing data, it has become possible to look at biology in a different way (Varshney *et al vol 1, Springer (2007)*). Meanwhile the genomic era has undergone a transformation in modern post-genomic times. Nowadays researchers do not need to approach biological questions in a hypothesis-driven way but instead can collect and analyse data in a more non-biased and broader fashion (Rounsley and Briggs *1999*; Brent *2000*). New types of scientific questions can be asked and new kinds of experiments can be performed at an unprecedented pace. Recent technological advances and the rapid development of novel tools now permit the interrogation of a complete genome all at once and in a single experiment.

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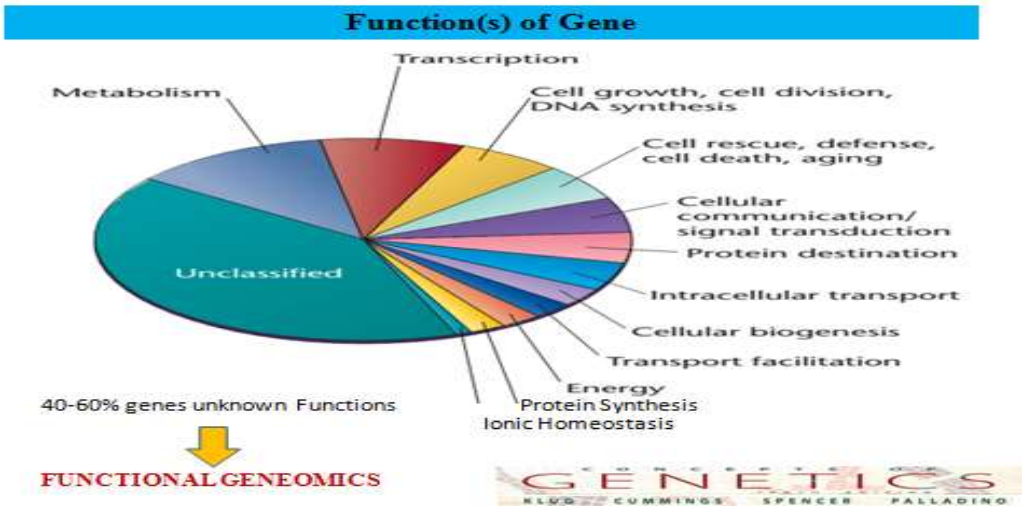
## PROTEOMICS:

Proteomics deals with study of the structure and function of entire set of proteins, present in an organism and hence is essential for studying the whole metabolic pathway. This involves separation, identification, and determination of function and functional network of proteins allowing the integral study of many proteins at the same time. There has been extensive research over last few years to study the technical aspects of proteomics in plants and studies have been conducted in several plant species e.g. rice and Arabidopsis, maize and chickpea. Proteomics enable not only the study of protein–protein interaction but also helps in identification of multisubunit complexes. Furthermore, proteomics can act as a powerful approach to organize and identify the proteome through development of 2-DE gel protein reference maps of sub-proteomes in different plant species.

## FUNCTIONS OF GENE:

In a cell there will be around 40-60% genes with unknown functions. Rest all genes will be known and will be having their specific functions in a cell. So to know these unknown genes and their function one can use the techniques and tools of functional genomics.




## IMPORTANCE OF FUNCTIONAL GENOMICS TO CROP IMPROVEMENT:

**Why functional genomics is important to agriculture?**

The world's population reached 7.3 billion in mid of 2015 and from the last 12 years 1 billion people have been added aprox and there was 2.5 % decline in crop production in world wide. In India crop production deficit observed 1.4% compare to the previous years. The Indian population reached to 1.271 billion. Our agriculture output is less. The current GDP is 14.3%. About 260 million tonnes is our crop production in the year 2015 and we have need of about 400 million tonnes of crop production to full fill the demand of the increasing population. So the with the help of functional genomics we can overcome this problem as this is a globe wise or system wise approach.Knowing the exact sequence and location of all the genes of a given organism is only the first step towards understanding how all the parts of a biological system work together. In this respect functional genomics is the key approach to transforming quantity into quality to crop improvement. Functional genomics is a general approach toward understanding how the genes of an organism work together by assigning new functions to unknown genes.

