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Metabolomics of drought stress tolerance in plants

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Abstract
Metabolites are more closer to the phenotype of an organism than the gene or protein component, as it takes into account the environmental interaction. It is ultimately the condition of the environment, which determines the actual potential of expression of a trait. Drought stress is one of the most important sought after problems in the context of climate change, the arid and semi-arid zones being extremely vulnerable. There is serial changes in the physiological process and accumulation of different metabolites within a plant after receive of stimuli. There is severe reduction in carbon dioxide assimilation rate associated with stomatal closure under drought stress. There is also over-production of ascorbic acid, glutathione, several amino acids including leucine, isoleucine, valine, proline etc. and polyphenols. These compounds serve as antioxidant machinery against the oxidative stress caused due to drought. There is also accumulation of carbohydrates and their alcohols in response to water stress. ABA plays a key role in drought stress signaling pathway. Hence, the characterstic metabolite markers can be used to trace out tolerant genotypes or rootstocks to wide range of abiotic stresses. GC-MS and NMR techniques are used as aid for metabolite profiling, which helps in identification and quantification of all low molecular weight molecules in a plant system. Therefore, metabolomics can be a promising approach to over-ride the constraints of achieving higher productivity due to stress factors.

Keywords: Metabolomics, drought, GC-ms, NMR, antioxidant

Introduction

Globally, biotic and abiotic stresses are the serious threats 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. Drought and salinity are the two major abiotic stresses that affect 1/3rd of the global population in terms of human health and farm productivity. Drought and salinity affects more than 10% of arable land, which results in rapid increase in desertification and salinization world-wide (Ashraf *et al.,* 2009). The present challenges like global climate change, water and soil pollution, less water availability, urbanization etc. adds up to the situation of nutritional demand by the ever growing population of country. In combination with elevated temperatures, decreased precipitation could cause reduction in availability of irrigation water and increase in evapo-transpiration, leading to severe crop water-stress conditions. The severe drought condition prevalent in many parts of the world as well as India has emerged as the greatest hurdle on the way of obtaining higher productivity per unit area.

Several environmental stresses disrupts metabolic homeostasis and causes serial changes in growth pattern and physiology of plants. In the initial stages of acclimation, the environmental change is sensed by the plant and further activates a network of signaling pathways. In succeeding phases, the signal transduction pathways being activated and makes production of different proteins and compounds, that achieve a new state of homeostasis (Shuladev *et al*., 2008) [11]. To understand the exact underlying mechanism of drought tolerance is really a difficult task, as the physiology and various signaling networks functioning under abiotic stress conditions often intersects each other giving rise a very complex network pathway. Drought stress can induce three different types of pathway in plants: (a) altered expression (over-, under-, or co-expression) of the genes, which are involved in the life sustenance of plants (Batlang *et al*. 2013), (b) dynamics in protein production and degradation as a direct response to drought stress, which may cause damage to the plants (Mohammadi *et al*. 2012) and (c) alterations in the metabolic pool to channelize the production of new biochemically related metabolites, which may confer resistance or tolerance to drought stress (Babita *et al*. 2010; Kumari *et al*. 2013).

Drought is an extended period when a region receives a deficiency in its water supply, whether atmospheric, surface or ground water. Climatic factors such as high temperature, high wind velocity and low relative humidity are associated with drought.

Basically there are two types of Drought-

- Atmospheric drought
- Soil drought

Drought induce numerous morphological, physiological and biochemical changes in all plant organs.

1. Morphological Changes-

Reduces leaf size, stem extension and root proliferation, disturbs plant water relations.

Physiological disorder-

- Defoliation in Apple.
- Firing in Cherry.
- Tip Burning in Pear.
- “Hard-end” and “Ishinashi” in Pear.(Hayashi *et al*., 1955)

2. Physiological & Biochemical changes

- Closing of the stomata.
- Limits Gas Exchange
- Photosynthetic activity
- Endogenous contents of auxins, gibberellins and cytokinin usually decrease, while those of abscisic acid and ethylene increase (Nilsen *et al*., 1996).
- Increase proline content.
- Oxidative damage
- Generation of ROS takes place react with proteins, lipids and DNA, causing oxidative damage (Farooq *et al.*, 2008)
- Plants adopt to survive under drought stress by the induction of various morphological, biochemical and physiological responses.

