International Journal of Psychosocial Rehabilitation, Vol. 23, Issue 05,
2019 ISSN: 1475-7192

Development of Proteomics in the Assessment of Genetically Modified Tomato

[1]Swarna Prava Chhuria, [2]Subhalaxmi Ray

Abstract--- *Variations in the membrane proteome of the pericarp tomato fruit have been investigated during maturation using label-free mass spectrometry proteomics. The ' substantial equivalence ' is currently the only widely accepted criterion to assess whether or not a transgenic crop is in food view, fully consistent with the ' traditional ' food from which it is derived. Even though, there is no exact comparison defined which deals with the comparison between the chemical compositions of the two foods. One of the most suited methods for the simultaneous screening of many components without previous identification, proteome analysis, can be used for a more in-depth analysis. Food security, with the provision of food to 9 billion people in 2050, is a key global challenge in plant biotechnology. New crop species must be tolerant of environmental stresses caused by climate change, better yields, more nutrition and less resource consumption to achieve this change in agricultural production. In order to supply these new crop varieties, genetic changes and marker-assisted screening are necessary along with the system level characterization that is important for evaluating these varieties both in terms of food safety and the efficient designing of traits. For the shotgun proteomics of tomato fruit, an optimized protocol was developed, which is a re-calcitrant tissue due to a high percentage of sugars and secondary metabolites.*

Index Terms--- *Marker-assisted screening, Proteome analysis, Re-calcitrant tissue, Spectrometry proteomics, Shotgun proteomics, Secondary metabolites.*

I. INTRODUCTION

The critical and analytically demanding side of modern biology is quantitative proteomics. This is especially true in plant and food applications, where the abundance of proteins is small and a wide range of proteins is available. A broad scope of scientific knowledge, embodied by nutritionists, microbiologists, toxicologists, genetic engineers, food technology specialists, customer representatives and ethicians, is important to evaluate food safety and particularly in the context of novel foodstuffs. During the 1990s, the FAO, OECD and WHO (World Health Organization) raised the question of the handling of novel foods, notably those from genetic modification (GM food), as well as of the organization of Food and Agriculture.

The proteome represents the connection between the transcriptome and metabolome, thereby providing a key level of cellular regulation [1]. According to various experimental studies, various methods have been developed for the study of protein levels in plants which could be classified into the two different methodologies such as: i) utilizing the

Department of Agriculture, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, swarnaprava@soa.ac.in, subhalaxmi@soa.ac.in

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019

labeling techniques which helps in employing stable isotopes [2], and ii) utilizing the label-free techniques. Most widely used techniques for stable isotopic labeling include the modification of peptides with isobaric labels for relative and absolute quantitation and the in vivo labeling of proteins with isotopic tags of affinity and the use of heavy proteins such as 15N or 13C, which are integrated as organisms evolve, as well as the use of heavy isotopes such as 15N or 13C. There are two different strategies that are commonly applied in the case of label-free solutions. Firstly, quantitation based on detection frequency; a method usually referred to as spectral counting. This approach is essentially based on the count of the total number of spectrums for a protein identified in an MS / MS experiment. The other approaches quantify based on peak strength, in accordance with the concentration of this specific protein which derive peptides in the chromatographic peacemaking areas of peptides.

Tomato (Solanum lycopersicum L.) is the most commonly used model for the study of different aspects of the production and maturation of fresh fruits, produced worldwide that are of high economic and nutritional value. It is a good model for fleshy fruit maturation due to high-quality genome sequences, mutant sets, healthy wild families, simple transformation, etc. The molecular basis of tomato is commonly used for fruit maturation at the transcriptome and metabolome stages [3]. The significant linkage between the transcriptome and metabolome is achieved by the Proteome [4]. Protein profiling recognizes the regulatory components that mediate different pathways and the proteins that can help to enhance food quality, fragrance, disease resistance / tolerance, shelf-life, etc., as the protein functionally reflects the Genome.

