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Metabolomics as a Tool in Agriculture

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Abstract

Metabolomics is a study through which can be obtained a better understanding of the complexity of the biological systems, through the chemical composition and relations with the physiology of the plant. The literature describes a lot of information on this support in areas of medicinal plants, chemosystematics, adulteration of plants, etc., but it is scarce in agriculture. At present, agriculture plays a crucial role in human beings. The demand of foods has increased due to the continuous increase in the population, and this requires an increase in the production of crops, besides the crops being affected by the climatic change, attack of pest and diseases and resistance to conventional agrochemicals. At present, scientists are doing some practices or studies of genetic improvement of crops to increase their production and avoid the problems pointed out. It is an important part of the genomic studies; the results could be the basis for a genetic improvement based on the chemical composition of the crops, and in the metabolomics studies represent a crucial role in their quality for human consumption. The aim of the chapter is to review the literature from 10 years behind emphasizing the importance of metabolomics in crops of economic and feed value.

Keywords: agriculture, metabolomics, crops

1. Introduction

Plants are a large source of natural products with a large chemical diversity and unimaginable properties; therefore, the interest in their study is increasing. Nowadays, the interest in agricultural crops has increased due to their economic importance and their projected importance in the next years in relation to world food security.

The usage on the plant species destined to agriculture is varied, from traditional foods to those with some desirable traits, such as nutritional value and the industrial products derived from them such as polymers, fibres, latex, industrial oils and packaging materials, in addition to basic chemical building blocks and fuels [1].

The objectives of the use of metabolomics tools in agriculture are to know the biochemistry and the functions of the species involved to apply that knowledge to food and environmental security; to use their potentials as tools in the improvement of nutrition, diets and health [2]; and to use them for genetic improvement in plants based on chemical composition, that is, taking into account some notable trait.

Nowadays, agriculture plays a crucial role in human feeding. Food demand has increased considerably due to the continuous increase of population centres, and thus, the demands on the production and diversity of crops, especially basic crops, are increasing. To this problem, we can add the noxious effects of climate change on crops, the attack of pests and diseases, as well as the emergence of new diseases and the development of resistance by them towards commonly used pesticides. Currently, trials or studies of genetic improvement of crops are being carried out to increase the quantity and quality of yield, avoid damages caused by pest and diseases and develop resistance to several factors, especially environmental ones. There are some crops that are considered as basic or important to world food, such as rice, maize, potato, avocado, tomato and citrus fruits, among others; these crops are placing themselves among priority crops because they support human consumption and thus have been the subject of the aforementioned studies or trials.

2. Metabolomics

Metabolomics represents a field of study with which we can gain a better understanding of the complexity of biological systems. It deals with the identification and quantification of the metabolites present in such systems [3, 4] with molecular weights less than 1500 Da [5], although the range could be occasionally wider (30–3000 Da) [6]. The group of small molecules of metabolites that are in a cell, in an organ or organism, is called metabolome [6, 7]. It is made up of a large variety of molecules such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, flavonoids, polyphenols, alkaloids, minerals or any other chemical compound that is used, metabolized or synthesized by a cell or by a given organism [7]. The importance of the metabolites resides in that they are an essential part of the behaviour of the individual that contains them; these compounds are the final products of the regulatory processes of the cell, and their presence represents the response of biological systems to environmental or genetic changes. It is due to this that metabolomics is considered as the link between genotype and phenotype [8, 9]. In the last years, metabolomics has managed to position itself as an area of research that is essential and complementary to proteomic, genomic and transcriptomic studies.

Metabolomics is a very important complement of genomic studies, and its results could be the basis of genetic improvement based on the chemical composition of crops, be it in nutritional or functional aspects, or in the participation of some chemical compounds in the resistance of some plant species to the factors that have already been mentioned.

It is important to know the current state of metabolomics studies of the most important crops in the world to use it as the basis for future studies destined to improve their production, or to know the diversity of the chemical composition that we have available, its benefits and its possible uses. Moreover, it would be important to know the advantages and disadvantages of using current analytical techniques, in addition to their current state and possible improvements in future analyses.

