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Role of metabolite profiling in investigating stress tolerance in fruit crops

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Abstract

Metabolites reflect the integration of gene expression, protein interaction and other different regulatory processes and are therefore closer to the phenotype than mRNA transcripts or proteins alone. Amongst all *-omics* technologies, metabolomics is the most transversal and can be applied to different organisms with little or no modifications. It has been successfully applied to study the response of plants under different kinds of stresses in order to find particular patterns associated to stress tolerance. These studies have emphasized the essential involvement of primary metabolites, primarily includes sugars, amino acids and Krebs cycle intermediates as direct markers of photosynthetic dysfunction as well as effectors of osmotic readjustment. On the contrary, secondary metabolites are more specific of genera and species and respond to particular stress conditions as antioxidants, Reactive Oxygen Species (ROS) scavengers, coenzymes, UV and excess radiation screen and also as regulatory molecules. In addition, the induction of secondary metabolites by several abiotic stress conditions could also be an effective mechanism of cross-protection against biotic threats, providing a link between abiotic and biotic stress responses. It is also known that the metabolomics has become a powerful tool in agriculture and food science and has been used to characterize metabolic changes in plants after biotic and abiotic stresses as well as biotic contamination of foods.

Keywords: Metabolite profiling, investigating stress tolerance, fruit crops

Introduction

Globally biotic and abiotic stresses are the serious threat to food security and India will be among the worst affected countries considering its large population under below poverty line. Abiotic stress under the climate change scenario will affect maximum to the perennial horticultural crops. Metabolites reflect the integration of gene expression, protein interaction and other different regulatory processes and are therefore closer to the phenotype than mRNA transcripts or proteins alone. Amongst all *-omics technologies*, metabolomics is the most transversal and can be applied to different organisms.

What is metabolite profiling?

- The term metabolite profiling has been defined as the identification and quantification of all low molecular weight metabolites in a given organism, at a given developmental stage and in a given organ, tissue or cell type (Arbona *et al.*, 2009).
- The most popular metabolomics techniques focus on metabolites with similar and specific chemical properties and are globally known as metabolite profiling only covering up a fraction of the metabolome.
- The primary metabolites showing involvement in the photosynthesis, respiration, osmotic adjustment; whereas the secondary metabolites respond to stress conditions as antioxidants, ROS scavengers, coenzymes, & regulatory molecules.

Omics Technologies: Transcriptomics, Proteomics and Metabolomics

The analysis of gene transcripts is probably the most developed field. Indeed, there are several platforms available: the extremely accurate qRT-PCR that allows only a limited number of genes to be analyzed the gene microarray technology allowing the analysis of thousands of genes at a time and RNA Sequence oriented to the performance of unbiased analysis of RNA transcripts which generates gigabyte size readouts with all the RNA transcripts of a given cell, organism or tissue.

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The increasing amount of data generated pushed researchers to create databases of experiments covering different organ, tissues and cell types, developmental events and environmental cues allowing to take a sneak peek into the expression of gene candidates in advance.