Plant breeders are increasingly using a variety of genes to alter crops that could lead to new metabolic pathways. As a result, it becomes increasingly difficult to evaluate the significant equivalence and examine single compounds almost pointless. New methods that allow the simultaneous screening of many components without a prior identification are therefore important to develop. A systematic comparative study can be supported by molecular profiling. This approach includes various technologies such as DNA microarrays [5], proteomics, mRNA profiling and metabolism. Plant genome studies provide pertinent information about protein synthesis ability, but not about actual protein expression. Generally, the association between mRNA and protein levels is low, as degradation rates for each mRNAs and protein differ. In addition, glycosylation and phosphorylation may lead to an altered protein activity and stability of the ingested gene product. The biological dynamics of the plant cell have to be further developed by using proteomics, a method that simultaneously analyzes several proteins. Actually, the technique of choice for protein expression studies is two-dimensional Polyacrylamide Gel electrophoresis (2- DE) [6]. Excision of protein spots from gel, fragmenting into proteases and subsequent analysis by mass spectrometry (i.e. fingerprints of peptide mass) enables protein to be detected when combined with data from the genetic sequence or protein knowledge of the mass of peptide fragments in particular. Other technologies, such as protein chips, are of significant use and are being developed nowadays. Both mRNA and proteomics rely on genomic databases in order to identify and characterize the function.

The fruit maturation is a highly coordinated genetically programmed permanent phenomenon that corresponds with the seed maturation of the plant development phase and includes a number of physiological, biochemical and organoleptic changes that lead to changes in “color, texture, taste, aroma and nutritional status” [7]. It is believed to be

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019

the main hormonal regulator in the ripening of climatic fruit while the other hormone including auxin and abscisic acid (ABA) participates in it. This cycle is regulated by significant hormonal changes. When the fruit is grown, glucose, amino acid and biological acids are reduced immediately and partially restored during fruit maturation with increased sugar levels, hydrolysis of the starch, decreased acidity, pulp softening and color development [8].

The genome from Te tomatoes has recently been sequenced and a variety of post-genomic methods have been used to gain insights into fruit production and maturation molecular networks. According to a recent study, the analyses for the "high-throughput proteomics" have been published. In addition, analyses were performed on the fruit transcriptomes and metabolome as well as multi-level studies that combine transcriptomics and metabolomics or profles transcriptomics and enzymes and transcriptomics, proteomics, and metabolomics. Such experiments have provided an enormous amount of information that enhances the knowledge related to the molecular events.

While many enzymes are soluble proteins, they make proteomics research more difficult to cover membrane- or membrane-associated proteins. In this work, at the first and last stage of the ripe process, we got a proteome profiling of the microsomal tomato pericarp fraction, such as the mature green (MG30) and the red ripple (R45)14 [9], in order to eluciate the most significant biochemical pathways related to the start of the ripening procedure on the one hand, and the final mature stage, with the particular. With this method, 1315 proteins were accurately identified, of which 145 differed considerably during the deux phases of fruit maturation. In fact the most significant changes in microsome protein depth between MG30 and R45 are mainly related to the cell wall reconstruction, vesicle trafcking, and lipid metabolism and ethylene biosynthesis, as well as to glycolysis, gluconeogenesis and TCAs [10]. Figure 1 shows the experimental design performed using the independent biological replicates for each fruit stage.

Recently, shotgun proteomics is one of the most preferred methods for proteome profiling. In profiling complex mixtures of proteins, 2D-LC in conjunction with MS / MS, MudPIT (Multidimensional Protein Identification Technology) is used. Although several labeling techniques for proteome profiling are available, their inherent limitations, including high costs and inaccurate labeling, have made label-free quantification for researchers more viable [11].

Due to the complexity of the samples, and the large dynamic protein abundance, it is important to optimally detect proteins by optimizing LC and MS parameters. Data acquisition is the most commonly used method of shotgun proteomics and involves a set of data collection MS parameters. In tomato fruits, proteomic shotguns were used to obtain proteome profiles in few studies where GeLCMS was used with purified chromoplast proteins or Strong cation chromatography with nano LCMS protein. Nevertheless, no justification for preferring any such methods was given. Considering the problems which are involved in the extraction of proteins, the effect of several LC conditions on proteomic coverage and MS parameter were analyzed for profiling of tomato plant.