3. Extraction methods in metabolomics

There are numerous extraction methods used to extract and isolate the compounds of interest, but is important to keep in mind to use a simple method, robust, low consume time, repeatable and cheap. The selected solvent should extract diverse groups of metabolites. Traditional extraction methods as percolation, maceration, Soxhlet extraction, steam distillation or hydro-distillation are convenient to extract a broad class of metabolites; they are cheap, simple and

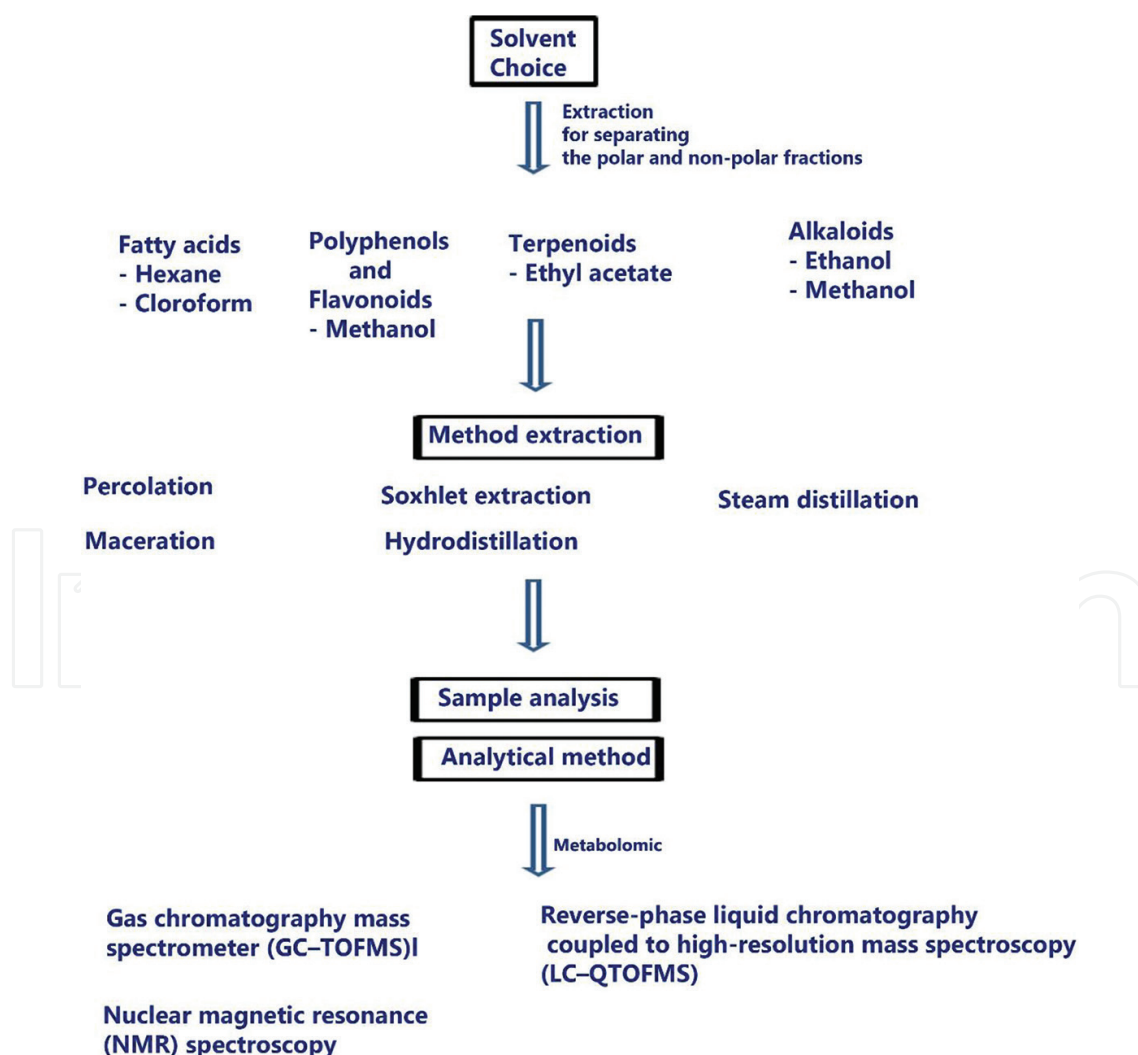


Figure 1. Extraction process and analysis of a sample. Modified from Ref. [92].

