Metabolomics and Plant Abiotic Stress

Subjects: Agronomy

Contributor: Juan Cevallos-Cevallos

Metabolomics is a technology that generates large amounts of data and contributes to obtaining wide and integral explanations of the biochemical state of a living organism. Plants are continuously affected by abiotic stresses such as water scarcity, high temperatures and high salinity, and metabolomics has the potential for elucidating the response-to-stress mechanisms and develop resistance strategies in affected cultivars.

Keywords: metabolite profile ; drought ; heat stress ; salinity stress

1. Introduction

Global agriculture is threatened by climate change as variations in rainfall, heat waves and global CO_2 levels are responsible for several types of abiotic stresses causing a negative impact on food production [1]. Production losses of about 50% have occurred due to abiotic stress [2] and the study of crops capable of withstanding abiotic stress is considered a priority [3][4]. The characterization of the effects and biochemical responses caused by abiotic stresses in different crops can contribute to understand the mechanisms of plant resistance to stress and favor the development of appropriate stress mitigation strategies including the development of abiotic stress resistant crops [4][5]. Among several alternatives, metabolomics—the study of the metabolites present in a biological system—is considered a key tool for assessing biochemical changes occurring in plants affected by abiotic stress [6].

Metabolomics is considered a fundamental branch of systems biology [7] and a powerful tool to investigate the response of organisms to external factors at the metabolite level [8][9][10][11]. Metabolomics is essential for understanding chemical signals as plants grow and develop [12]. However, the full importance of metabolomics for assessing plant responses to stress is difficult to estimate because, unlike the transcriptome and proteome, the metabolome is not necessarily associated with the plant genome [11].

Metabolites, analyzed by metabolomics, are defined as small molecules developed or modified during cellular metabolism [8]. The orderly identification and quantification of metabolites can provide the chemical fingerprint of a phenotype [13] and the biochemical response of an organism to specific conditions [14].

Plants process a wide variety of primary and secondary metabolites with diverse chemical structures. Primary metabolites are essential for plant growth and development, but secondary metabolites have more specific purposes and both types of metabolites are part of plant stress response mechanisms [15]. Primary metabolites, including amino acids, sugars, and lipids, have highly conserved structures, but secondary metabolites, such as alkaloids, polyphenols, and terpenoids, are more diverse and can vary greatly among plant species [15][16]. With current metabolomics tools, about 14,000 plant metabolites can be identified, although more than 200,000 molecules are expected in plant samples [11].

Large-scale metabolomic studies have made it possible for researchers to obtain a wealth of global data on metabolites and relevant metabolic pathways in an unprecedented manner [17]. Metabolomics has evolved into a powerful tool in many research areas, such as molecular epidemiology, toxicity assessment, biomarker discovery and identification [14][15][16][17][18] and plant research [12][17]. Currently, metabolomics has ventured into broader studies such as functional metabolomics, which is responsible for investigating the functions of specific metabolites [19]. Metabolomics has also been responsible for improving crop yield and quality [11][17][20]; and the fusion of metabolomics with other technologies related to genetic modification, transcriptomics, proteomics and quantitative genetics have boosted plant breeding [17]. Additionally, metabolomics has forayed into the observation of morphological, phenotypic and physiological responses of plants to environmental perturbations and interactions with other organisms [21].

2. Metabolomic Assessment of Abiotic Stress in Plants

Abiotic stress causes innumerable transformations in plant metabolisms, such as disturbances in enzyme activities, high requirement for various metabolites, high levels of reactive oxygen species (ROS) or a composition of them [22][23]. Consequently, abiotic stresses alter cellular structures and impair key functions of plant physiology [24]. Among the predominant alterations, low photosynthetic capacity, attenuated development, decrease in fertility and interruption of reproduction are found, causing a decrease in crop yield [22][23]. Stress influences each species in different ways, and even under some circumstances can be positive to obtain a desired crop response [25][26]. There are an abundance of plant response mechanisms against environmental stresses [22].

Metabolomics is able to assist prominently in the analysis of stress biology in plants [15] and has the potential to elucidate the mechanisms for tolerance to abiotic stress in plants [27]. Qualitative and quantitative studies of the metabolites of plants subjected to biotic and abiotic stresses are not only descriptive, but may also reveal deep genetic and biochemical mechanisms as responses of stressed plants, as well as distinguish the ability of plants to resist and tolerate stress [15]. Plants under stressful abiotic factors can react with tolerance or adaptation. Most metabolomics studies have focused on comparing the response of stress-susceptible and stress-tolerant cultivars. In most studies, the role of amino acids as osmoprotectants was confirmed but the importance of other metabolites including organic acids, sugars and phenolic compounds in abiotic stress have been proposed for various plants [28][29]. In general, the stressful situation appears to activate proline production, while proline catabolism improves during stress recovery [22].

