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Review

Functional Metabolomics – a Useful Tool to Characterize Stress-Induced Metabolome Alterations Opening New Avenues Towards Tailoring Food Crop Quality

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Abstract: The breeding of stress-tolerant cultivated plants that would allow for a reduction in harvest losses and undesirable decrease in quality attributes requires a new quality of knowledge on molecular markers associated with relevant agronomic traits, on quantitative metabolic responses of plants on stress challenges, and on the mechanisms controlling the biosynthesis of these molecules. By combining metabolomics with genomics, transcriptomics and proteomics datasets a more comprehensive knowledge of the composition of crop plants used for food or animal feed is possible. In order to optimize crop trait developments, to enhance crop yields and quality, as well as to guarantee nutritional and health factors, that provides the possibility to create functional food or feedstuffs, the knowledge about the plants' metabolome is crucial. Next to classical metabolomics studies, this review focusses on several metabolomics based working techniques, such as sensomics, lipidomics, hormonomics and phytometabolomics, which were used to characterize metabolome alterations during abiotic and biotic stress, to find resistant food crops with a preferred quality or at least to produce functional food crops are highlighted.

Keywords: Plant stress, abiotic stress, biotic stress, metabolomics, phytometabolomics, sensomics, phytohormonics, LC-MS/MS, NMR, targeted metabolomics, untargeted metabolomics, functional food.

1. Importance of metabolomics for agricultural research

In their environment plants are often exposed to an enormous number of biotic and abiotic stress factors, such as pathogen and insect infestation as well as extreme temperatures, drought, salinity, pollutants, heavy metals or nutritional deficiencies, that lead to harvest or quality losses, such as the formation of a pronounced bitter off-flavor, and sometimes causes toxicological problems as well as huge global economic losses [1]. The breeding of stress-tolerant cultivated plants that would allow for a reduction in harvest losses and undesirable decrease in quality attributes requires a new quality of knowledge on molecular markers associated with relevant agronomic traits, on quantitative metabolic responses of plants on stress challenges, and on the mechanisms controlling the biosynthesis of these molecules. Thereby, the knowledge of the biologically active metabolites, especially, secondary metabolites as well as metabolic networks of primary and secondary metabolites that were affected upon plant stress conditions are inalienable.

The metabolome of plants represents the complete set of low-molecular weight metabolites (such as primary metabolites including all necessary metabolic intermediates, hormones and other

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signaling molecules, as well as secondary metabolites) in a given organism, a biological cell, tissue, or organ at a certain point in time and development. Especially, environmental stress influences the metabolome composition of plants. Stress conditions are sensed by the plant, activate a network of signaling pathways, including the participation of phytohormons, and lead to changes on the one hand in the primary metabolism and on the other hand in the up-regulation of phytochemicals [2].

For example, during fungi infestations antifungal phytochemicals, especially metabolic pathways leading to isoprenoids, phenylpropanoids, alkaloids, fatty acids, and polyketides are up-regulated [3]. Many phytoalexins, which partly have been described as flavor-active, nutritional components and as a source for development of health-promoting food products, have been well documented in crop plants in the field of plant defense [3]. In contrast, primary metabolism is documented to be mainly influenced by abiotic stress challenges [2, 4–6]. For instance, in *Arabidopsis* leaves, next to the amino acid profiles, oligosaccharides, γ-amino butyrate and the metabolites from the tricarboxylic acid cycle are influenced during drought conditions [7].

Analysis of those changes in the molecular composition of plant material by means of mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR) based techniques is called metabolomics, which can profile the impact of time, stress, nutritional status and environmental perturbation on hundreds of metabolites simultaneously. It is an essential technology for functional genomics and systems biology which can visualize and answer questions about biological systems [8, 9]. Kushalappa and Gunnaiah [10] already predicted, that while in the past genetically tools were mainly used for crop improvement on yield, flagged as green revolution, in the next decades, functional genomics to identify genes that enhance crop yields without losses in nutritional values but with minimizing loss due to stress will be the main topic. Metabolomical working techniques are especially helpful to discover metabolites up-regulated during stress conditions. These metabolites then can be tested for their biological function. Thereby, the combination and understanding of information received from genomics, transcriptomics, proteomics and metabolomics studies is a complementary tool for functional genomics and systems biology investigations. Although nowadays, next to the human genome [11], the genome of several plants such as *Arabidopsis thaliana* [12], rice [13, 14], tomato [15], and barley [16] have been decoded by means of automated nucleotide sequencing, the knowledge about the regulation of gene expression, about the action of these genetic products, and especially about the metabolic networks resulting from catalytic proteins is rather fragmentary [8].

For a long time, primary and also secondary metabolites were simply considered as one of the end-products of gene expression and protein activity. Nowadays, it is increasingly accepted that low-molecular weight molecules modulate macromolecular processes through, e. g. feedback inhibition and by signaling phytohormons [8]. Therefore, Dixon et al. [8] conclude that, metabolomic studies are “intended to provide an integrated view of the functional status of an organism”.