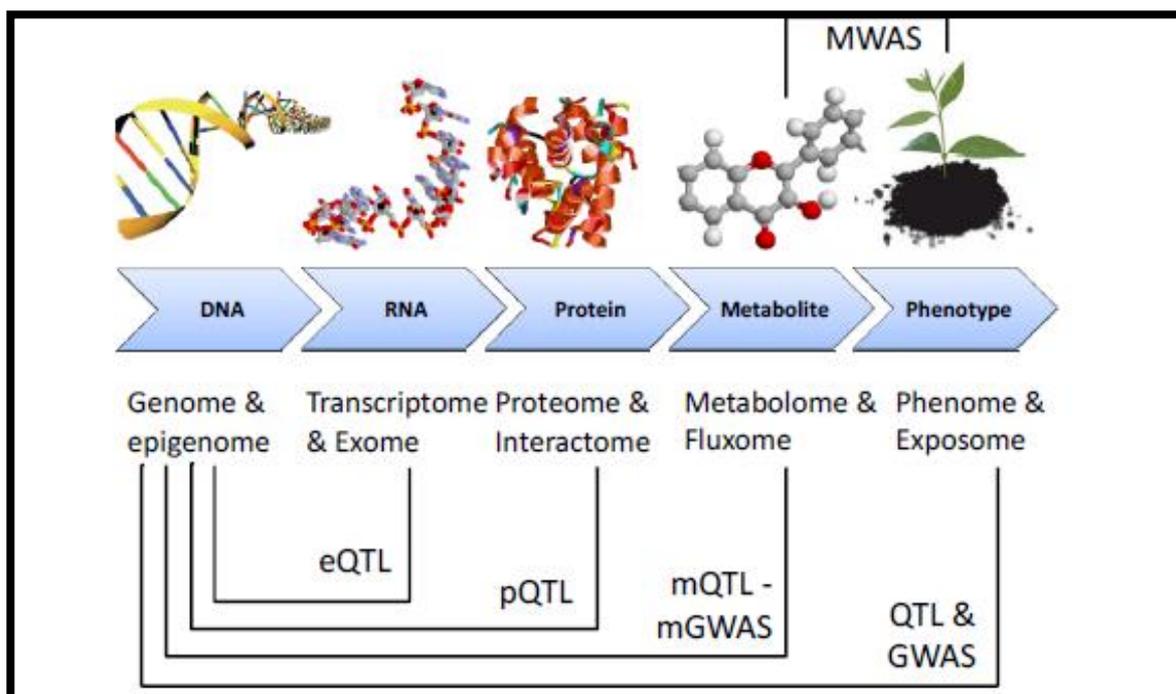
Following transcript analyses, proteins are the second most important aspect in defining an organism's phenotype, as the product of gene expression. To cover up all the translational events from mRNA to functional proteins, analytical platforms necessarily need to be able to evaluate not only the presence/absence of a given protein but the potential post-translational modifications (e.g., phosphorylations, glycosylations or prenylations) and also the potential to assess protein-protein interactions. Available platforms include the traditional 2D gel electrophoresis (combined or not with fluorescent dyes, as in the DIGE technique) which is useful for protein fingerprinting when coupled to mass spectrometry (MS) for protein identification and, finally, the shotgun proteomics approach, based on nano-liquid chromatography (nanoLC) separation of matrices and MS detection, which offers a deeper and less biased coverage of the proteome including low abundant proteins.

The term metabolomics has been defined as the identification and quantitation of all low molecular weight metabolites in a given organism, at a given developmental stage and in a given organ, tissue or cell type. This is a challenging task due to the wide array of molecules with different structures and chemical properties. For instance, it is estimated that a single accession of *Arabidopsis* contains more than 5000 metabolites, most of them yet uncharacterized. Unlike transcriptomics, there is no single approach to detect all compounds and the adequate combination of extraction and detection techniques is key to increase the coverage of the technique.

The most popular metabolomics techniques focus on metabolites with similar and specific chemical properties and are globally known as metabolite profiling only covering up a fraction of the metabolome. To achieve a comprehensive coverage of the vast range of metabolites present in the plant kingdom several analytical techniques

consisting of a separation technique coupled to a detection device (usually MS) are combined. However, there are alternatives that dismiss the use of a separation technique such is the case of flow injection analysis coupled to MS (FIA/MS) or use different analyzers such as nuclear magnetic resonance (NMR) or Fourier Transform Infrared spectroscopy (FTIR) that are used only for fingerprinting purposes. The separation part provides the selectivity needed for certain groups of metabolites. For instance, gas chromatography (GC) is mainly intended for volatiles and primary metabolites (e.g., sugars, aminoacids or tricarboxylic acid (TCA) cycle intermediates) after derivatization. On the other hand, although LC is very flexible and can be adapted to a vast array of compounds, it has been mainly used for secondary metabolites without prior derivatization. In this sense, capillary electrophoresis (CZE) provides similar characteristics as LC but with the advantage that ionic metabolites can also be properly separated. Among all analyzers that can be used with the separation techniques mentioned, the most popular in metabolomics are MS analyzers and, particularly, those providing accurate mass measures such as hybrid quadrupole/time-of-flight or orbitraps. However, more targeted techniques are still extensively used for the quantitation of several plant metabolites and hormones due to their enhanced sensitivity and specificity.

As mentioned above, a serious drawback is the handling of the great amount of data generated. In addition, metabolites need to be properly annotated to obtain consistent and useful results. Whereas, for primary metabolites, it is much facilitated due to the availability of several public libraries for GC/MS studies such as it is still a challenging task in the case of secondary metabolites, since no comprehensive database exists up to date. Therefore, a future objective to achieve in these techniques is the standardization and annotation of data from multiple metabolomics technologies in public databases. The future challenge is the integration of all three aspects within a single framework that will allow a better understanding of how plants respond to a changing environment.