In the coming paragraphs, we briefly comment on topics about metabolomic studies on important abiotic stress factors (drought, temperature, salt and oxidative stresses) with emphasis on the most recent years.

3. Water Scarcity and Drought Stress

Drought stress is one of the most damaging stresses in plants, particularly in regions with rain-based plant irrigation, generating dramatic changes in metabolism. Under water scarcity, physiological adjustments tend to reduce water loss and increase water uptake, which leads to metabolic consequences. Thereby, among biochemical responses is found the accumulation of osmoregulators in order to maintain cell turgor [30], including sugars, poyalcohols, polyamines and amino acids, mainly proline [29]. Thus, several metabolomic studies have been carried out in leaf tissues regarding drought stress and proline accumulation in dehydrated leaves resulted frequent [31][32][33]. Proline accumulation has been also reported in a wide variety of plants under stresses which may lead to low water availability, such as high salinity, heavy metals and low temperatures [22][32][34][35].

Most of the metabolomics analyses in plants were performed on aerial parts, mainly leaves. Dehydration at the metabolomic level has been well studied in *Arabidopsis thaliana* L. Under dehydration, ABA is generated, and the aerial part of this species accumulated amino acids and polyamines under an ABA-dependent manner, as well as raffinose, which was produced independently of this hormone [32]. Together with those biomarkers of drought stress, under water scarcity, *A. thaliana* aerial parts also accumulated flavonols and anthocyanins, which could indicate that these molecules can alleviate such stress [36]. Leaves are more affected by drought stress than other parts of the plant. For example, drought led to stronger changes in composition of corn leaves when compared to other organs [37][31]. In corn, the metabolites that accumulated most in the leaves were the ringed amino acids (proline, tryptophan, phenylalanine and histidine), while pyruvic and quinic acids decreased.

Metabolome affectation under water stress is dependent on genotype. Thus, although some metabolite contents showed similar alterations in leaves of two wine cultivars (Shiraz and Cabernet Sauvignon) [35], more metabolites were affected in the cv. Shiraz [35], which showed less-adjusted stomatal regulation than the other cultivar. Nicotinate was the only organic acid that increased in both cultivars, whereas glycerate and galactonate decreased. Furthermore, both genotypes experienced a marked increase in certain amino acids (the drought stress-associated proline, and also threonine, tryptophan, valine, leucine and phenylalanine) and changes in the phenylpropanoid pathway. Nevertheless, glutamine increased in Shiraz and decreased in Cabernet Sauvignon.

Metabolomic tools have permitted the characterization of the response to stress of specifically drought-susceptible (DS) and drought-tolerant (DT) cultivars and have allowed the identification of potential biomarkers related with this type of stress. Thus, metabolomics was used to compare DT and DS in wheat varieties. After drought stress treatment, thymine, the aminoacids L-cysteinylglycine and fructoselysine, as well as a series of phenolic compounds, accumulated higher in leaves in the DT variety than in the DS one, whereas increased proline levels were observed in the DS variety only [38]. Another study on leaves of wheat cultivars under water scarcity [37] showed that tryptophan, valine, and the TCA-cycle acids citric, fumaric and malic appeared in higher levels in the DT cultivar when compared to the DS cultivar. Similarly, the

DT cultivar significantly accumulated alpha-tocopherol and the acids 3-hydroxy propanoic, gluconic, glycolic, citric and isocitric, whereas the DS cultivar accumulated both alpha- and gamma- tocopherols as well as gluconic and malic acids. Regarding sugars, the concentrations of nigrose, seduheptose and galactose were enhanced in DS, whereas glucose, fructose and galactose levels increased in DT. Similarly, in both leaves and roots of peanuts submitted to drought stress, metabolomics analysis showed that pentitol, phytol, xylonic acid, d-xylopyranose, stearic acid and D-ribose were important drought-response metabolites [39]. Agmatine and cadaverine were present only in DT during drought. Additionally, polyphenols such as syringic acid and vanillic acid were more accumulated in DT than in DS peanuts, while catechin production was higher in DS than in DT during drought.