Although for a single plant like *Arabidosis thaliana* no more than round about 5,000 metabolites have been assumed, the plant kingdom is reported to contain between 200,000 and 1,000,000 metabolites all of them having different structural features and polarities [6, 17–20]. This wide structural diversity with different chemical properties is a challenging task during sample work up and analysis. Therefore, monitoring the whole metabolome with exclusively one technique is not possible [21]. In addition, the knowledge about secondary metabolites, having an antifungal, a

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toxically, or an off-flavor activity or having promising human health promoting activities, is not yet sufficient.

Therefore, an important aspect of the use of crop plants for human consumption is the characterization and determination of their nutritional value as well as their individual flavor-active, health-promoting, non-toxic constituents. The aim of crop plant research should be to find a 'perfect' functional pheno-/genotype as golden standard with high resistance and best possible quality criteria for future breeding experiments (cf. Figure 1). Analytical marker compounds can help to support molecular breeding efforts to obtain phenotype knowledge based plant populations, no matter which analysis method is used, such as bulk segregant analysis, mapping by sequencing or QTL analysis is used [22, 23].

Next to the aim to avoid quality or yield losses induced by abiotic or biotic stress conditions, there are exciting new ways of thinking that plant stress also can also be used to produce phytoalexin-enriched functional foods [3] or to enhance the flavor quality of crop plants by stress or elicitor challenges to produce new functional foodstuffs with high quality [24]. Dixon et al. [8] additionally suggest that the long-term goal should be the application of new knowledge of functional metabolites for "rational custom-designed breeding by classical methods as well the application of genetic engineering techniques to improve and develop new aromatic plants". Therefore, publicity open databases, containing all spectroscopic, spectrometric and bioactivity data as well as the biosynthetic pathways of worthy metabolites are necessary in the future [8].

Targeted as well as non-targeted metabolomic based working techniques, such as phytometabolomics, sensomics, lipidomics or hormonomics can help to unravel metabolic pathways, signal transmissions as well as metabolites with different bioactivity which can be simultaneously up- or down-regulated by stress conditions. After decoding marker compounds by means of these non-targeted approaches, marker metabolites can be isolated from the plant material and characterized by means of 1/2D-NMR or MS based structure identification techniques as well as by biological assays (e.g. using antifungal test systems). Although the last-named structure identification techniques and the testing of the metabolite's bioactivity do not belong to the metabolomics approach, its application is mandatory to understand biological processes.

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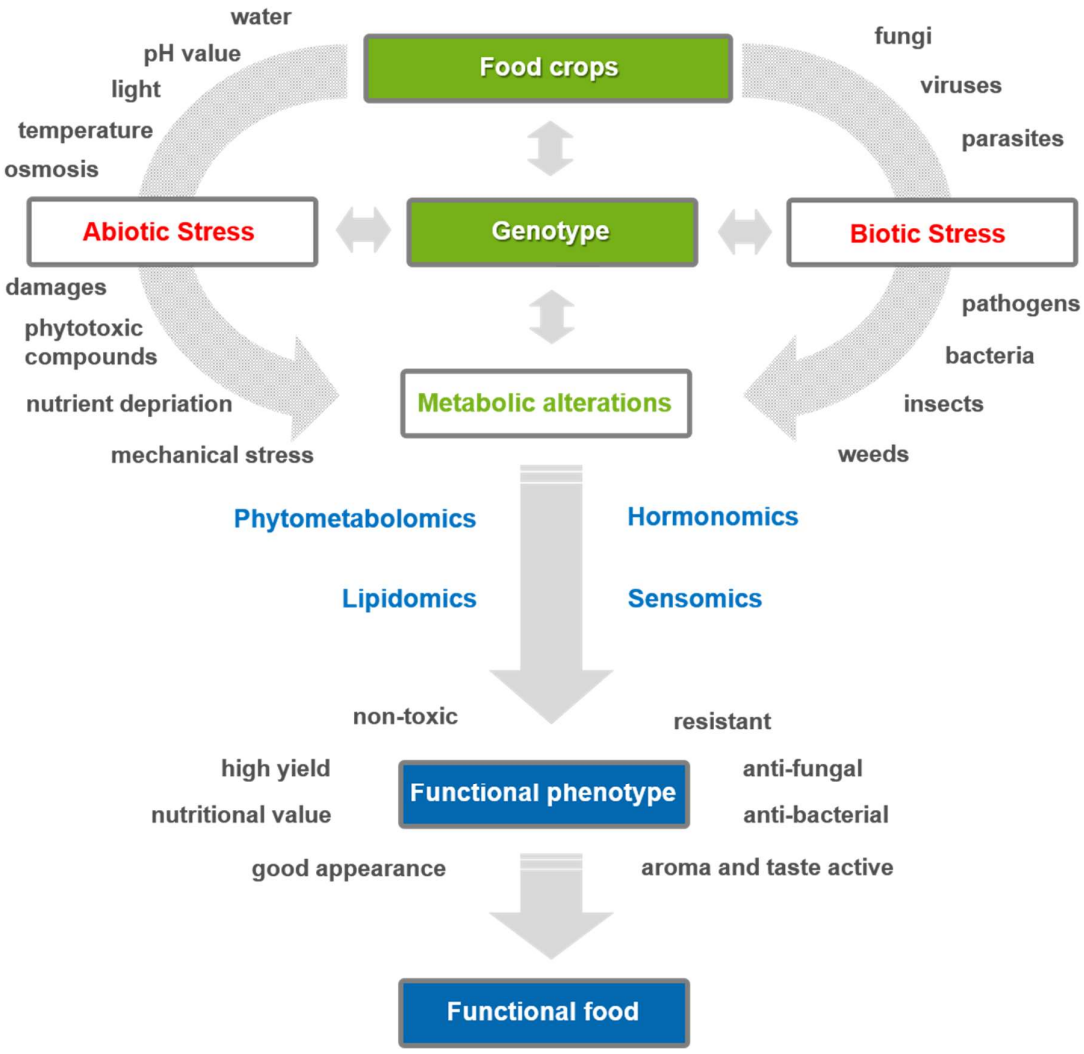