repeatable and can be used for raw plant extraction; and the amounts to be processed depend on the source of plant material, and the amounts to be processed can be since a few grams to a higher amounts, but depends of the source of plant material, but they are time consuming (**Figure 1**). At present, they can complemented with modern techniques as ultrasonication (sonochemistry), microwaves, supercritical fluid extraction or accelerated solvent extraction; they are simple, repeatable and mainly the extraction time is short, but the cost of these equipments is high. It is also important to consider the effect of temperature provided by the selected method, because some components of the sample can be decomposed if the temperature is high. Roesnner and Dias [10] described a recent review where they notice all the details that should be consider to get a good result with the extraction and isolation of the compounds of interest. Once the sample is extracted properly, is ready to submit for the analysis on GC-MS, LC-MS, NMR or MS.

4. Analytical techniques

Today we have a wide variety of analytical techniques that are getting better and better, and which help us to obtain reliable data about the behaviour of a plant species, or about its response to diverse environmental factors, both biotic and abiotic. During the last years, there has been a boom of genetic studies of many plant species, and the generated information can be used in a wide range of applications, for example, genetic improvement. A very important aspect that could help us to improve the understanding and to enrich the information of such studies would be the inclusion of metabolomic studies. Metabolomics has been considered as the link between genotype and phenotype, that is, with this kind of studies, gene expression would be better understood, and hence, the behaviour of the plant species in the face of the aforementioned factors.

An essential aspect of the metabolomic study is the qualitative and quantitative analysis of metabolites, which allows us to know the biochemical state of an organism; that information can be used to monitor and evaluate gene function and the multiple responses of the organism to the conditions where it develops [8]. In spite of the technological advances, it is practically impossible to determine the entire composition of even a single cell in a single study or with a single analytical technique [9]; in order to do that, coupled techniques have been developed, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) coupled with mass spectrometry (MS).

The analyses that have already been mentioned are carried out using analytical techniques specialized in separation, identification and quantification. Such techniques must have high resolution, be very precise and very sensitive, and be able to analyse a wide variety of compounds of different chemical nature and origin because the structural complexity of many molecules makes their study difficult. Several analytical technologies can be used, depending on the chemical nature of the compounds. Some of them are nuclear magnetic resonance (NMR), GC and HPLC coupled with MS, as well as capillary electrophoresis coupled with MS [7].

It is known that a single analytical technique is not enough to visualize whole metabolome [11], and thus, it is necessary and important to carry out separate studies using different techniques, or using the aforementioned coupled systems.

NMR has several uses in metabolomics which can be applied or adapted to agriculture. For example, in quality control, chemotaxonomy (classification and characterization), analysis of genetically modified plants and the study of interactions with other organisms and the environment, besides the study of diseases in humans. Currently, the study of the metabolome based on NMR is accepted as an efficient analytical tool to study biological systems [6].

The main advantages of the NMR over the rest of the analytical techniques are that it can detect a wide range of chemical compounds of different nature; quantification does not pose any problem; it is highly reproducible and metabolite identification is simple [12]. Perhaps the most important benefit is that the method is quick and simple, and the damage of the existing compounds during the preparation of the extracts is minimal. However, the technique is not very sensitive [13, 14].

As it was already mentioned, chemotaxonomy is one of the main applications of NMR, since through the metabolomics profiles obtained by this technique it is possible to classify and identify plants and their derived preparations. There are several examples of the application of NMR in chemotaxonomy. The classification of *Ilex* species [15], *Cannabis sativa* [16], *Ephedra* [17], and the metabolomic differentiation and classification of species of *Verbascum* [18], discrimination of commercial preparations of *Matricaria* [19] and commercial samples of catuaba [20], among others.

5. Applications of metabolomics in agriculture

Specifically in agriculture, metabolite content is related to developmental and differentiation processes, fruit maturation processes, resistance to adverse environmental factors, stress-related problems and pathogen attack, among others.

The wide range of compounds is analysed through several analytical techniques; some of which are specific to certain compounds. Liquid chromatography coupled with mass spectrometry is a technique that can be used to analyse a wide range of compounds such as vitamins (hydrophilic and hydrophobic), coenzymes, phenylpropanoids, polyketides, terpenoids, amino acids and amines, lipids, carbohydrates, phenolic compounds and alkaloids, among others. With GC-MS, fewer compounds can be analysed due to the type of compounds that this technique can detect; although by using derivatization reactions, the number of metabolites detected by this technique increases considerably. With this technique, it is easy to analyse essential oils and fatty acids as well as terpenoids, alkaloids, monosaccharides and steroids, among others. Capillary electrophoresis, for its part, helps in the detection of oligosaccharides, hydrophobic vitamins, coenzymes, prosthetic groups, nitrogenous bases, nucleotides and nucleosides, among others [21].

