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GLOBAL APPROACHES FOR IDENTIFICATION OF MARKERS OF SEED QUALITY

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ABSTRACT: ***The term 'Global or Genome Wide' means the study of expression of thousands of genes at once to create global picture of cellular function. The study of '-omics' is important to know the structure, functions & regulation of genes, to understand evolution & relationships of genomes and to interpret interactions between the genes and its environment. Studies using '-omics' approaches support the finding that a main contributor of seed germination success is the quality of the messenger RNAs stored during embryo maturation on the mother plant. Use of global approaches such as transcriptomics, proteomics or metabolomics analysis could also result in the identification of new markers of seed quality. Transcriptome analysis are being used to understand the molecular mechanisms involved in dormancy loss by comparing the transcriptome of dormant and after-ripened embryos using a tissue-specific microarray approach. Proteomics have been used to describe the effect of different factors involved during germination and different developmental stages. The developmental changes triggered by internal programming and response to environmental factors induce metabolic transitions that result in modifications of final metabolite concentration. The characterization of biomarkers of seed vigor for seed improvement via breeding programs and/or technological and biotechnological approaches will allow the production of seeds of the highest possible quality with the goal of improving crop yields, particularly under stressful environmental conditions. There is need to identify areas in seed science that need attention from transcriptomic, proteomics and metabolomic based researches.***
Keywords: seed quality, transcriptomic, proteiomic, metabolomic

INTRODUCTION

Seed quality is determined by a number of physiological principles related to important plant developmental processes, such as embryogenesis, growth, stress-resistance and the transition from a seed to seedling. Seed quality attributes include germination (percentage, rate and uniformity), dormancy, seed and seedling vigor (germination/growth under stress conditions), seedling dry weight, and normal embryo and seedling morphology, as well as the ability to develop into a healthy plant (Corbineau, 2012; McDonald, 1998). The molecular-genetic dissection of these seed processes and their relationship with seed and seedling phenotypes will ultimately identify the regulatory genes and signaling pathways and, thus, provide the means by which to predict and enhance seed quality. Research to date has tended to focus on the nutritional quality of crops as economic values of seed quality rather than on genetic mechanisms regulating seed quality traits. The issue has grown in importance in the light of proven associations between mother plant traits and seed metabolism and vigor on a genomic scale. These integrative –omics approaches could facilitate the understanding of the underlying causes of complex traits of seed quality. Another promising expectation is that the Genralized Genetical Genomics (GGG) model could lead to a better understanding of environmental and stress responses of seeds as part of integrative seed quality traits, including seed performance under biotic and abiotic stress (Kazmi et al., 2013).

The study of omics is important to understand the evolution, structure and relationships of genomes. It also helps to isolate genes, finding their structure and function and to understand interaction among genes and between the genes and its environment. The global approaches such as genomics, transcriptomic, proteomic and metabolomic aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms (Syrenne et al., 2012). Global molecular profiling can be monitored at the three levels of gene expression, transcripts, proteins, and metabolites.A better understanding of the biochemical, cellular and molecular mechanisms involved in the acquisition of seed vigour during seed development, in the germination process and in seed deterioration during ageing could suggest various markers of seed quality besides

the classical physiological tests. Post-genomics studies have provided important new information about mechanisms controlling germination. Therefore, the main focus will be on transcriptomic, proteomic and metabolomic approaches for the identification of markers of quality. Genome means one complete copy of the genetic information or one complete set of chromosomes (haploid) of an organism whereas genomics is sub discipline of genetics devoted to the mapping, sequencing, and functional and comparative analyses of genomes(Catusse et al., 2008; Syrenne et al., 2012).

Biochemical Markers

Several studies have been carried out to characterize different cultivars for quality and agronomic properties. In particular, the use of biochemical analysis to identify cultivars has been ongoing for many years. The ability to distinguish between and identify cultivars of species is a fundamental to the operation of seed testing and to modem crop production. Most electrophoretic systems employ either starch, polyacrylamide or agarose gels for protein/ isoenzyme separation based on molecular size and charge density. Another approach is the use of isoelectrically focused gels that separate proteins/ isoenzymes based on their position within a pH gradient (Smith and Register., 1998; Della Vecchia and Da Silva, 1998; McDonald, 1998). Electrophoresis has been used in isoenzyme studies to analyze not only changes in physiological seed quality, but also in genetic and biochemical regulations (International Seed Testing Association [ISTA], 1992). Since then, enzyme markers have been highlighted as valuable tools, because they assist in the identification process of the physiological state of seeds and may also help to understand factors that result in reductions in vigor and viability (Veiga et al., 2010). Protein/ isoenzyme electrophoresis can be successfully used in more than 80% of the cases where genetic purity determinations are required for seed testing(Cruz et al., 2013; Smith, 1984). The successful exploitation of electrophoresis for plant variety identification relies on the fact that proteins are the products of structural genes. By considering sufficient protein markers a large portion of the genome can be an analysis of gene expression and can be used as an ideal means of varietal discrimination (Cooke, 1992). Seed storage proteins have been less conserved in discriminating than other plant proteins although isozymes have got wider acceptance (Siva and Krishnamuthy, 2005; William et al., 1993; Ramiro et al., 1995).