Figure 1. Functional metabolomics – a useful tool to discover metabolome alterations during abiotic and biotic stress to find the perfect functional pheno-/genotype to produce functional food.

2. Analytical techniques used to characterize stress induced metabolome alterations in plants

Enormous technological progress has been achieved since the early days of plant metabolome characterization. Starting with thin-layer chromatography (TLC) and gas chromatography coupled to flame ionization detector (GC-FID) to characterize the first primary and secondary metabolites in plant material, nowadays depending if high-throughput, sensitive techniques using gas chromatography, liquid chromatography as well as capillary electrophoresis coupled to mass spectrometry (GC-MS, LC-MS/MS, UHPLC-TOF-MS or CE-MS) or nuclear magnetic resonance spectroscopy (NMR) together with bioinformatics tools are state-of the art metabolomics working techniques [8]. With the development of mass spectrometry imaging approaches, including MALDI or DESI ionization procedures coupled to high resolution mass spectrometer, it is possible to perform metabolomics analysis *in situ*, too [25]. Although NMR based metabolomics techniques are used in

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plant metabolomics, it is less common in plant-stress metabolomics. However, new working techniques including NMR-technology, such as differential off-line LC-NMR (DOLC-NMR), show to be promising to characterize metabolites up- or down-regulated during biotic or abiotic stress influences on plant [26].

Although the term plant metabolomics is defined as the identification and quantification of all low-molecular weight substances in an organism, at a defined point of harvest time and development stage and in a given organ, tissue or cell type, there is no single work-up protocol or standard technique available to detect all metabolites using just one platform. Therefore, usually several analytical separation and detection techniques are combined to visualize abiotic and biotic stress induced metabolome alterations of different plants induced by abiotic and biotic stress [21].

In the last four decades, different methods have been established for the targeted and non-targeted characterization of plant metabolites (cf. the following reviews: Shualev et al. [2]; Urano et al. [5]; Obata and Fernie, [6]; Arbona et al. [21]). Nowadays, we differentiate between the non-targeted, sometimes also called untargeted, and the targeted metabolomics approach. While the non-targeted approach represents a comprehensive analysis of all measurable compounds, including unknown metabolites, targeted metabolomics is the measurement of defined groups of chemically characterized and biochemically annotated substances [27]. For both, targeted and non-targeted, analyses GC-MS, UHPLC-TOF-MS, as well as LC-MS/MS are the most common technique to characterize stress induced metabolome alterations in plants [6].

Especially, primary metabolites, such as carbohydrates, amino acids or fatty acids and all volatile compounds are mainly characterized by means of targeted GC-MS with or without derivatization [28]. Due to the fact that standard compounds are commercially available and spectrometric data are published in several public libraries, such as NIST, the identification and exact quantitation of primary metabolites by means of GC-MS analysis is straightforward. In contrast, structure identification of secondary metabolites exclusively by means of MS data is rather challenging ([21, 29]. Metabolomics approaches mainly identify secondary non-volatile compounds by means of non-targeted UHPLC-TOF-MS techniques. In contrast to GC-MS, LC-MS libraries for structure identification are less developed, because instrument-type dependent mass spectra and MS fragmentation patterns, as well as retention time shifts depending on used LC columns complicate the comparison of structure identification results using different instruments [30]. In the future, a world-wide concept of raw data processing, extensive mass spectral libraries and powerful database management systems that can store and provide both raw and meta data are desirable.