Crop or plant species	Compound/analytical technique	Topic	References
Avocado	Mannoheptulose carbohydrate)/LC-MS	Development and growth	[86, 87, 91]
	Perseitol (carbohydrate) /LC-MS	Development and growth	[85, 86, 90]
	Stearic, palmitic, oleic, Linoleic, linolenic and palmitoleic acids (fatty acids), carbohydrates /FID-GC, LC	Development of rapid method	[88]
	Persone A and B/HPLC, NMR	Targeted metabolomics	[53]
	Cyanidin 3-O-glucoside (anthocyanin)/HPLC	Fruit ripening	[84]
	Sugars, protein, oil/LC	Fruit ripening	[54]
Tomato	1-Methyl-tryptophan/UHLC-MS	Plant-pathogen interaction	[50]
	Flavonoids/GC-MS, LC-MX	Metabolic engineering	[51]
	Sugars, amino acids, organic acids/NMR, GC-TOF-MS	Growth and development	[49]
	Organic acids/NMR, GC-MS	Metabolic engineering	[89]
	Enzymes/GC-MS	Fruit development in transgenic plants	[90]
Maize	Amino acids, carbohydrates, organic acids/NMR	Salt stress	[27]
	Total nitrogen, protein/MS-MS	Development and growth	[85]
	Protein/MALDI-TOF-MS	Metabolic changes under phosphorus deficit	[26]
Potato	Glycoalkaloids, fructans/GC-TOF-MS, LC-MS	Genetically modified plants	[37]
	Fatty acids, amino acids, organic acids/NMR, HPLC-UV	Genetic modifications to metabolic pathways	[39]
	Sugars, amino acids, organic acids/FIE-MS, GC-MS	Total composition analysis and quality trait	[36]
	Fatty acids, organic acids, alkaloids and amino acids	Plant-pathogen interaction	[40]
	Amino acids, organic acids, sugars and sugar alcohols	Phytochemical diversity	[34]

Table 1. Metabolomics studies in agricultural crops.

Currently, there are many examples of metabolomic studies that have been applied to agriculture with various purposes (**Table 1**). Some of the main objectives of metabolomic studies in agriculture are as follows: know the metabolic responses towards any type of stress; generate metabolic profiles for genetic mapping and generate metabolic profiles to study the impact of

heredity. Generate metabolic profiles to determine the impact of geographic location and season, study the phenotype of natural variations of certain plant species, evaluate transgenic varieties, study metabolic variations of different cultivars and carry out functional characterizations (functional genomics). Analyse metabolic changes during growth, development and differentiation, elucidate biosynthetic pathways, analyse population differentiation, carry out chemotaxonomic analyses, carry out nontargeted studies and characterize cultivars [21].

Nowadays, human world food depends on certain crops that are indispensable due to their nutritional value, and the access that we have to them.

Maize (*Zea mays* L.) has been subject of numerous metabolomic studies, many of which are related to genomic studies. For example, it has been obtained the metabolomic profile of its leaves to carry out genetic mapping using GC-MS [22]. On the other hand, the genetic basis of the metabolic diversity in its grains was analysed in a study that involved 702 genotypes collected from different geographic zones and growing conditions; in that study, 983 metabolites were quantified [23]. Another study that included the analysis of genetic background (heredity), different geographic locations and environmental conditions through GC-MS was the one carried out by Röhlig et al. [24]; in that study, they could distinguish the chemical composition of the genotypes under analysis, which helped to determine notable chemical characteristics that varied depending on the region of origin.

The development of maize, as well as of other crops, under ideal nutritional conditions is paramount in order to obtain good yield and quality; hence, the importance of carrying out studies that involve nutrient analysis. The effect of nitrogen (N) deficiency on the development of maize has been studied using metabolomic studies [25], and the effect of phosphorous (P) deficiency has been analysed through MALDI-TOF MS [26]. Stress caused by any factor limits the growth and development of any plant species. The effect of salt stress on the growth of maize seedlings was studied through ¹H-NMR; the metabolic profile obtained in the study can be used to improve the growing conditions [27].