Genome mapping of molecular phenotypes. The levels of organization are depicted in the *x*-axis: from DNA to phenotype. Mapping of the molecular phenotypes onto the genome is achieved by quantitative trait loci (QTL) mapping and genome-wide association (GWAS) techniques. All profiling techniques but metabolome-wide association studies (MWAS) require genetic data.

The study of the metabolome represents the integration of the genetic background and the influence of the environmental conditions, thus describing more accurately the phenotype of a given plant species. In response to adverse abiotic stimuli, plants orchestrate an array of responses oriented to stress avoidance, defense or resistance, depending on the particular stress tolerance. Whereas, stress avoidance involves modifications in growth habits and seasonal quiescence, defense and resistance are necessarily associated to strong metabolic modifications. Among all metabolic responses, alterations in the primary metabolism are the most evident and involve changes in levels of sugars and sugar alcohols, aminoacids and TCA cycle intermediates, showing general trends in response to abiotic stress. However, changes in the secondary metabolism are more specific of a given species and are highly specific of the particular stress condition. The integration of genome and metabolome for phenotype prediction is particularly interesting in crop breeding, since the selection based solely on genetic markers is strongly biased by the influence of the environment. The development of mQTL and MWAS markers for crop selection and improvement of abiotic stress tolerance in crops will help to overcome the problems derived from differing environmental conditions. This field will take advantage of the new plant genomes recently issued and the modern and more powerful metabolite profiling tools.

Primary Metabolites & Osmoprotectants

Carbohydrates

- Carbohydrate metabolism plays an important role in the stress tolerance conditions as it is directly linked to photosynthetic performance.
- During the stress period, plants use starch and fructans as a source of energy instead of glucose (Kaplan *et al.*, 2004) [8].
- These simple sugars can act as osmolytes maintaining cell turgor, stabilizing cell membranes and preventing protein degradation (Sharp *et al.*, 2004) [10].
- Other sugars with no energetic role, such as the oligosaccharides raffinose and stachyose accumulate in different plant species in response to a broad range of abiotic stress conditions such as drought, salinity or extreme temperatures.
- Moreover, high amounts of non-reducing disaccharides such as trehalose can accumulate in tolerant plants subjected to desiccation.
- The simple sugars can act as osmolytes maintaining cell turgor, stabilizing cell membranes and preventing protein degradation.
- Indeed, under water deficit the concentration of soluble carbohydrates such as glucose and fructose increases in roots of stressed plants whereas sucrose is transported to the root tips promoting growth and contributing to the increase in root-to-shoot ratio.

Amino Acids

- Among amino acids, Proline is the main effector contributing to around 50% of the osmotic adjustment in plant root tips (Nishizawa *et al.*, 2008) [9].
- Indeed, increases in Pro content have been reported in response to different abiotic stress conditions like salt stress, soil flooding, drought or extreme temperatures.
- The biosynthesis of Pro is activated under dehydration whereas rehydration induces the opposite pathway.
- Besides the known activity of Pro as a compatible solute several researchers have also claimed its role in ROS scavenging and DNA, membrane and protein stabilization (Arbona *et al.*, 2003) [3].
- However, in response to different abiotic stress conditions, over accumulation of Pro in leaves of several citrus genotypes and model plants was associated to sensitivity.
- Nevertheless, transformation of citrus with a P5CS gene under the control of Cauliflower mosaic virus 35S rRNA promoter led to an increased tolerance to drought and an improved ability for osmotic adjustment.

Polyamines

- Polyamines (PA) are nitrogenous aliphatic molecules of low molecular weight and positively charged which are present in most living organisms.
- The most common PAs found in higher plants are putrescine (Put), spermidine (Spd) and spermine (Spm) and can be present as free and conjugated forms.
- Exogenous application of PA to plants subjected to drought alleviated stress pressure by reducing H₂O₂ and MDA levels through the increase in peroxidase and catalase enzyme activity and Pro levels (Alacazr *et al.*, 2010 and Cuevas *et al.*, 2009) [5].
- This improved stress tolerance in plants with high Put levels was correlated with a reduced stomatal aperture and lower transpiration rate.
- PAs such as Spm and Spd, have been associated to the induction of nitric oxide (NO) which is involved in signalling under abiotic stress conditions

Secondary Metabolites: Antioxidants, Defence Compounds, and Regulatory Metabolites

The array of secondary metabolites is specific to plant species and their biosynthesis is tightly regulated by the developmental stage, tissue or cell group and of course by several stress situations.