At the moment biological active compounds are unequivocally identified by means of MS and 1/2D NMR experiments after their isolation by means of preparative HPLC techniques. But their MS spectra obtained were mostly not shared with the metabolomics community due to a missing standardized data handling concept. Once identified, sensitive quantitation methods using stable isotope dilution assays (SIDA) are carried out by means of LC-MS/MS or GC-MS techniques. Therefore, nowadays, determination of the exact concentration of the isotopically labled internal standards can best be achieved by means of quantitative NMR spectroscopy (q NMR) [31].

Moreover, a major challenge for combining all omics-techniques is the integration of detailed metabolomics data into transcriptomic and proteomic profiling data sets [8]. Also Urano et al. [5] summarized, that holistic omics analysis and their data interpretation are inalienable to identify the

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broad function of metabolite regulatory networks during the responses to plant stress. Therefore, bioinformatics knowledge and tools and knowledge should be used more often in the future to combine the information from both research fields.

3. Phytometabolomics – From plant stress to metabolic response

Plants continuously encounter various biotic and abiotic environmental stresses during its growth and development phases. While biotic stress is caused by pathogens, parasites, predators, and other competing organisms, abiotic stress arises from inappropriate levels of physical components of the environment, such as temperature or water extremes [4]. Both stress factors lead likewise to yield, and quality losses in plant crops used in human nutrition. On the one hand to avoid, those immense quality and yield losses to ensure feeding the world's growing population and on the other hand to develop new functional foods and to reduce insecticides and pesticides used in agriculture the knowledge about molecular networks and pathway activation of plants during their stress response is inalienable.

Only recently Mithöfer and Boland [32] asked the question "Do you speak chemistry?". They highlighted that plants when they recognize and respond signals from their environment always response by means of chemical compounds. Moreover, they pointed out that plants use metabolites as chemical sensing and communication systems. On the one hand signaling molecules and phytoalexins are formed, on the other hand it is suggested that plants "cry-for-help" by producing secondary metabolites as indirect defense system. Those cry-for-help compounds usually belonging to the group of volatile organic compounds attract predators or herbivores helping the plant to get rid of other stress triggers [32, 33]. Thereby, it is important to notice that stress-specific as well as common metabolites, so-called generalist and specialist, can be formed or their amounts can be up-regulated [6].

The understanding of metabolic stress pathways is just possible if comparative metabolite analysis by means of metabolomics working techniques of stressed and non-stressed plants followed by structure identification experiments are performed. In the following three subsections a first view into the literature knowledge about biotic and abiotic stress studies is given:

3.1. From abiotic plant stress to metabolic response

It is well-expected that climatically changes due to the global warming leads to massive abiotic stress factors influencing the metabolome spectra of all plants in the future. Next to water stress, including soil flooding and drought stress, salt stress, temperature stress, light stress, sulfur and phosphorus stress, oxidative stress, as well as heavy metal stress will play an important role for food crop producers. To understand and avoid quality and yield losses induced by abiotic stress factors several metabolomics studies have been achieved (cf. the following reviews: Shulaev et al. [2]; Kaplan et al. [4]; Urano et al. [5]; Obata and Fernie [6]; Arbona et al. [21]).

Among them, Arbona et al. [21] already summarized in their review "Metabolomics as a tool to investigate abiotic stress tolerance in plants" that the plants' metabolic responses to abiotic stress can mainly be found in the primary metabolism. Next to metabolic responses in mono- and disaccharides, sugar alcohols, amino acids, especially proline, polyamines and members of the tricarboxylic acid

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cycle were found to be influenced by abiotic factors. Thereby, especially the carbohydrate metabolism is directly influenced by stress conditions, because during stress instead of glucose plants use fructanes and starch as energy source [34].

In their review Obata and Fernie [6] compared several metabolic fingerprints of *Arabidopsis* leaves influenced by dehydration, salt, heat and cold, high light and sulfor limitation, UV, light quality change, low nitrogen amounts, and potassium limitation with each other. Thereby, they highlighted that specific compounds and compound classes are generally accumulated during abiotic stress factors (except of light treatment), such as sucrose, raffinose, proline, other branched chain amino acids or GABA. However, the amounts of such up-regulated "generalists" varies from stress to stress. They concluded, that the stress-specific plant response is the result of an inhibition or activation of a defined metabolic pathway. Especially, enzymes activities are known to be influenced by temperature or ion concentrations in plants. In contrast, other metabolites, such as trehalose accumulated only under specific conditions.