Among various –Omics approaches, metabolomics is the most transversal and can be applied to different organisms with little or no modifications. Transcriptomics is a suitable approach for the identification of candidate genes for adaptive traits and molecular markers that are linked to phenotypic variation under drought. Sequencing and transcriptome analysis are new techniques used to study expression patterns under drought. Muller *et al*. (2012) assembled a set of 170,859 putative unique transcripts from 12 normalized cDNA libraries with 3.6 million reads in genetically diverse, drought-stressed seedlings of Douglas-fir (Pseudotsuga menziesii).Proteomics branch deals with the expression pattern analyses of various proteins, which are up-regulated or down-regulated following drought stress conditions. Metabolomics has been successfully applied to study the response of plants under different kinds of stresses in order to find particular patterns associated with stress tolerance. These studies have emphasized the essential involvement of primary metabolites, primarily includes carbohydrates, amino acids, lipids, and different polyamines 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. From the standpoint of metabolomics, at least three different types of compounds are important for these processes: (1) compounds involved in the acclimation process such as antioxidants or osmoprotectants; (2) byproducts of stress that appear in cells because of the disruption of normal homeostasis by the alteration (s) in growth conditions; and (3) signal transduction molecules involved in mediating the acclimation response.

Drought condition prevalent for a longer period drastically reduces the photosynthetic ability of plants. This hamper of photosynthetic machinery of plants may be either due to stomatal closure or due to metabolic impairment (Lawson *et al*., 2003) [5]. However, stomatal limitation was generally accepted to be the main determinant of reduced photosynthesis under drought stress (Cornic, 2000) [2]. There is a drought-induced root-to-leaf signaling, which is promoted by soil drying through the transpiration stream, resulting in stomatal closure and this signal is now known to be mediated by abscisic acid (ABA). The limitation of photosynthesis under drought through metabolic impairment is a more complex phenomenon than stomatal limitation. Drought generally reduces the biochemical capacity for carbon assimilation and utilization (Reddy *et al*., 2004) [10]. The rate of photosynthesis also depends on the synthesis of RuBP. Under drought stress conditions, decrease in chloroplast volume might also lead to desiccation within the chloroplast, causing for conformational changes in rubisco. Drought stress conditions also cause acidification of the chloroplast stroma, resulting in inhibition of rubisco activity and thus hampers photosynthesis (Meyer and Genty, 1999) [8].

Drought also induces oxidative stress in the environment around the plants. During drought, there is every chance for increased accumulation of superoxide and hydrogen peroxide resulting from the increased rate of O_2 photoreduction in chloroplasts (Robinson and Bunce, 2000). Reactive oxygen species (ROS) attack the most sensitive biological macromolecules in cells to impair their function. The enhanced level of ROS increases the expression of genes for antioxidant functions and thereby activates the antioxidative systems to impart tolerance mechanism during drought (Mano, 2002) [7]. The synthesis of xanthophyll pigments, zeaxanthin, and antheraxanthin, at the expense of violoxanthin in water stressed plants has also been seen as a common phenomenon (Alonso *et al*., 2001) [1]. Various osmo-protectants are also produced such as; organic solutes including nitrogen-containing compounds, i.e. proline and other amino acids, polyamines, sucrose, polyols, sugar alchohols (pinitol), oligosacharides and quaternary ammonium compounds like glycine betaine (GlyBet) (Tamura *et al*., 2003) [12]. Osmolytes have main function of stabilizing membranes and maintaining protein conformation at low leaf water potentials. The synthesis and accumulation of osmolytes depends on the plant species as well as different cultivars of the same species. Osmolytes play a major role in osmotic adjustment and also protect the cells by scavenging ROS (Pinhero *et al*., 2001) [9]. Proline is known to be an important osmolyte, which is upregulated under drought stress and involved in reducing the photodamage in the thylakoid membranes by scavenging and/or reducing the production of $^1O_2$.