The process of traditional plant breeding needs more time for development of new variety so with the help of tools (MAS) of functional genomics we can reduce the time barrier and can increase the efficiency in accurate selection of plant which gives the better result as compare to traditional or conventional plant breeding.


# TECHNIQUES OF FUNCTIONAL GENOMICS

## A. CLASSICAL TECHNIQUES/ TOOLS:

- ✓ **INSERTIONAL MUTAGENESIS:**

Insertional mutagenesis is mutagenesis of DNA by the insertion of one or more bases Insertional mutations can occur naturally, mediated by virus or transposon, or can be artificially created for research purposes in the lab.

- ✓ **SEQUENCE BASED MUTAGENESIS:**

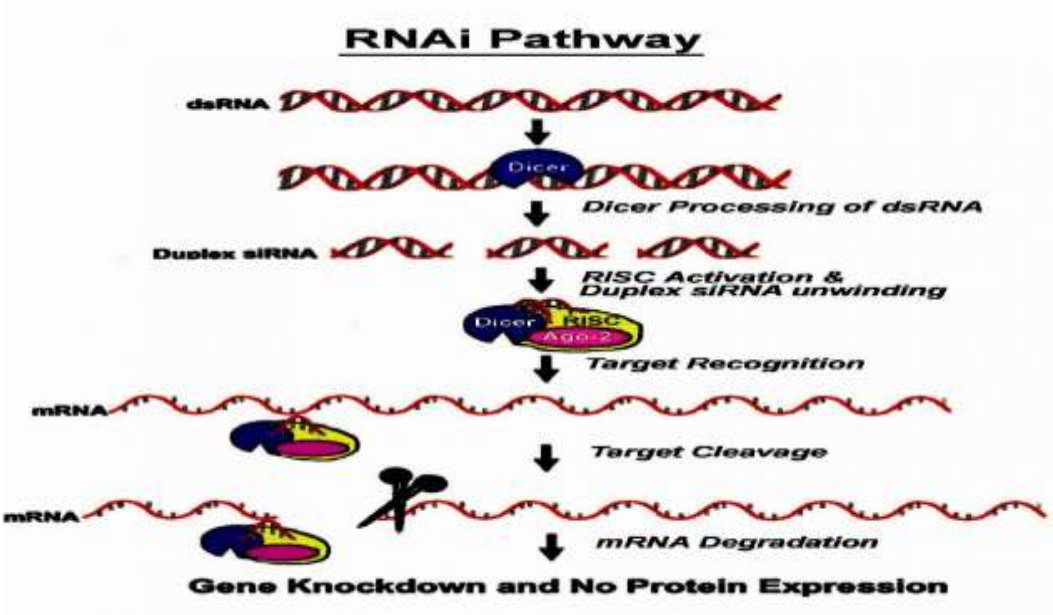
This can be achieved by using Physical & Chemical Mutagens.

- ✓ **TARGET GENE MUTAGENESIS:**

### 1. RNAi TECHNIQUE:

RNA interference (RNAi) methods can be used to transiently silence or knock down gene expression using ~20 base-pair double-stranded RNA typically delivered by transfection of synthetic ~20-mer short-interfering RNA molecules (siRNAs) or by virally encoded short-hairpin RNAs (shRNAs). RNAi screens, typically performed in cell culture-based assays or experimental organisms (such as C. elegans) can be used to systematically disrupt nearly every gene in a genome or subsets of genes (sub-genomes); possible functions of disrupted genes can

be assigned based on observed phenotypes.




**RNAi and Plant Functional Genomics:**

A major challenge in the post-genomic era of plant biology is to determine the functions of all genes in the plant genome. Compared to other techniques, RNAi offers specificity and efficacy in silencing members of a gene or multiple gene family. In addition, the expression of dsRNAs with inducible promoters can control the extent and timing of gene silencing, such that essential genes are only silenced at chosen growth stages or plant organs.

There are several ways of activating the RNAi pathway in plants. The various RNAi techniques have advantages and disadvantages with respect to how persistent their effects are and the range of plants to which they can be applied. These include the use of hairpin RNA-expressing vectors, particle bombardment, Agrobacterium- mediated transformation and virus-induced gene silencing (VIGS).