Rice (*Oryza sativa* L.) is one of the most important crops in the world, especially in Asia. Several studies have been carried out in this crop, where the main objective has been to characterize cultivars through the metabolic profiles obtained from several plant structures, mainly the leaf, using capillary electrophoresis coupled with mass spectrometry [28]. This crop has also been subject of studies where genetic and metabolic studies are combined using techniques such as GC-FID, GC-MS and GC-TOF-MS, in what is called functional genomics [29, 30]. Naturally, in modern times, studies on transgenic individuals could not be absent, where their metabolic profiles have been determined [31].

Potato (*Solanum tuberosum* L.) is a crop that has been widely studied in several areas, among which is metabolomics. In such studies, the objectives have ranged from the elucidation of the metabolic profile to know the chemical characteristics of the tubers through GC-MS [32, 33] and the characterization of some cultivars [34, 35], to the characterization of the relationship between composition and quality traits [36].

As it was mentioned in maize and rice, there have been analyses in transgenic potato crops to know their metabolic profile; such studies have been carried out in tubers through techniques such as GC-MS [37, 38] proton NMR and HPLC-UV [39].

One of the main limitations in yield and quality of crops is the effects caused by pathogens. It is important to study the interaction between pathogens and crops to gain a better understanding of its effects. In this crop, the interaction with *Rhizoctonia solani* has been studied using FT-ICR/MS and GC-EI/MS [40].

Metabolomics in agriculture can be used to obtain a chemotaxonomic classification, where differences and similarities can be distinguished between related species or cultivars, as it was the case between genetically modified potato crops and conventional potato crops, where the study was carried out using GC TOF-MS and FIE-MS [37].

Species in the family Solanaceae, such as potato and tomato (*Solanum lycopersicum* L.), are among the most important crops for agriculture and food and thus are subject of different types of studies. With respect to metabolomic studies, metabolomic profiles of tomato have been obtained for genetic mapping of fruits and leaves of both, conventional and wild genotypes [41, 42]. It is known that tomato has a large number of chemical compounds with important uses. This chemical variability has been elucidated through metabolomic studies. An important group of compounds that have been studied is the volatiles from fruit, peel and pulp, which were analysed through UPLC-QTOF-MS [43, 44]. The profile of carotenoid compounds from the fruit has also been characterized by proton NMR [45]. Changes in chemical composition during growth, development and differentiation are very important to determine the conditions where these events are occurring. It has been obtained chemical profiles from tomato plants in several stages of development through LC-MS [46]. Something similar was carried out by Moco et al. [47], where they obtained databases from the metabolome from the fruit using LC-MS and ¹H-NMR. The simple characterization of fruits from different cultivars was carried out through MALDI/TOF-MS [48].

During the day, any plant species has changes in its chemical composition due to the action or effect of the environment, something that [49] determined when detected 70 metabolites in tomato leaves and 60 in the fruit using NMR and MS. Also, with the aid of metabolomics, it is possible to identify particular compounds with some specific function, important to the survival of a species. With respect to this, 1-methyltryptophan was identified as the metabolite involved in the response of the plant to *Botrytis cinerea* and *Pseudomonas syringae* [50].

On the other hand, it can be altered the levels and composition of flavonoids in tomato fruits through the modification of biosynthetic pathways using regulatory and structural genes [51].

Throughout time, about 95% of the genetic and chemical diversity of tomato has been lost owing to its domestication; therefore, it is important to know the entire metabolic profiles of both commercial and surviving wild tomatoes to determine their chemical and genetic variability and use that information as the foundation to obtain better genotypes or varieties [52].

One of the most important fruit trees, economically speaking, due to its particular characteristics as flavour, texture and chemical composition, is the avocado (*Persea americana* Miller).

It has also been subject of different studies where its main chemical traits have been determined. It is a species with important properties and benefits to health, based on several chemical compounds.

The study of the chemical compounds present in avocado is very important, and it will help us to lay the foundations to use the chemical information of this fruit tree, for example, in genetic improvement or it can be used to increase the interest in those varieties or races that are not widely consumed and find new applications to them. In this approach, the chemical composition of avocado is addressed using techniques and strategies that combine the identification and quantification of cellular metabolites through sophisticated analytical techniques, and the use of statistical and multivariate methods to analyse and interpret data. In the development of analytical methods throughout time, there have been important technological advances, which have resulted in improvements in the way biological systems are seen, analysed and interpreted [13].