Tools Used In Metabolomics

Two techniques dominate metabolite profiling strategies:

1. mass spectrometry (MS); and
2. nuclear magnetic resonance (NMR).

Gas-chromatography-mass-spectrometry (GC-MS), gas-chromatography-time-of-flight-mass-spectrometry (GC-TOF-MS) and liquid-chromatography-mass-spectrometry (LC-MS) are currently the standard mass-spectrometry methods for metabolite analyses.

NMR approaches, which rely on the detection of magnetic nuclei of atoms after application of a constant magnetic field, are the main alternative to MS-based approaches for metabolite profiling and this is mainly associated with computer softwares.

Mechanism of drought tolerance

1. Morphological mechanisms-

a. Escape

- Escape from drought is attained through a shortened life cycle or growing season.
- Allowing plants to reproduce before the environment becomes dry.
- Like in Coffee (*Coffea arabica*) and cacao (*Theobroma cacao*) flower and fruit when rains follow a drought period (Alvim, 1985).

b. Drought Avoidance

- Efficient water absorption from roots.
- Reducing evapotranspiration from aerial part.
- Like in Ber, Bael, Custard apple, Phalsa, Datepalm, Tamarind etc. can be grown in xerophytic Conditions.

c. Drought Tolerance

- Some plants have the ability to tolerate dehydration or maintain turgor pressure through an osmotic adjustment.
- An increase of anti-oxidative defense system. (Reddy *et al*., 2004)

Physiological mechanism

d. Osmotic Adjustment

Osmotic adjustment is a mechanism to maintain water relations under osmotic stress.

It involves the accumulation of soluble solutes like sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions etc. (Gomes *et al*., 2010)

e. Plant Growth Regulators

ABA, brassinosteroids, jasmonate, salicylic acid, polyamines and nitric oxide increase under drought stress (Acharya, 2009)

f. Antioxidant Defense System

Most efficient mechanisms against oxidative stress.

Enzymatic components- Superoxide dismutase, Catalase peroxidase, Ascorbate peroxidase and Glutathione reductase.

Non-enzymatic components- Cysteine, reduced glutathione and ascorbic acid.

Abiotic stress and plant system's biology

While most of the scientists today are busy in identifying the gene and developing the resistant varieties, but it would be a more wise choice if we look for simultaneous realization of the actual potential of a target gene to express under different sets of growing conditions. As India is bestowed with wide climatic and geographical variations, so we also cannot be universal in our findings, rather we have to test its practical feasibility under different contexts. The data collected from transcriptomics, proteomics and metabolomics needs to be combined to achieve a better understanding of the plant as a system. There should be integration of all such informations into a single pipeline in order to achieve greater accuracy. We can opt for transcriptomic-proteomic or metabolomics-proteomic approaches to unveil several genes and processes underlying complex traits. In order to facilitate the integration, several software packages have been developed such as Map Man (Wochniak *et al*., 2006) or, more recently, Met Gen Map (Joosen *et al*., 2011). These computer programs have been successfully applied to identification of genes and metabolic pathways involved in germination, diurnal cycles (Kempa *et al*., 2008) [4] and seed dormancy (Luo *et al*., 2009) [6].

Conclusion

The present day practice of monoculture and hi-tech cultivation rapidly erodes the genetic diversity present in the nature, hence it reduces the adaptive capability of plants to different stress conditions. There is also wide gap exists in knowledge about the level of stress tolerance to be developed in crops intended to be grown in targeted environment. There should be identification of low inputs responsive crops/cultivars suitable for location specific conservation horticulture. Development and standardization of bio-regulator mediated drought stress tolerance technology for plants can also be helpful to a great extent.

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