Molecular Markers

The development of a molecular marker linked to a trait of interest using different molecular marker techniques allows the monitoring of similarity/dissimilarity among different genotypes at the very early stages of plant development, independent of environmental effects. Molecular marker assisted identification with high power of genetic resolutions has emerged as a robust technique for cultivar fingerprinting, identify profiling, estimating and comparing genetic similarity and variety protection. This knowledge can directly be used in a marker assisted selection program in plant breeding to identify a desirable genotype in a segregating population.

DNA-based markers allow direct comparison of the genetic material of two individual plants and have been used quite extensively. Molecular markers provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors. Assays based on the Polymerase Chain Reaction (PCR) are considered to meet both the technical and genetic requirements for the characterization of plant and animal genetic resources (Wolfe and Liston, 1998). RAPD markers have been demonstrated to be useful for the studies of taxonomic identities, systematic relationships, population genetic structure, species hybridization and parentage identifications (Fahima et al. 1999; Wachira et al., 1995). The use of seed genomic DNA for developing RAPD fingerprints based on arbitrarily primed PCR reactions can be a time-saving and cost-effective technique for genetic purity testing of seed lots for seed certification (Crockett et al., 2002; Smith and Register, 1998; Ballester et al., 1998).

Among available DNA markers systems, PCR based co-dominant Simple Sequence Repeats (SSRs or microsatellites) are preferred for genotyping because of their reproducibility, abundance and amenability to high throughput screening. The SSR markers are of great importance for rapid assessment of hybrid and parental line seed purity (Kumar et al., 2014; Hipi et al., 2013; Dongre et al., 2011, 2012; Liu et al., 2007; Sundaram et al., 2008). Sequence tagged microsatellite (STMS) markers have also been used for rapid genetic purity assessment of the hybrid and parental lines (Rana et al., 2007; Mohapatra et al., 2003).

Transcriptomics

Seed development is associated with enormous differential gene expression depending on growth stage, setting the importance of -omics in context for seed development and improvement. With the availability of high precision -omics tools for biological research, lots of investigations are undertaken globally to answer the physiological questions underlying seed germination and invigoration. The increasing -omics datasets constitute important resources for the delivery of new seed vigor markers and advancing new seed vigor manipulation opportunities. Transcriptomics is the study of the RNA transcripts of a cell, tissue, or organism i.e. the transcriptome. The transcriptome can be seen as a precursor for the proteome, that is, the entire set of proteins expressed by a genome. Transcriptomics have allowed the development of analyses that relate the abundance of mRNA molecules, or transcripts, to gene expression and regulation under varying environmental conditions. As a result, transcriptomics is the dynamic link between genomics and proteomics and thus can elaborate on the complex cellular processes responsible for adapting to environmental conditions (Singh and Nagaraj, 2006; Syrenne et al., 2012).

Transcriptomic study help to get an understanding of genes and pathways involved in biological processes and help elucidating the function of unknown genes based on their spatial and temporal expression. In recent years, RNA-seq technology has rapidly developed into the most important method for transcriptome profiling. Plant transcriptome analyses could provide considerable information on highly expressed genes, differentially expressed genes and new genes. Transcriptome sequencing has been widely applied in studies on field crops, such as transcriptome profile analysis of young floral buds of fertile and sterile plants, transcriptome profiling of resistance to pathogen and research to narrow down the number of candidate genes in identified quantitative trait locus (QTL) regions (Kaur et al., 2012; Agarwal and Rakwal., 2012; Syrenne et al., 2012; Wan et al., 2008; Yu et al., 2014; Ligterink et al., 2012; Zhu et al., 2013; Fedoruk et al., 2013; Pradhan et al., 2014; Xue et al., 2012; Liu et al., 2012). To study gene expression, there are several approaches such as Serial Analysis of Gene Expression (SAGE) and Massively Parallel Signature Sequencing (MPSS); however, the most widely used one is Microarray. This is a high throughput technology that allows

detection of thousands of genes simultaneously. It provides a tool to broadly analyze the expression of several thousand genes during seed development and to identify tissue/ stage specific expression patterns. This technique is used to identify candidate genes for more detailed analysis. (Sharma et al., 2007; Zhu et al., 2001)