In the last years, the content of acetogenins in avocado has attracted attention. These long-chain fatty acid derivatives have important medicinal properties; they are considered anticancer agents. The profile of acetogenins in the peel, seed and pulp of avocado fruits has been determined to obtain a chemotaxonomic model using a linear discriminant analysis [53]. Also, changes of sugars, total protein and oil in 'Hass' avocado have been determined using GC-FID and LC [54]. On the other hand, important proteins from the pulp have been identified through the use of nano-LC-MS/MS [55]. The use of coupled analytical systems has helped to get better resolution, higher sensitivity, higher speed of analysis and wider diversity of applications. A study in avocado was carried out to investigate fruit maturation through its metabolomic profile using GC-APCI-TOF-MS. Such technique showed that it is a valuable and powerful tool to improve the understanding of the process of fruit maturation [56].

Through the years, the metabolomic knowledge has been applied to agriculture through various planned objectives. For example, some studies have been carried out to elucidate the biosynthetic pathways that produce metabolites in herbs in order to discover the mechanisms responsible for the evolution of these pathways and to understand the function of a given natural product within the physiology of the plant where it is found [2]. Such studies have been carried out in ginger (*Zingiber officinale* Roscoe) [57], turmeric (*Curcuma longa* L.) [58] and basil (*Ocimum basilicum* L.) [59]. These plants have an enormous potential for the development of products with some application, mainly industrial or in medicinal.

A metabolomic study, whatever it may be, is a process with some key stages to know the real status of a plant. Such stages are collection and extraction of the sample, and the analysis, identification, and quantification of the chemical compounds of interest, according to the objective of study. Another case is the analysis of the extracts of *Pisum sativum* L. to define the impact of the environment and the genetic diversity based on the metabolic profile [60, 61].

Several metabolic studies have been carried out in cultivable plant species, where all the tissues have been used and all the analytical techniques employed. Within the cereal group, the

leaves and roots of barley (*Hordeum vulgare* L.) have been studied to analyse the metabolic responses to salt in two cultivars using GC-MS [62]. In oat, the metabolic profile of leaves was obtained using ^1H -NMR to evaluate metabolic variation in European cultivars [63]. Several studies have been carried out also in legumes. For example, in pea (*Pisum sativum* L.), the metabolic fingerprint of transgenic varieties was obtained from the leaves using ^1H -NMR [61]. In *Medicago truncatula* Gaertn, the functional characterization of glycosyltransferases of terpenes in cell culture was obtained using HPLC-MS and also the metabolomic profile for functional genomics in cell culture [64, 65]. In the seeds of *Vigna radiata* (L.) R. Wilczek, the metabolic changes that occur during sprouting were investigated through GC-MS [66].

The tobacco (*Nicotiana tabacum* L) has been subject of study in functional genomics to study the changes induced by jasmonates in the biosynthesis of metabolites in a cell suspension culture, using GC-MS [67]. Also in this family, the metabolic profile from the fruit of chili (*Capsicum* sp.) was determined to study its diversity, using HPLC-PDA/LC-PDA-QTOF-MS-MS [68].

Fruit trees have also been studied to obtain their metabolic profiles for several purposes. In grape berries (*Vitis vinifera* L.), the metabolic variation in several cultivars was evaluated using ^1H -NMR [69]. Also, the metabolic profile of raspberry fruits (*Rubus idaeus* L.) was characterized to identify beneficial compounds using LC-MS [70]. In strawberry (*Fragaria × ananassa* hort.), metabolic studies in fruits and flowers were carried out to know the chemical composition at several developmental stages [71, 72]. Finally, in muskmelon (*Cucumis melo* L.), the metabolic profile of the fruit was obtained using GC-MS [73]. Medicinal plants are also subject of study to find medicinal chemical compounds. In *Catharanthus roseus* (L.) G. Don, there has been hundreds of studies to understand and improve the biosynthetic pathway of indole alkaloids in cell culture; many of the studies have used LC-MS and ^1H -NMR.

The analytical techniques have many advantages for metabolomic analyses; however, their application is not universal. It is important to know the limitations and the processes that are being developed to improve them.