Regarding metabolic pathways, a metabolite profiling analysis in leaves of wild soybean suggested that the TCA-cycle was enhanced in DT whereas it was inhibited in the common DS under drought stress [40]. ɣ-aminobutyric acid (GABA), asparagine and methionine increased significantly in DT but not in DS. Organic acids, such as galactonic, glucoheptonic, malonic and glycolic acids, increased significantly in DT. Unsaturated fatty acids, including linolenic and linoleic acids, accumulated significantly in DT. In addition, secondary antioxidant metabolites, including 5-methoxytryptamine and fluorine, accumulated significantly in DT. Furthermore, the aromatic aminoacid phenylalanine and phenolic compounds (ferulic acid, salicylic acid and 4-hydroxycinnamic acid) increased significantly in DT. Other metabolites, including glucose-1-phosphate, D-fructose 1, 6-bisphosphate, pyruvic acid, D-glyceric acid, oxalic acid and 2-methylfumarate, increased significantly in both DT and DS cultivars. Similarly, amino acids, including proline, glycine, serine, valine, beta-alanine, threonine and isoleucine, accumulated significantly in both DT and DS.

The temporal dynamics of metabolite reprograming of DT and DS cultivars under drought stress has also been studied by metabolomic tools. In Tibetan hulless barley, both types of cultivars responded to drought stress by accumulating flavonoids and glycerophospholipids compounds within one hour [41]. However, the number of differentially accumulated metabolites decreased in DS over time but increased in DT. In DT, the differentially accumulated metabolites after 8 h of drought stress were quite different from those identified at the previous hours suggesting a specific metabolite reprograming aimed to cope with drought stress.

Under osmotic stress (associated to drought stress), various plant species such as barley usually show an increase in L-proline together with other metabolites, including various osmoprotectants like mannitol [42]. Interestingly, a DS barley genotype showed the highest increase in the levels of most metabolite-except mannitol-and decreased levels of maltose, when compared to the stable contents in other genotypes. In both DS and DT wild genotypes tested, there was a decrease of the TCA cycle metabolite 2 α–-ketoglutaric – acid from which proline proceeds- and xylitol.

The nature of drought (cyclic vs. acute), as well as its frequency and severity, may also affect the degree of osmotic adjustment and the nature of the organic solutes that are accumulated. In *Populus deltoides* L. leaves, whereas cyclic drought induced the largest responses in primary metabolism (soluble sugars, organic acids and amino acids), acute onset of prolonged drought induced the greatest osmotic adjustment and largest responses in secondary metabolism, especially populosides (hydroxycinnamic acid conjugates of salicin). [43]. In rice, a combination of metabolomics with proteomics revealed that, under abrupt drought-flood alternation stress, energy metabolism pathways and reactive oxygen species (ROS) changed strongly, leading to grain yield reduction [44].

Metabolomics has been the basis of several mixed omics studies. The combination of metabolomics and proteomics allowed to assess the recovery of *Eucaliptus globulus* L. [45] or the response of DT and DS spring-wheat cultivars [46] to water stress. In this case, leaves of the DS cultivar showed increased levels of amino acids such as proline, methionine, arginine and lysine. However, only two pathways were affected in the DT cultivar, one of them being purine metabolism. Similarly, transcriptomics and metabolomics studies revealed that ABA, aminoacids and organic acids accumulated in leaves of both DS and DT sesame varieties but the DT variety showed higher levels of ABA, proline, arginine, lysine, aromatic and branched chain amino acids, GABA, saccharopine, 2-aminoadipate and allantoin than the DS under the stress condition [47].

From a practical point of view, drought favors the obtaining of several secondary metabolites like complex phenols, terpenes and alkaloids. For example, under drought stress, phenolics increased in *Hypericum polyanthemum* [48], *Oryza sativa* [49], *Salvia officinalis* [50] and *Hordeum vulgare* [51], and monoterpenes or terpenoids rose in these last two species, respectively [51][52]. However, the stress generally caused a reduction in the plant biomass.

Drought stress usually leads to heat stress due to the decrease of transpirational cooling. Most of the physiological, biochemical and metabolic changes observed under drought and heat stresses were reversed upon recovery in *Eucalyptus globulus* Labill [45] and rice (*Oryza sativa* L.) [53]. In rice flag leaves at the flowering statage, many compounds with after-stress altered concentration reached original levels after rehydration. Thus, 60 h after rewatering, some primary organic acids (such as the TCAs isocitric, and citric, and glyceric acid) increased, and glucose, raffinose,

glycine, N-carboxiglycine and proline decreased. Proline decreased more in drought-tolerant cultivars. The behavior of several metabolites was generally shared in early grain-filling flag leaves after stress and rewatering (the TCAs isocitric and citric, and proline).