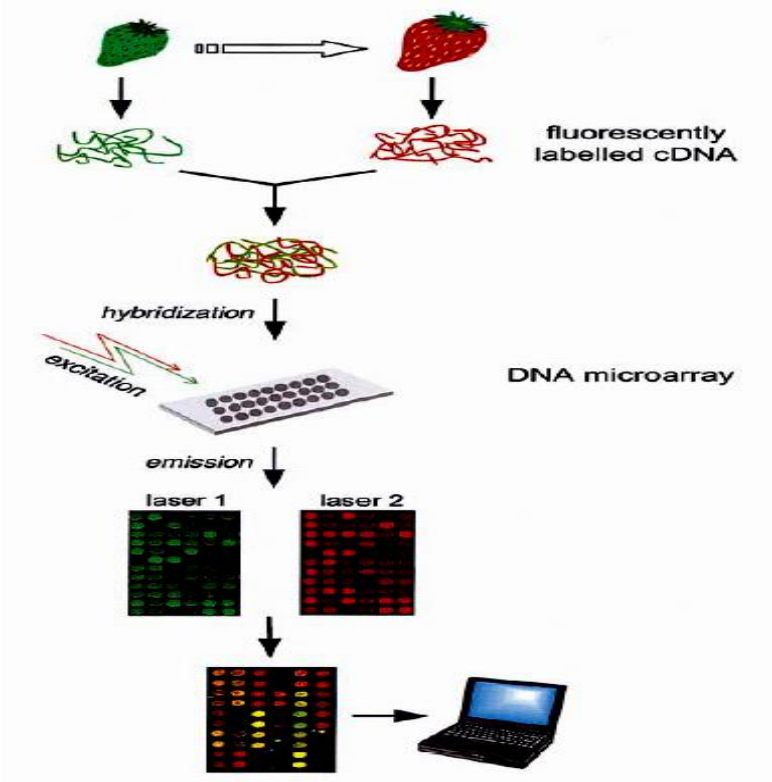

Fig.1 Overview of microarray analysis

Proteomics

The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. Proteomics is the large-scale study of proteins, particularly their structures and functions. Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. Proteomics analysis is critical in improved seed development for incorporation into modern plant breeding strategies because proteins directly affect phenotypic traits as opposed to gene sequence (Singh and Nagaraj 2006; Fu et al., 2005).

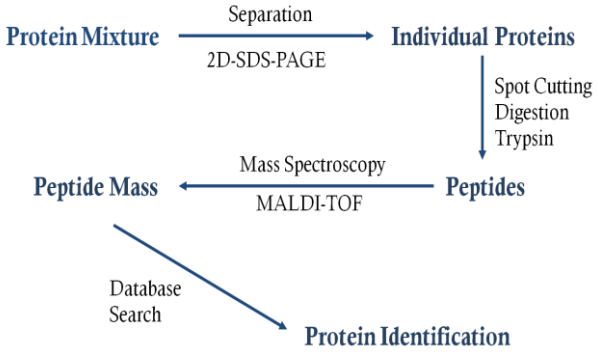

Fig. 2 Schemetatic diagram for proteome analysis

The ability to detect and measure protein molecules has traditionally relied on several methods to separate and observe protein structure: two-dimensional PAGE (2-DGE) and mass spectrometry (Liu et al., 2010; Zhang et al. 2010; Gerber et al., 1993). 2-DGE separates proteins based on two distinct properties: mass and isoelectric point (pI). Basically, the protein is first separated based on its isoelectric point; a property wherein proteins will remain charged at all pH values other than their pI. This is accomplished through use of a pH gradient. After proteins are separated based on isoelectric point, they are washed and treated with sodium dodecyl sulfate (SDS) to denature the protein and yield a linear, negatively charged polypeptide. Separation of the protein is then done by applying an electric current to the gel, where proteins are separated by size similar to traditional gel electrophoresis (Fu et al., 2005; Righetti et al. 2008).

Applications of Proteomics

- Mining: identification of proteins
- Protein-expression profile: identification of proteins in a particular state of the organism
- Protein-network mapping: protein interactions in living systems
- Mapping of protein modifications: how and where proteins are modified

Metabolomics

Metabolome is the complete set of small-molecule metabolites to be found within a biological sample. Metabolites are the intermediates and products of metabolism (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites). Metabolomics is the scientific study of chemical processes involving metabolites like the transcriptome and the proteome, the metabolome is dynamic, changing from second to second. Metabolites can be associated with specific genetic markers, mRNA transcripts, and enzyme activities, allowing a linkage between variation from genetic to biochemical levels that is more complex for lessdefined or more pleiotropic phenotypes, such as seed quality (Taubiana and Fait, 2012; Koornneef et al., 2004; Keurentjes et al., 2008; Keurentjes and Sulpice, 2009).