Metabolomic approaches have been divided in two groups, targeted and untargeted metabolomics. In principle, both approaches can be applied in all analytical platforms as LC-MS and GC-MS. The main difference between them is the identification of the analytical signals. In the first one, the metabolites under study are known. In the second one, no specific metabolites are chosen and all the signals detected are taken in account. Clearly, both techniques have advantages and disadvantages. The main benefit of untargeted approach is a more holistic view of the behaviour of metabolite composition with the chance of low probability of missing key metabolites. If it already known what specific metabolites are key for the research, an optimized targeted approach could be most successful. Through the use of internal standards, analysis can be undertaken in a quantitative or semi-quantitative form.

These tools have been useful in pre-harvest and postharvest issues of food constituents and are related with food safety and quality control. In fruits as mango prone to a pre-harvest fungal

disease or the postharvest contamination of onions have been assessed by GC-MS, in which the sample is trapped by head space and then submitted for the GC-MS analysis. In fact, metabolomics techniques may find their greatest application in the food industry in monitoring quality control of different batches. It is described that fruit juice adulteration is quite common; it is not easy to detect the adulteration, but the LC-MS has helped to control the adulteration. The distinction between fresh squeezed juices and those come from pulp washes also has been determined by ^1H NMR, with a high accuracy of the method [4]. Recently, Canela et al. [83] described a review on foodomics imaging by mass spectrometry and magnetic resonance (IMS and MRI). They pointed out that these tools have advantages over fluorescent microscopy or immunochemistry to localization and chemical identity of small molecules. These can determine the presence of many compounds in a single multi-detecting measurement. In the review described some examples where these techniques can support the visualization of the compounds present in a plant tissue used for evaluation of quality control.

In general, there are many uses of metabolomics in agriculture, and there are no limitations in the study of the multiple species. It is possible to work with any plant structure, with any crop, for almost any purpose, and the array of techniques available makes it easy to carry out these studies. In the end, the results of metabolomics from agriculture will have an impact on other areas of study, such as medicine, food quality control, nutrition, and genetic improvement, among others. Finally, an essential part of metabolomic studies is the statistical analysis and bioinformatics resources used to interpret the results, which are important to take into account in a wide review of metabolomics.

6. Plant-pathogen interaction

Plants are always influenced by different factors, mainly environmental ones; those factors force them to adapt and change parts of their functioning to protect themselves, in most cases. One of the main interactions that exist in plants is that with micro-organisms, which also cause changes in the physiology and development of plants, like the environment does. Normally, there is an adverse effect to the plant, but in some occasions, both obtain a benefit, in what is called symbiosis. Pathogen attacks can lead to yield losses although occasionally the micro-organisms may help the plant to improve nutrient uptake. The most characteristic example is the interaction of nitrogen-fixing bacteria with legumes.

When the interaction between a plant and a pathogen occurs, one of the changes that happen in the plant is the production of several kinds of compounds that act as a type of defence as attractants, repellents, feeding inhibitors or the production of chemical compounds that are beneficial to human health.

Metabolomics, through its variants, can help to determine the physiological and biochemical changes that happen during the interaction and provide a general overview of the whole system.

Primary and secondary metabolites are a key in the response of the plant towards pathogen attacks. There are many modifications that could happen during such interaction, such as

molecular and physiological modifications. The modifications range from changes in primary metabolism, where basic processes like photosynthesis can be affected, modifications in the cell wall and in some organs of the plant, to the production of secondary metabolites that can be toxic or trigger defensive signals in the plant. Depending on the type of interaction, resistance, tolerance or susceptibility could occur [74].

One example is the plant-fungus interaction, where a study was carried out using mass spectrometry using electrospray ionization to detect changes in the levels of lipids and hormones. In this study, the researchers had predicted that those molecules were involved in the interaction between *Brachypodium distachyon* and *Magnaporthe grisea*. A variation in the level of phospholipids was detected, which was the main response of the plant to the attack of the fungus [75]. One advantage of the use of metabolomics is that targeted and nontargeted studies can be performed at the same time, as in the case of the interaction between *Lupinus angustifolius* with the fungus *Colletotrichum lupini*. The response of this interaction was characterized focusing on the metabolites of the cuticle using GC-MS (nontargeted study) and on the flavonoids using LC-MS (targeted study). In this case, it was found that there was a higher variation in the kinds of flavonoids when the interaction took place and that variation was more marked in the extract from the plant than in the extract from the fungus [76].