**Examples of novel plant traits engineered through RNAi.**

| **Trait** | **Target Gene** | **Host** | **Application** |
|---|---|---|---|
| Enhanced nutrient content | Lyc | Tomato | Increased concentration of lycopene (carotenoid antioxidant) |
| | DET1 | Tomato | Higher flavonoid and b-carotene contents |

| | | | |
|---|---|---|---|
| | SBEII | Wheat, Sweet potato, Maize | Increased levels of amylose for glycemic management and digestive health |
| | FAD2 | Canola, Peanut, Cotton | Increased oleic acid content |
| | SAD1 | Cotton | Increased stearic acid content |
| | ZLKR/SDH | Maize | Lysine-fortified maize |
| Reduced alkaloid production | CaMXMT1 | Coffee | Decaffeinated coffee |
| | COR | Opium poppy | Production of non-narcotic alkaloid, instead of morphine |
| | CYP82E4 | Tobacco | Reduced levels of the carcinogen nornicotine in cured leaves |
| Heavy metal accumulation | ACR2 | *Arabidopsis* | Arsenic hyperaccumulation for phytoremediation |
| Reduced polyphenol production | s-cadinene synthase gene | Cotton | Lower gossypol levels in cottonseeds, for safe consumption |
| Ethylene sensitivity | LeETR4 | Tomato | Early ripening tomatoes |
| | ACC oxidase gene | Tomato | Longer shelf life because of slow ripening |
| Reduced allergenicity | Arah2 | Peanut | Allergen-free peanuts |
| | Lolp1, Lolp2 | Ryegrass | Hypo-allergenic ryegrass |
| Reduced production of lachrymatory factor synthase | lachrymatory factor synthase gene | Onion | "Tearless" onion |

### 2. VIGS:

Virus-induced gene silencing (VIGS) is a technology that exploits an RNA-mediated antiviral defense mechanism. In plants infected with unmodified viruses the mechanism is specifically targeted against the viral genome. However, with virus vectors carrying inserts derived from host genes the process can be additionally targeted against the corresponding mRNAs. VIGS has been used widely in plants for analysis of gene function and has been adapted for high-throughput functional genomics.Creation of engineered viruses carrying sequences corresponding to the host gene to be silenced.

- ✓ Infection leads to synthesis of viral dsRNA.
- ✓ Results in down regulation of the host gene transcript.
- ✓ It provides robust silencing, has a broad host range, can infect meristematic tissue, and produces only mild disease symptoms.

**Examples:-**

- ✓ Silencing of phytotene desaturase gene (PDS) in *N.benthamiana* plants.
- ✓ Recently, the turnip yellow mosaic virus has been adopted for VIGS in Arabidopsis and provides silencing following mechanical inoculation with a plasmid carrying engineered virus. (Pfliger *et al.*2008).

## PROTEIN LEVEL: (PROTEIN PROTEIN INTERACTION):

### 1. AP/MS:

Affinity purification and mass spectrometry (AP/MS) is able to identify proteins that interact with one another in complexes. Complexes of proteins are allowed to form around a particular "bait" protein. The bait protein is identified using an antibody or a recombinant tag which allows it to be extracted along with any proteins that have formed a complex with it. The proteins are then digested into short peptide fragments and mass spectrometry is used to identify the proteins based on the mass-to-charge ratios of those fragments.

### 2. YEAST TWO-HYBRID SYSTEM:

A yeast two-hybrid (Y2H) screen tests a "bait" protein against many potential interacting proteins ("prey") to identify physical protein–protein interactions. This system is based on a transcription factor, originally GAL4, who's separate DNA-binding and transcription activation domains are both required in order for the protein to cause transcription of a reporter gene. In a Y2H screen, the "bait" protein is fused to the binding domain of GAL4, and a library of potential "prey" (interacting) proteins is recombinant expressed in a vector with the activation domain. In vivo interaction of bait and prey proteins in yeast cell brings the activation and binding domains of GAL4 close enough together to result in expression of a reporter gene. It is also possible to

systematically test a library of bait proteins against a library of prey proteins to identify all possible interactions in a cell.

## CONCLUSION:

- Functional genomics is a field of molecular biology that attempts to make use of the vast wealth of data produced by genomics and transcriptomics to describe gene (and protein) functions and interactions.
- It focuses on the dynamic aspects such as gene regulation, transcription, translation, gene expression and protein-protein interaction
- It attempts to answer questions about the function of DNA at the levels of genes, RNA transcripts, and protein products

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