With the recent developments in analytical methods and data mining, metabolomics has rapidly evolved to provide a global picture of molecular plant organization at the metabolite level (Lin et al., 2014). Various biological issues have been successfully studied using this holistic approach. Growing number of examples indicate that profiling approaches can be used to expose significant sources of variation in the composition of crop and model plants caused by their genetic background, breeding method, growing environment, genotype-environment interactions, and crop cultural practices (Marti et al., 2012; Fernie et al., 2009). In metabolomics, unlike genomics and proteomics, a single analytical technique capable of profiling all of the low molecular weight metabolites of the cell does not exist (Dunn 2008). Among the different techniques enlisted for metabolome analysis, both MS and Nuclear Magnetic Resonance (NMR) represent key methods, each with advantages and disadvantages. Both detection methods can also be hyphenated with chromatography; however, only MS, mainly in gas chromatography (GC)-MS or LC-MS hyphenation, is used to profile plant tissues. LC-NMR is powerful for providing structural information on single constituents of complex mixtures (Wolfender, 2010). However, profiling data obtained by LC-NMR has not been directly used for differential metabolomics, mainly because of the lack of sensitivity, cost, and low throughput of the method. The use of microNMR methods in direct relation with HPLC, mainly by at-line hyphenation, is however, a powerful way to identify de novo biomarkers highlighted by metabolomics(Eugster et al., 2013).

Applications of Seed Metabolomic

- To assess genotypic and phenotypic diversity in plants
- Metabolomics allows probing of rapid physiological changes or events that are not as easily detected by transcriptomic or proteomic approach
- Impact of induced mutation on crop metabolites
- In biotechnology and plant breeding
- To study the nutritional quality of seeds

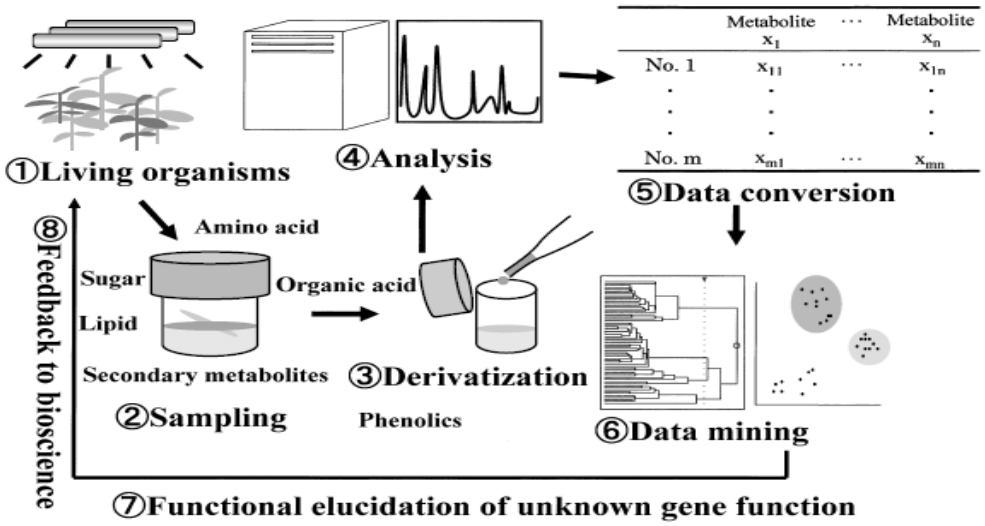

Fig. 3 General scheme of metabolomics

Conclusion

A global analytical and chemometrics approach was developed and used to compare compositional changes in tomato flesh and seeds from the same fruits of the same plants which revealed similarities and differences between the two tissues during fruit development. A map of the different metabolite concentrations at different stages of development flesh and seed of tomato fruit was established which will be useful for further analyses of genotype and environment effects on fruit or seed quality.There is need to identify areas in seed science that need attention from transcriptomic, proteomics and metabolomic based researches. To identify and characterize transcripts and stored proteins, whose action is required following imbibitions. There is need to compare gene

expression profiles, protein patterns and metabolite composition in good and bad quality seed lots, aged, primed, dormant or non-dormant seed lots. Further developing a systems approach to the germination process and seed vigor will greatly help in unraveling the principal biochemical and molecular mechanisms controlling such complex traits that are unique to plants. The characterization of biomarkers of seed vigor for seed improvement via breeding programs and/or technological and biotechnological approaches will allow the production of seeds of the highest possible quality with the goal of improving crop yields, particularly under stressful environmental conditions**.**

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