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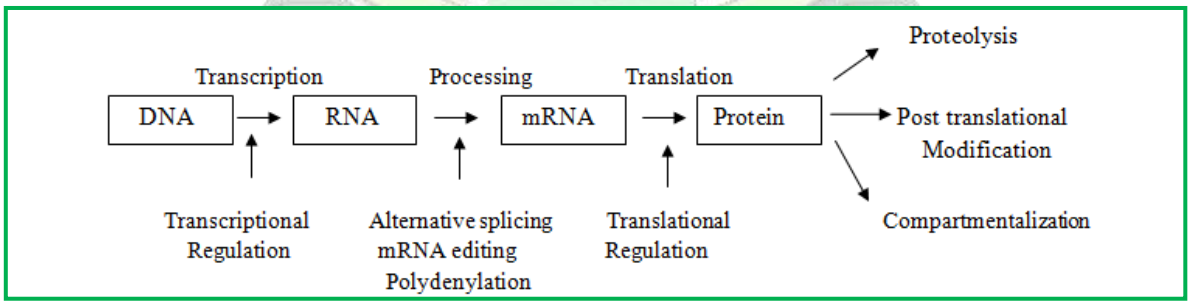
Role of Proteomics in Crop Improvement

(*Mohana Sundaram K)
Department of Plant Breeding and Genetics, Krishna College of Agriculture and Technology, Usilampatti, Madurai, Tamil Nadu
*Corresponding Author's email: kmsagri@gmail.com

Proteomics is the study of proteins and their interactions in a cell. It is the large scale study of proteins particularly their composition, structures, functions, and interactions of the proteins directing the activities of cell (Wilkins *et al*.,1995). Proteomics has become a useful tool and the emphasis is shifting from genomics to the protein compliment of the human organism. The main goal of proteomics is to study, know and understand "how", "where", "when", and "what for" are the several hundred thousand of individual protein forms produced in a living organism, how do they interact with one another and with other molecules to construct the cellular building, how can they be modified and work in order to fit in with programmed growthand development, and to interact with their biotic and a biotic environment (Smith *et al*.,2013). The terms 'proteomics and proteome' was coined by Wilkins *et al.* in 1995. The of analysis of proteins from tryptic digests using mass spectrometry combined with good separation techniques is capable of providing rapid and confident protein identifications (Buts *et al*.,2014).

Necessary of proteomics

- Proteomics having complete sequences of genome is not sufficient to elucidate biological function.
- It involved in the regulation of a wide range of biological processes, and affect *e.g.* protein structure, activity and stability.
- It is necessary to determine the protein expression level
- The localization of gene products can be determined experimentally
- Protein-protein interactions
- Modifications of proteins can be determined only by proteomic methodologies



Types of Proteomics

1. **Functional Proteomics:** The identification of protein functions, activities or interactions at a global or organism wide scale
2. **Expressional Proteomics:** The analysis of global or organism wide changes in protein expression

3. **Structural Proteomics:** The high throughput, or high volume expression and structure determination of proteins by X ray, Nuclear Magnetic Resonance spectroscopy (NMR) or computer based methods

Proteomic technologies

1. Two-dimensional polyacrylamide gel electrophoresis: Two Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) is used to separate proteins from a mixture providing information such as: molecular weight, isoelectric point, presence or absence of proteins in a sample and PTMs (Lodha, *et al*., 2013). Nowadays, 2-D PAGE analysis is often coupled with the MS technology and it has provided with higher resolution, improved reproducibility, and higher loading capacity for preparative purposes with the intent of subsequent spot identifications (Kim *et al*., 2013)

2. Mass Spectrometer for protein characterization: A relatively new and rapidly evolving development in proteomics research has been the application of mass spectrometry (MS) which, in conjunction with the development of comprehensive protein databases (Kamal *et al*.,2015). Biomolecules and synthetic polymers have low volatility and are thermally unstable, which has limited the use of MS as a means of characterization. These problems have been minimized through the development of MALDI-TOF, which allows for the mass determination of biomolecules by ionization and vaporization without degradation.