Phenolics

- Phenolics constitute the most diverse array of secondary metabolites found in plants and includes phenylpropanoids (cinnamic, coumaric, caffeic and ferulic acids) and its derivatives such as polyphenolics, namely flavonoids, anthocyanins and tannins.
- Under different adverse environmental conditions, the increase in PAL activity as well as other enzymes of the phenylpropanoid pathway has been reported (Wahid *et al.*, 2007) [11].
- These play a role in the side effects derived from environmental changes, such as increase in insect predation.
- It is known that heat induces PAL activity and the production of phenolics and, at the same time, reduces their oxidation contributing to heat stress acclimation.

- In *Arabidopsis thaliana*, UV-B treatment increased the concentration of flavonol (naringenin, kaempferol and quercetin hexosides) and derivatives (cinnamoyl and coumaroyl) that may act as UV-B radiation screen.

Carotenoids & Other Terpenoid Derivatives

- Carotenoids and xanthophylls are lipophilic compounds synthesized in plants from isopentenyl pyrophosphate (IPP) via the plastidial methyl erythritol phosphate (MEP) pathway.
- These metabolites exert a positive effect against heat stress through the stabilization of the lipid phase of the thylakoid membranes.
- In addition, high irradiation and especially UV radiation, has an impact on the composition of this kind of protective compounds.
- Citrus limonoids and particularly the triterpenoid limonin, which is assumed to be responsible of the delayed bitterness phenomenon, occurs in juice sacs of citrus as a result of physical damage or field freeze (Dhuique-Mayer *et al.*, 2009) [6].

Metabolomics & Biotic Stress

Secondary metabolites defend plants against herbivores and pathogens. They protect plants against being eaten by herbivores and against being eaten by microbial pathogens. They serve as defence compounds that increase reproductive fitness of plants by warding off fungi, bacteria, and herbivores.

Terpenes

These are toxins and feeding deterrents to many plant-feeding insects and mammals. For example, the monoterpene esters called Pyrethroids, that occur in the leaves & flowers of *Chrysanthemum* species show very striking insecticidal activities. Many plants contain mixtures of volatile monoterpenes and sesquiterpenes, called essential oils, that lend a characteristic odour to their foliage. Ex: menthol in peppermint oil, and limonene in lemon oil. These essential oils have well-known insect repellent properties. They are frequently found in glandular hairs that project outward from the epidermis and serve to advertise the toxicity of plant, repelling potential herbivores even before they take a trial bite.

Among the non-volatile terpene antiherbivore compounds are the limonoids, a group of triterpenes wellknown as bitter substances in citrus fruit.

Isoflavonoids have anti-microbial activity. These are mostly found in legumes and have several different biological activities. Some such as the rotenoids, have strong insecticidal actions.

Tannins deter feeding by herbivores. Tannins bind the collagen proteins of animal hides, increasing their resistance to heat, water and microbes.

Effect of abiotic stress on plant biochemistry

- The actual metabolite composition of a given plant species is the result of a particular gene expression profile.
- When a certain metabolic pathway is activated, precursors and intermediates are channeled to produce a bioactive molecule: an antioxidant, a signaling compound, a cell structure biosynthesis intermediate or even a storage compound.

- Considering the metabolome, the balance between defense, signaling & damage- metabolites can be used to assess plant tolerance to a certain stress situation.

Uses of Metabolomics

- Evaluation of the impact of stress/treatment on plant metabolism
- Tracking of a certain compound or compound category within particular biosynthetic or degradation pathway
- Classification of samples

Conclusion

In response to adverse abiotic stimuli, plants orchestrate an array of responses oriented to stress avoidance, defense or resistance, depending on the particular stress tolerance. Whereas stress avoidance involves modifications in growth habits and seasonal quiescence, defense and resistance are necessarily associated to strong metabolic modifications. Among all metabolic responses, alterations in the primary metabolism are the most evident and involve changes in levels of sugars and sugar alcohols, aminoacids and TCA cycle intermediates, showing general trends in response to abiotic stress. However, changes in the secondary metabolism are more specific of a given species and are highly specific of the particular stress condition.

Future Prospects

- The integration of genome and metabolome for phenotype prediction is particularly interesting in crop breeding, since the selection based solely on genetic markers is strongly biased by the influence of the environment.
- The development of mQTL and MWAS markers for crop selection and improvement of abiotic stress tolerance in crops will help to overcome the problems derived from differing environmental conditions.
- This field will take advantage of the new plant genomes recently issued and the modern and more powerful metabolite profiling tools.

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