Next to the carbohydrates additionally the lipid composition of plants is influenced by abiotic stress factors. Lipids in plants are a crucial and diverse class of biomolecules that forms the so called plant lipidome. Lipidomics is defined as the identification and quantification of all lipids within a biological system at a defined stage of development [35]. Within the field of omics-analysis lipidomics represents a subunit of targeted and non-targeted metabolomics approaches, mainly using MS and NMR based techniques. The development of lipidomics and its increased promise in systems biology over the past two decades has been the subject of several reviews (e.g.: Blanksby and Mitchell [36]). Changes in the lipidome, induced by modifications of its biosynthesis, regulation, adaption, remodeling, function, role, and interaction, as well as membrane lipid remodeling are relevant responses from plants cells to counteract biotic and abiotic stress challenges [35]. Especially, the oxidation of polyunsaturated fatty acids is one of the most fundamental reactions in lipid chemistry, which for example leads to off-flavor (perceived rancidity) or to phytohormon precursor formations [37]. Different groups of lipids, such as fatty acids, phosphatidic acids, inositol phosphates, diacylglycerols, oxylipins, shingolipids, and *N*-acetylethanolamine are involved in signaling systems during stress conditions, too [38–43]. Those compounds are shown to be directly synthesized after abiotic stress influences by a wide range of enzymes, like fatty acid amide hydrolases, phospholipases, acyl hydrolases, diacylglycero kinases or phytoshingosine kinases [43]. In addition, it is known that in response to abiotic stress factors, lipids migrate into cell walls to repair damages by membrane remodeling [43–45]. Moreover, a combination of transcriptomics and lipidomic profiling exhibited that cold stress induces the prokaryotic pathway and suppresses the eukaryotic pathway for glycerolipid biosynthesis [46]. Next to cold stress, light and temperature stress, as well as nutrient starvation as typical abiotic stress factors were studied by means of lipidomic approaches in *Arabidopsis* (for details cf. the following review: Tenenboim et al. [35]). Only recently, lipidomics studies led to the discovery of the role of leaf lipids in thyme plant response to drought stress [43].

In the future, more stress related lipidomic studies are expected, due to the fact that also in nutritional research lipid profiling plays an important role [35]. On the one hand, food industry shows great interest in the use of lipidomics to characterize defined crop plant genotypes. For example, due to the fact that a replacement of palm kernel fat by a natural alternative is wanted, new plant genotypes having the same triglyceride pattern is needed. On the other hand, although most

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genes annotated as lipid-related are functionally characterized their corresponding metabolites are not assigned. That would be an interesting field of research in future, too.

In sum, although integrated omics data of model plants such as *Arabidopsis* have markedly increased our knowledge of understanding the mechanisms involved in plants' response to various abiotic stress factors [5], the knowledge about secondary metabolome alterations in abiotic stressed food crops is rather fragmentary. Solely few studies indicated abiotic stress influenced the amounts of phenolic compounds, glucosinolates, carotinoides terpene derivatives and phytohormons in plants [21, 35]. Urano et al. [5] emphasize again and again that phytohormon levels, especially those of abscisic acid, varies a lot in different plant compartments. Next to knowledge gaps in the stress' influence on the secondary metabolome, more studies are needed to characterize stress combinations, too [6].

3.2. From biotic stress metabolomics to metabolic response

During biotic stress exposure plants use qualitative and quantitative measures to resist pathogen attacks [47]. While, in the past the qualitative resistance, based on monogenetic inheritance, has been successfully used to elite cultivars to improve resistance, the quantitative resistance, which is presumed to be durable, non-race-specific, and effective against a large bouquet of pathogens, is mainly unknown [10]. However, a huge number of quantitative trait loci (QTLs) associated with qualitative resistance against pathogen-associated molecular patterns (PAMs), found for example in soy beans (*Glycine max*), and with quantitative resistance, found for blast in barley (*Hordeum vulgare*), or powdery mildew in wheat have already been identified for biotic stress challenges [10, 48–50]. But both, the mechanisms of resistance and the metabolites up-regulated during plant stress controlled by the QTLs are mainly unexplored [48]. Especially, targeted metabolomics approaches enables the possibility to monitor marker compounds up-regulated during plant stress, which then can be isolated, identified and biologically characterized, for example an antifungal test can be approved.

Non-targeted metabolomics in comparison with bioinformatics and database research can also give an idea, which metabolic pathways are activated during plant's stress response. Moreover, targeted analysis of known bioactive, e.g. anti-microbial or anti-fungal, compounds can help to identify QTLs, by using targeted metabolomics techniques for phenotyping experiments in breeding crossing lines. Thereby, next to breeding improvement, metabolomics helps to understand biological functions, and to study host-pathogen interactions [10].

It is already known from plant-pathogen interaction studies that signaling molecules, such as ethylene, salicilic acid, jasmonic acid or inositol, as well as antifungal/antimicrobial phytochemicals and cell wall compartments are formed or their concentrations are up-regulated during plant stress [10]. Numerous studies already indicate that a complex network of secondary metabolites is influenced by biotic stress challenges. Next to phenylpropanoids, including flavonoids [51–53], alkaloids [54, 55], terpenoids [52], and fatty acids [56] are known to be up-regulated [10]. In addition, lipids act under pathogen or herbivore attack as mechanical barriers. The plants' wax layers form the first line of defense against pathogens and herbivores [35, 57]. Additionally, some lipids serve as signaling molecules, namely oxylipins and jasmonates, participating in immunity answer cascades, and phytoalexins, a group of fatty acid degradation products, have antimicrobial and antifungal

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activities [35]. In addition, only recently Caroline Gutjahr's lab could show that next to carbohydrates, also fatty acids are transported from the plant host to fungi [58].