II. IDENTIFICATION AND PROTEIN ENRICHMENT OF THE TOMATO MEMBRANE:

The tomato fruits tested at 30 and 45 days post - anthesis (DPA) and corresponding to the maturing phases MG30 and R45, were formed as a full microsome. Such phases lead to the first stage of maturation (MG30) and to the mature

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019

fruit (R45). In the Te Protocol sequential steps were taken (figure 1) with the help of a low ion resistance buffer to ensure that membrane assigned supramolecular (e.g. metabolons) complexes were organized and change in membrane-related processes were better evaluated in the development. The enrichment of the membrane proteins in total microsomal fraction was analyzed by using the Western blotting technique. While the initial homogeneity of the nuclear, chloroplastic and mitochondrial markers showed presence, both MG30 and R45 samples showed negligible presence; the enrichment was instead shown by Golgi and ER (figure 2A) [12].

The sample specificity was minimized by subjecting the MG30 and R45 protein fractions to a monodimensional SDS-PAGE and the nano-LC-MS / MS analyzes were in-gel digested in ten slices. The MaxQuant software package for the ITAG v 2.3 protein databases has carried out protein recognition and quantification. The MaxQuant software package against the ITAG v 2.3 protein databases was performed for protein recognition and quantification. In total, a FDR<0.01 and a minimum of one specific peptide were classified as separate protein / protein group. For high quality datasets of 1315 proteins for quantitative proteomic analysis, strict criteria have been applied.