Drought and heat stress affected soybean (*Glycine max* L.) leaf metabolomics [54]. The levels of glycolisis intermediates (pyruvate, glucose, dihydroxyacetone phosphate) were dramatically reduced, whereas those from TCA-cycle metabolites (malate, succinate, alpha-ketoglutarate, citrate) were significantly decreased. Pyruvate decrease can be the cause of the affectation of the biosynthesis of phenylalanine, tryptophan, tyrosine, isoleucine and alanine. Nevertheless, the oxaloacetate (a metabolite from TCA cycle) and its amino-acidic derivatives methionine and lysine also decreased. Similarly, sugar alcohols such as mannitol and galactitol were reduced as a result of temperature stress. In maize, the combination of drought and cold stresses was assayed and led to a multi-faceted metabolic response that revealed an ABA-dependent acclimation mechanism [38].

4. Temperature Stress

Plants are committed to a wide variety of factors, and one of the primary agents that constitute the organization and fate of plants is temperature stress, which comprises both high and low temperature stresses and is judged to be the major abiotic stress for seedlings [55][56]. Temperature stress is of interest to plant researchers due to climate change, which adversely affects crop productivity worldwide [57][58]. The increase in temperature is capable of damage essential physiological issues like the balanced relationship between primary and secondary metabolites with hormones, or the correspondence between water and membrane consistency, respiration and photosynthesis [59]. The generated disruptions impair metabolic development reducing plant growth and altering its development, and ultimately results in little economic benefit.

Various studies have been carried out recently on metabolomic responses to cold stress (tomato, wheat, maize, miscanthus) [60][61][62][63][64], including several medicinal plants [65], but *Arabidopsis thaliana* is the species traditionally more studied on such respects. In this grass, cold stress generates increases in most of its metabolites, including proline, sugars and TCA-cycle intermediates [66]. A biomarker candidate of cold tolerance in Arabidopsis resulted to be raffinose [67][68], although the general response depends on ecotypes. In this species, most of its heat shock metabolite responses were shared with those caused by cold, such as increases of amino acids derivated from pyruvate and the TCA-cycle [69].

Heat is an important abiotic stress continuously affecting crops in many countries, as worldwide temperatures are increasing. Heat stress in plants causes the overproduction of phenolic metabolites, phenylpropanoids and flavonoids [70]. In tomato (*Solanum lycopersicum* L.) leaves, both the suppression and overexpression of the heat stress factor B1 (HsfB1) enhanced the plant thermotolerance by different means. The overexpression of HsfB1 caused the accumulation of products of the phenylpropanoid and flavonoid pathways, including several caffeoyl quinic acid isomers [60]. However, tomato leaves with suppressed HsfB1 showed an accumulation of the polyamine putrescine and the sugars sucrose and glucose. Therefore, heat-tolerance was not specific to one metabolite group. Thus, affectations in respiratory pathways at substrate level were detected by means of metabolomics and transcriptomics, which revealed the differential response of *Arabidopsis thaliana* leaves to heat stress caused by prolonged warming or by heat shocks. Prolonged warming enhanced the glycolysis pathway but inhibited the TCA-cycle, while a heat shock significantly limited the conversion of pyruvate into acetyl coenzyme A [71], then also negatively affecting TCA-cycle, but not, in such a measure, glycolysis. Moreover, in wheat (*Triticum aestivum* L.) subjected to high temperature stress, the flag leaves after 10 days of anthesis showed a general increase in pipecolate and L-tryptophan, whereas anthranilate and drummondol decreased. The metabolic pathways most impaired by high temperature stress were the biosynthesis of secondary metabolites and the aminoacyl-tRNA pathway [72].

Similarly, metabolomic tools have permitted the assessment of the metabolite dynamics of heat-tolerant and heat-sensitive plants in response to heat stress. A recent metabolomic study showed a two-step response of *Pinus radiata* to heat stress with the metabolite profile of leaves significantly changing after a 3-day heat-stress to activate a long-term plant response to stress. Cytokinins (CKs), fatty acid metabolism and flavonoid and terpenoid biosynthesis were the most important pathways involved in heat-stress response. Additionally, L-phenylalanine, hexadecanoic acid, and dihydromyricetin were identified as potential biomarkers for heat toletrant plants.

In spite of the described metabolic changes, it seems that pollen is more resistant to heat stress than tissues. In tomato pollen heat stress did not lead to the detection of differential metabolites [61].

Heat stress has been studied in combination with salt stress in lablab bean (*Dolichos lablab*) seedlings, which were pretreated at 35 °C and 100 mM NaCl and then exposed to 45 °C for 5 h and restored to 25 °C. Such treatment generated higher levels of proline, ascorbate peroxidase, glutathione reductase, peroxidase, ascorbate, glutathione, and sugars [73].

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