Moreover, due to the fact that plants produce a diverse array of over 100,000 low molecular weight natural products [3], countless studies are available using targeted quantifications of phytoalaxins in plants (cf. Journal of Phytochemistry, Journal of Agriculture and Food Chemistry or Natural Product Chemistry).

Already 10 years ago, Boue et al. [3] published a forward-looking paper called "phytoalexin-enriched functional foods" in which they proposed to use stress conditions to enhance the amounts of phytoalexins in food plants to produce functional foods. Several phytoalaxins are known for their health beneficial properties, including antioxidant activities, anti-inflammation activities, cholesterol-lowering abilities, and also anticancer activities [3]. Especially, flavonoids, which are ubiquitous in many food plants have been linked to important health-promoting activities. For example, consumption of legumes, especially soy, have been linked to the reduction of cancer risks and coronary heart diseases just because of its high flavonoid yields [3]. Therefore, next to resistance and yield stability, additionally, functional food ingredients from the phytoalexin family could be a possible target for plant breeders. In addition, mild stress condition would maybe help to enhance the amounts of bioactive secondary metabolites and to produce functional foods [3].

Next to aspects in nutrition, diet and health, especially, food and environmental safety can be monitored by means of metabolomics [8]. On the one hand, toxic compounds are formed as stress response by plants, such as furanocoumarins in celery and glycoalcaloides in potatoes, on the other hand especially fungi produce human toxic mycotoxins [59]. Therefore, versatile, sensitive, reliable and fast targeted LC-MS/MS and GC-MS methods using stable isotope dilution techniques have been developed in the past to monitor food quality [60, 61].

In sum, future more versatile and sensitive multiple targeted MS quantification method to evaluate food and crop quality are required.

3.3. Functional phytometabolomics – characterization approach of plant stress metabolites

In conclusion, the science-driven breeding of stress-tolerant cultivated plants that would allow for a reduction in harvest losses and undesirable decrease in quality attributes requires a new quality of knowledge on molecular markers associated with relevant agronomic traits, on quantitative metabolic responses of plants on stress challenges, and the mechanisms controlling their biosynthesis. The field of "functional phytometabolomics", therefore, using targeted and non-targeted MS or NMR techniques to quantitatively assess key metabolome alterations in plant-derived crops and foods induced by biotic stress challenges as well as abiotic stress conditions, is a promising field of research.

In the phytometabolomics approach metabolites up-regulated during stress challenges are visualized by means of metabolomics based working techniques. Markers previously not published in literature, e.g. visualized by retention time and mass to charge ratio in UHPLC-TOF-MS analysis, are isolated in purities higher than 98% from the plant material using MPLC and preparative HPLC techniques and are identified by means of LC-MS, LC-MS/MS, UHPLC-TOF-MS and 1/2D NMR experiments. Testing of different biological activities (anti-fungal, anti-bacterial, anti-oxidant activities etc.) of those compounds allow first insights into their biological functions. To translate the knowledge on how stress-resistant traits master their successful defense against stress conditions into

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breeding programs, genotype specific metabolome alterations have to be characterized and gene clusters controlling the biosynthetic pathways of key stress metabolites have to be identified by means of genome-wide association studies. This research will help to navigate breeding programs and to optimize post-harvest treatment of plant-derived food products from producer to consumer/processor towards the production of high quality food products.

4. Phytohormone profiling by means of plant hormonomics

Phytohormones are a class of low molecular weight, structurally diverse, but highly bioactive compounds in plants, that acts as chemical messengers, triggering and controlling physiological processes during plant growth and development (e.g.: cell elongation, regulation of apical dominance, vascular differentiation, fruit development, latal and adventitious root formation) as well as in response to abiotic and biotic stress conditions [62, 63]. Next to ethylene, auxins, cytokinins, brassinosteroids, gibberellins, jasmonates, salicylates, polyamines, abscisates and signal peptides, strigolactones are part of the phytohormone family [63, 64]. During stress exposure those phytohormone classes interact with each other by means of synergistic or antagonistic cross-talks, resulting in each other´s biosynthesis or response up-regulation [65]). In the past, several studies gave evidence, that plant hormones are necessary for plants to adapt changing environments, especially, during abiotic stress factors by mediating a wide range of adaptive responses [65]. They play a key role in the plant's intricate signal networks and often immediately alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin–proteasome system [65, 66]. Although the analysis of plant hormones, such as auxins, especially their quantification by means of SIDA-LC-MS/MS revealed significant insights in their tissue- and cell-type-specific analysis, their distribution profiles in plant organs, tissues, and cells still remains elusive [63]. Although a wide variety of targeted GC-MS and LC-MS/MS methods have been published dealing with the quantitation of exact amounts of single members of the phytohormone family (e.g., cf.: Porifiro et al. [67]; Novák et al. [63]) during the last two decades, exclusively one method was described to characterize those physiologically important molecules in their network interactions in one single run [68]. To characterize the majority of known phytohormones as well as their biosynthetically precursors, that regulate diverse processes in plants by intricate signaling networks, only recently, Ondřej Novák and his team developed a new, versatile and sensitive targeted UHPLC-MS/MS method, which enables the simultaneous quantification of 101 phytohormone-related metabolites (phytohormones and their precursors) in less than 20 mg plant material [68]. With this newly-developed, targeted metabolomics approach, so called „plant hormonomics", Šimura et al. [68] were able to detect 45 and quantify a total of 43 endogenous compounds out of the 101 phytohormones in both, root and shoot samples, in salt-stressed and non-stressed, 12-day-old *Arabidopsis thaliana* seedlings. Subsequent multivariate statistical analysis and cross-validation with data obtained from transcriptomic studies enabled the identification of the main phytohormones involved in the adaption of *Arabidopsis thaliana* to salt stress. While the multivariate statistical analysis revealed that 23 of the quantified 43 metabolites significantly differed between the salt-stressed and non-stressed roots, in the shoots the concentrations of deviant 15 compounds differed. Those obtained hormone profiles were cross-validated with transciptomic data. Thereby, well in line with findings from Rhy and Cho [69] who found that jasmoic acid and abscisic acid to promote salt tolerance in *Arabidopsis*,