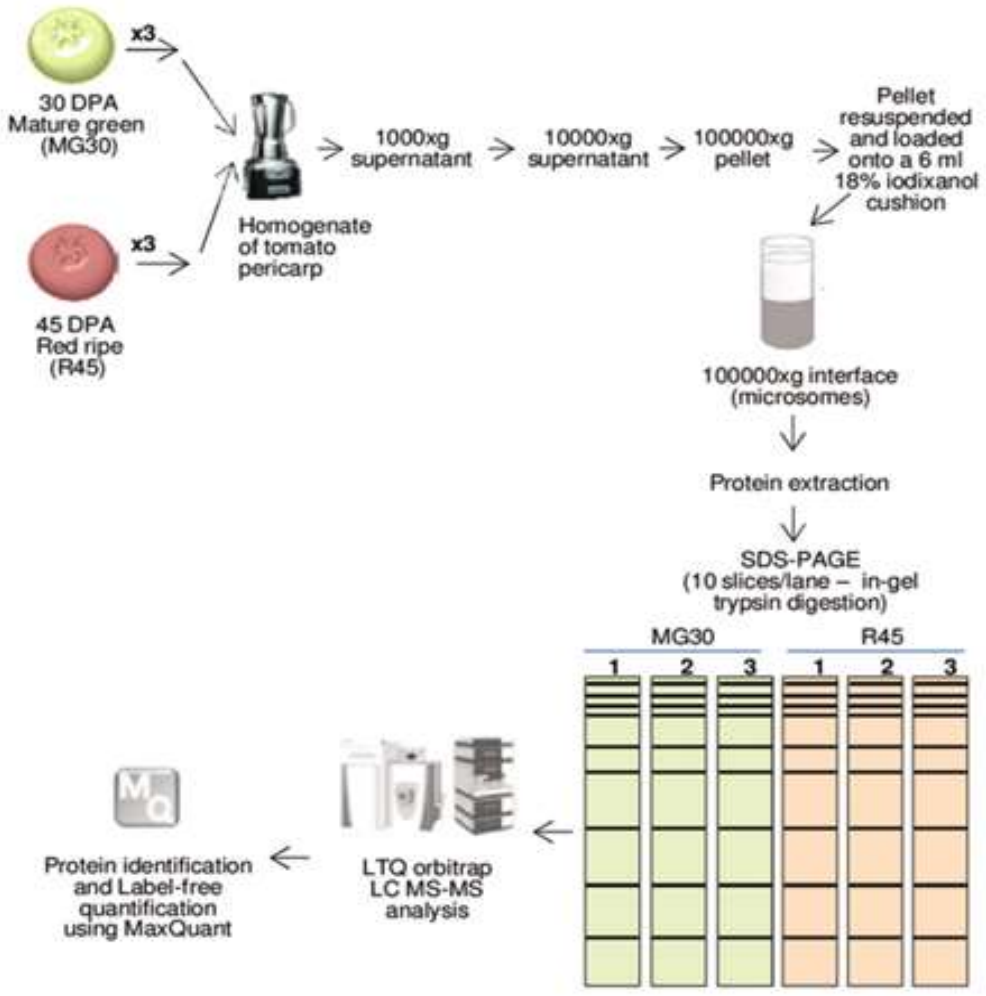

Fig.1: Experimental design. The experiment was performed using three independent biological replicates for each fruit stage. DPA, days post-anthesis.

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019

III. ANALYSIS OF THE FUNCTIONAL CLASSIFICATION AND EXPRESSION PROFILE:

The ITAG annotation for the tomato genome was used to annotate Te 1315 protein sequences. The high-confidence ER markers BiP and Golgi markers of the Coatomer subunit gamma were identified as proteins with the putative transmembrane region, with a signal peptide of 105 being translocated to the endoplasmic reticulum (ER) [13]. Moreover, the signal peptide only contained 91 proteins. In comparison with the total tomato fruit proteome, the proteins found were classified by the "PANTHER GO" classification system instead of "GO" term enrichment. The GO terms extracellular area, ribosomal subunits, ER to Golgi transport membranes, endosome as well as cytosol were found to be enriched in particular (figure 2B).

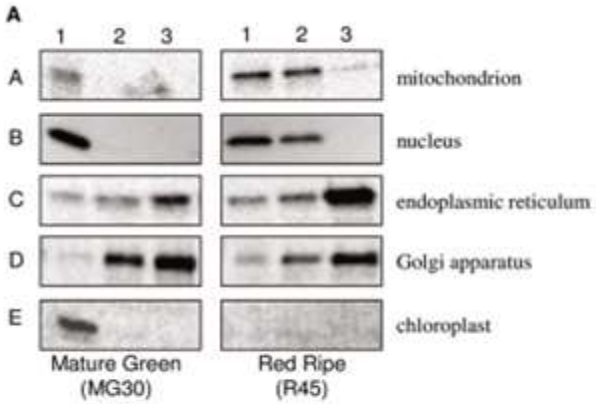

Fig.2A: Assessment of the progressive enrichment of Western blot analysis of protein extracts

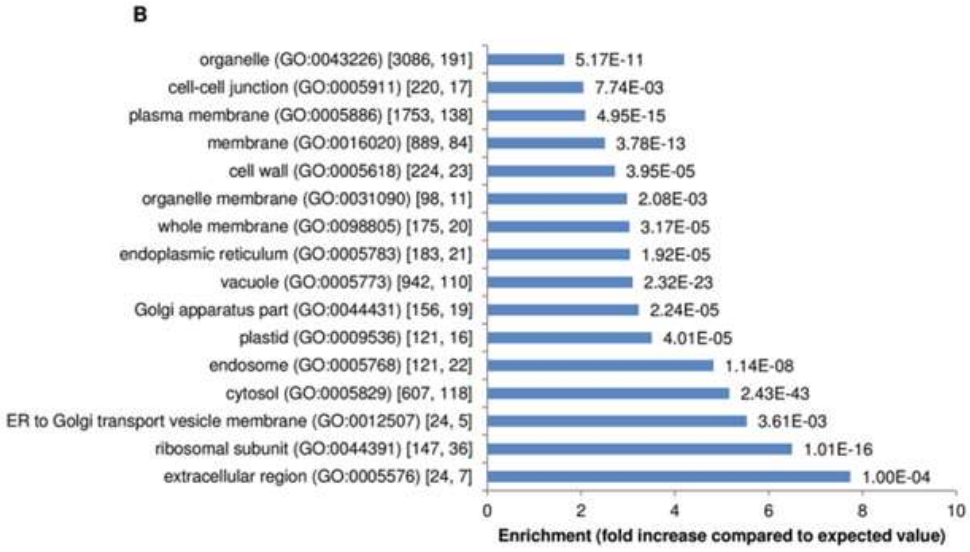

Fig.2B: Cellular component GO-term enrichment analysis for all the identified proteins using PHANTER analysis tool