3. Protein Identification by MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time of Flight): MALDI is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules (proteins, peptides and sugars) and large organic molecules (such as polymers and other macromolecules), which tend to be fragile and fragment when ionized by more conventional ionization methods. Ionization is triggered by a laser beam. The data are then processed through a series of computer algorithms that determine the sequence identity of the proteins, and to some extent, their state of modification. It has been reported that a reduction in loss of some post-translational modifications can be accomplished if MALDI is used instead of UV MALDI. MALDI/TOF spectra are used for the identification of microorganisms such as bacteria or fungi. The accurate determination of protein molecular weights is mainly achieved using a MALDI-TOF instrument. These peptide mass finger prints are compared with a data base of virtual peptide mass finger prints generated by the theoretical digestion of known proteins by specific proteases (Kamal *et al*., 2015).

Databases for Identification of mass-spectra

- **SWISS-PROT:** It is a database of annotated protein sequences; it also contains additional information on function of the protein, its domain structure, posttranslational modification(s), *etc*.;
- **TrEMBL:** It is a supplement to SWISS-PROT, which contains all protein sequences, translated from nucleotide sequences of the EMBL database;
- **PIR-International:** (Protein Identification Resource, National Biomedical Research Foundation, Washington, USA) is also an annotated database of protein sequences;
- **NCBInr:** (National Center of Biotechnological Information) is a database containing sequences translated from DNA sequences of GenBank and also sequences from PDB, SWISS-PROT, and PIR databases;
- **ESTdb:** (Expressed Sequence Tags database, NCBI, NIH).
- Programs operating with MS/MS only (SEQUEST, PepFrag, MS-Tag, Sherpa).

Protein identification software

- UCSF(http://prospector.ucsf.edu)
- PROWL (http://prowl.rockefeller.edu)

- MASCOT (http://www.matrixscience.com)

Uses of Proteomics

- Protein Mining – catalog all the proteins present in a tissue, cell, organelle, *etc*,.
- Differential Expression Profiling – Identification of proteins in a sample as a function of a particular state: differentiation, stage of development, disease state, response to drug or stimulus
- Network Mapping – Identification of proteins in functional networks: biosynthetic pathways, signal transduction pathways, multiprotein complexes
- Mapping Protein Modifications – Characterization of post-translational modifications: phosphorylation, glycosylation, oxidation, *etc*.,

Application of Proteomics in Crop Improvement

Proteomics has contributed to defining the specific functions of genes and proteins involved in plant-pathogen interactions. Proteomic studies have led to the identification of many pathogenicity and defense-related genes and proteins expressed during phytopathogen infections, resulting in the collection of an enormous amount of data. Resistance acquired upon induction of stress related proteins in intact plant leaves is mediated by potentiation of pathogens via signal elicitors. Stress related proteins identified must be followed through for studying the molecular mechanism for plant defense against pathogens.

Proteome profiling of soybean organs has been reported for leaves, root nodules, root hair cells, the cytosol of nodules and seeds. Extensive proteomic studies have been performed to unravel the difference in chloroplasts between bundle sheath and mesophyll cells in maize. Comprehensive analyses of rice leaf proteome in response to cold stress revealed that photosynthetic proteins are largely affected under cold stress. Proteomic profiles of salt responsive proteins have been generated in major crops like rice, wheat, grapevine, potato and soybean.

To meet the current challenges of food insecurity, genes and proteins that control crop architecture and/or stress resistance in a wide range of environments will need to be identified to facilitate the biological improvement of crop productivity. However there is need of integration of genomics, trancriptomics and proteomics to facilitate understanding of normal function, disease, and development.

Conclusion

Genes may be the stuff of life, but they're not the whole story. Scientists wrapping up the mammoth task of decoding the human genome say the next step will be understanding the "proteome", the proteins that genes help make.

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