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Šimura et al. [68] also observed in salt-stressed root samples increased levels of abscisic acid, its oxidation products phaseic acid, dihydrophaseic acid, and jasmoic acid, too. In addition, different responses of gibberillinic acid derivatives, especially its active form GA4, and its transcriptomic data were found by Šimura et al. [68]. In sum, this newly developed multiple parallel analysis of phytohormones, called „plant hormonomics" enables the real-time profiling of hormone networks of large collections of phytohormones, their precursors, transport forms and degradation products in single stressed or non-stressed samples [68, 70].

Although, Šimura et al. [68] were already able to quantify the main phytohormones in less than 20 mg of salt stressed *Arabidopsis* samples, special derivatisation reactions of the analytes prior to their analysis lead to a conceivable increased sensitivity and selectivity during MS analysis in the future [67, 70–72]. For example, the response/sensitivity during LC-ESI-MS/MS analysis of metabolites only present in very low concentrations in plants, like indole-3-acetic acid, a member of the auxin family, can be tremendously increased up to 200 times after methylation [71]. In addition, several aldehyde trapping derivatisation reagents are known to enhance the sensitivity during ESI-MS analysis of biological mixtures [73]. In the future, it could be an extremely important and advantageous step in hormonomics to analyze a combination of derivatised and non-derivatised phytohormone classes, simultaneously in one method.

5. Sensomics - a phenotyping tool to characterize crops flavor impression

International survey data reveal that more than 83% of consumers say that flavor influences their decision the most when purchasing any kind of food products or beverages [74]. Therefore, it is no surprise that, although aided by visual inspection, the final recognition and quality evaluation of food crops made by consumers are mainly mediated by its flavor perception. Human flavor perception is induced by the interaction of volatile odor-active and non-volatile taste-active molecules with ~390 odorant receptor proteins located in the *regio olfactoria* in the nose and ~40 taste receptor proteins on the tongue [75]. To meet the consumers' demand on continuous available, fresh foods with a premium quality, the "flavor blueprint" of a golden standard, which is a combinatorial code of the entire set of odor- and taste-active metabolites in their natural concentrations, has to be known [76]. To decode all flavor-active molecules of a crop, the so-called sensometabolome, several high-end working techniques, including combinations of state-of-the-art metabolomics and chromatography approaches in combination with human sensory science experiments, such as aroma or taste dilution analysis, have been developed in the past. This so called molecular sensory science or sensomics approach has especially been shaped by Peter Schieberle´s and Thomas Hofmann´s working groups during the last three decades [75, 76]. Today, it is well accepted that the presence of certain structural elements, so-called olfactophores and gustophores, as well as specific concentrations exceeding the sensory thresholds are important prerequisites of low molecular weight metabolites to become flavor-active [76]. Although a total of more than 10,000 volatiles occur in crop plants, the use of the so-called sensomics approach gave evidence that the typical aroma impression of a crop based food stuff is caused by a limited number of aroma-active volatiles [75]. In conclusion, only a comprising and surprisingly small group of so called "key odorants in food" contribute to the typical aroma profile of a crop plant. For example, only two compounds, namely ethyl (2*S*)-2-methylbutanoate and