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019

IV. CONCLUSION

It is widely accepted that an evaluation of transgenic foods is healthy as their corresponding wild-type is not appropriate in the present definition of a meaningful equivalence. The systematic review of various sample preparation protocols and MS parameters established an optimal procedure for the proteome isolation and identification of tomato fruits. The most effectively extracted proteins accompanied by TCA are used with buffer saturated phenol. GeLCMS has been the best method for fractionation of the protein. According to the studies, it has been demonstrated that to understand the GM foods, it is important to analyze the effect of every gene manipulation, which is the possible modification of its proteome. By drawing 2-DE maps of tomato seedlings protein, it has been demonstrated that the inclusion of a resistance trait in our hybrid does not lead to significant changes in the expression of proteins in comparison with the animals. As with all methods of profiling, the evaluation of important protemic equivalences requires further development and validation before routinal screening for unintended effects in transgenic plants can be carried out. It should nevertheless be taken into consideration that the general public will hopefully, after a routine proteomic analysis, accept even a genetically modified plant, free from a marker gene and not expressing a transgenic protein.

REFERENCES

[1] K. L. Eckel-Mahan, V. R. Patel, R. P. Mohney, K. S. Vignola, P. Baldi, and P. Sassone-Corsi, “Coordination of the transcriptome and metabolome by the circadian clock,” Proc. Natl. Acad. Sci. U. S. A., 2012.
[2] C. Holme, V. Gkinis, and B. M. Vinther, “Molecular diffusion of stable water isotopes in polar firn as a proxy for past temperatures,” Geochim. Cosmochim. Acta, 2018.
[3] P. Pesaresi, C. Mizzotti, M. Colombo, and S. Masiero, “Genetic regulation and structural changes during tomato fruit development and ripening,” Frontiers in Plant Science. 2014.
[4] N. E. Gapper, J. J. Giovannoni, and C. B. Watkins, “Understanding development and ripening of fruit crops in an ‘omics’ era,” Horticulture Research. 2014.
[5] C. Nguyen and X. Gidrol, “DNA microarrays,” in Nanoscience: Nanobiotechnology and Nanobiology, 2010.
[6] S. Magdeldin et al., “Basics and recent advances of two dimensional-polyacrylamide gel electrophoresis,” Clinical Proteomics. 2014.
[7] T. Tohge, S. Alseekh, and A. R. Fernie, “On the regulation and function of secondary metabolism during fruit development and ripening,” Journal of Experimental Botany. 2014.
[8] L. Su et al., “Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance,” BMC Plant Biol., 2015.
[9] H. V. Kilambi, K. Manda, H. Sanivarapu, V. K. Maurya, R. Sharma, and Y. Sreelakshmi, “Shotgun proteomics of tomato fruits: Evaluation, optimization and validation of sample preparation methods and mass spectrometric parameters,” Front. Plant Sci., 2016.
[10] D. Pontiggia et al., “Changes in the microsomal proteome of tomato fruit during ripening,” Sci. Rep., 2019.
[11] G. Kahl, “Shotgun proteomics,” in The Dictionary of Genomics, Transcriptomics and Proteomics, 2015.
[12] I. A. Brewis and P. Brennan, “Proteomics technologies for the global identification and quantification of proteins,” in Advances in Protein Chemistry and Structural Biology, 2010.
[13] O. Jouffroy, S. Saha, L. Mueller, H. Quesneville, and F. Maumus, “Comprehensive repeatome annotation reveals strong potential impact of repetitive elements on tomato ripening,” BMC Genomics, 2016.

Received: 10 Nov 2018 | Revised: 20 Dec 2018 | Accepted: 02 Jan 2019