1-(ethylsulfanyl)ethane-1-thiol, are necessary to explain the typical aroma impression of durian fruits [77], and to mimic the typical aroma impression of mangos only eight compounds are required [78]. While only a small number of aroma-active compounds contribute to the key odorants in food and interact with a huge number of odorant receptors, several thousands of non-volatile taste-active compounds are already known. Especially, the application of taste dilution analysis followed by dose/activity considerations led to the discovery of many bitter, sweet, umami, pungent, astringent, salty, or sour sensing molecules in several plants, such as carrots [79, 80], cocoa [81], asparagus [82–84], pepper [85], red currents [86], tea [87], stevia [88] or spinach [89] in the past. Due to the nowadays well-accepted fact that not only a single flavor-impact molecule, but a combinatorial code of multiple odor- and taste-active key compounds, each in its specific concentration, reflect the chemosensory phenotype and trigger the typical flavor profile of food products. In particular, for flavor improvement of crops, the analysis of aroma- and taste-active compounds present a major challenge for flavor improvement of crops [76]. Although it has been shown in the past, that the biosynthesis of several key odorants in food and tastants is controlled by genes whose expression is altered or even induced by biotic or abiotic stress challenges, in the past, crop production often has been targeted primarily toward field performance, yield, and storage characteristics, while ignoring quality trails, such as the flavor code [24, 90].

On the one hand, induced by several biotic and abiotic stress factors during growth in the field as well as during post-harvest storage, attractive sensory quality of miscellaneous crop plants is hindered by sporadic off-flavors, which is often the reason for consumer complaints and therefore a major problem for plant processors. On the other hand, mild stress factors can lead to an increase in in the concentration of flavor-active constituents and hence to a more intensive desirable aroma or taste impressions [24, 90].

Moreover, abiotic factors, such as mechanical stress, is reported to increase the amounts of the key bitter tastants, members of the C17-polycetylenes, present in native carrots (*Daucus carota* L.) to efficiently high concentrations, causing a bitter off-flavor is perceived, occurring especially often during the production process for infant diet carrot products [79, 80, 91–98]. In addition, a decrease in flavor quality accompanied by an increase in bitter taste has also been reported in raw hazelnuts (*Corylus avellana* L.) upon biotic stress challenges, such as upon infection by bugs, belonging to the hemipteran family, like *Gonocerus acuteangulatus* and *Coreus marginatus* [99].

Next to the non-volatile, taste-active metabolites, aroma-active compounds are also known to be influenced by individual stress factors [24]. Especially studies dealing with tea (*Camellia sinensis*), which is enjoyed as freshly brewed green, black, oolong, or decaffeinated tea infusion, and its quality changes induced by stress are available in literature. For example, the tea green leafhopper (*Empoasca (Matsumurasca) onukii* Matsuda) attacks, at pre-harvest stages, can decisively influence the unique aroma quality of tea leaves as a result of the upregulation of the linalool synthase (CsLIS1 and CsLIS2) causing higher concentrations of the key odorant, linalool [24, 100]. Next to linalool, further key odorants, such as (*E*)-nerolidol, can be influenced by a combination of low-temperature stress and mechanical damages [24, 101]. Therefore, Wüst [24] concluded, that the tea-related findings nicely illustrate how the use of stress response of plants within the sensometabolome can lead to an improvement of flavor of agricultural products. In addition, he pointed out, that the plants contact

with stress elicitors, such as methyl jasmonate instead of the actual biotic or abiotic stress factors could also lead to "stress induced" flavor improvement [24, 102].

In order to gain a more comprehensive knowledge on the chemical mechanisms involved in quality changes of cultivated crop plants in response to biotic or abiotic stress challenges or to improve the flavor quality by the application of moderate, well controlled stress, numerous volatile, aroma-active as well as non-volatile taste-active key metabolites, the so-called sensometabolites, of stressed and non-stressed plant genotypes should be comparatively characterized by means of a fast and robust high-throughput GC-MS systems with high peak separation capacity and sensitivity, such as comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC×GC/TOF-MS) or ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC-TOF-MS) metabolic profiling analysis in the future. This strategy aims at reducing the flavor deficiencies in modern commercial varieties as a "green" alternative to genetic engineering. The workflow for a successful implementation of this approach – from the identification of key odorants by molecular science techniques to the investigation of mechanisms controlling their biosynthesis – is complex and calls for interdisciplinary research [24, 90].

6. Conclusion

Plants continuously encounter various biotic and abiotic environmental stresses during their growth and development phases, which leads likewise to yield, and quality losses. To avoid, those previously listed economical losses, to reduce insecticides and pesticides used in agriculture, as well as to ensure feeding the world's growing population and to develop new functional foods the knowledge about molecular networks and pathway activation of plants during their stress response is inalienable.

This review emphasized the importance of metabolomics based working techniques to discover metabolome alterations during abiotic and biotic stress conditions. Thereby, metabolomics is a promising tool for knowledge-based targeted breeding programs. It also shows, that due to missing data bases and non-standardized LC-MS conditions pure metabolomics, lipidomics and phytohormonics strategies, without isolation and unequivocal structure identification experiments sometimes are not sufficient enough. Therefore, techniques using biological and molecular structural characterizations of single marker metabolites in combination with metabolomics techniques, such as phytometabolomics or sensomics approaches, are useful solutions to produce high quality phytoalexin-enriched functional foods in the future.

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