On the other hand, a metabolomic study of the plant-pathogen interaction can help to elucidate the genetic mechanisms originated during resistance. Through a nontargeted study using GC-MS, the resistance of sunflower (*Helianthus annuus*) to the necrotrophic pathogen *Sclerotinia sclerotiorum* was characterized; 63 metabolites were found, including sugars, organic acids, amino acids and secondary metabolites, such as chlorogenic acid, which are associated with tolerant phenotypes [77].

Many studies about plant-pathogen interactions have focused on relating chemical compounds with diseases using bacteria, fungi, oomycetes and even viruses, interacting with plants such as Arabidopsis, tobacco, sunflower, barley, rice, potato and grapevine, where changes have been detected in both primary and secondary metabolism [74]. Most of the studies that have been mentioned have used mass spectrometry (coupled with LC or GC) as the analytical technique that helps to characterize the metabolomic response of the interaction, but there are studies based on NMR to achieve the characterization. In a study of the interaction between tobacco (*Nicotiana tabacum* L.) and the tobacco mosaic virus, the alteration of the metabolic pathways in the leaves was proposed after they were infected with the virus. Healthy and diseased leaves were compared through 1D and 2D NMR [78].

NMR has also been used to characterize the compounds involved in the resistance of host plants to the western flower thrips (*Frankliniella occidentalis*). For this study, three plants of different types were used: Senecio (wild), chrysanthemum (ornamental) and tomato (cultivated). A resistant Senecio hybrid was evaluated because it showed significantly higher amounts of pyrrolizidine alkaloids (which are involved in plant chemical defences against herbivores in general), as well as some flavonoids. In the case of the resistant chrysanthemum, high amounts of chlorogenic acid and ferulic acid were found which, as phenolic compounds, are expressed during plant defence. Cultivated tomato was the most susceptible towards pathogen attack, but the resistant hybrid of this crop showed high levels of acyl sugars, which confer some protection against the attack of pathogens [79].

There are many examples of the application of metabolomics in plant-pathogen interactions that help us to know and interpret this interrelation through the physiological and biochemical changes that take place. For this, advanced analytical platforms of high sensitivity are used; these platforms allow the characterization of almost any molecule expressed during the interaction. Any kind of plant involved in any type of attack or interaction can be studied, and valuable information can be obtained that will help us to understand the reaction of the plant or both. From this type of interactions, and through applied analytical techniques, information about the best way to exploit such interaction can be obtained, in case one wants to obtain or increase chemical compounds with some use, or if one has interest in other aspects of life, such as health.

7. Future perspectives of metabolomics

Over the years, the field of metabolomics has gained interest in several disciplines such as functional genomics, biological and integrative systems, pharmacogenomics, and the discovery of biomarkers to predict diseases, diagnostics and therapy monitoring [80], besides the area of study of agriculture [2].

Modern challenges for metabolomics are diverse in all the fields mentioned, but they are particularly relevant in the discovery of biomarkers [82], especially in the field of diseases, because their detection, monitoring and treatment are important. One of the problems that researchers face these days is the difficulty in the identification and quantification of several chemical compounds at the same time in a reliable and ideal way, since their number can be huge, and some metabolites, which may be undetectable due to their low concentrations, may be relevant to some function or application. Nowadays, new analytical techniques are being developed or improved to solve these problems, widening the range of detectable metabolites based on their structural characteristics, and making the methods more sensitive, so they can detect very low concentrations of compounds.

Nowadays, the information obtained from analysed samples may not be correctly interpreted, or it may offer much more information than what is obtained, but some limitations related to the design of the experiment could have impeded the correct interpretation and use of that information.

A future challenge will be to improve this type of situations to use the information better and find possible applications for it. The validity of a metabolomic study is affected by the sizes of unbalanced samples (this aspect has to do with the design of the experiment), especially in studies with humans, mainly when statistical methods are used to interpret the data. This is mentioned because in some studies that have already been carried out, the number of control and diseased individuals is not associated, that is, it is not balanced [81], so it is necessary to balance these cases of study.

One of the challenges of metabolomics is to participate in more fields of study which could be waiting for this type of analyses; although the most important ones are already included, such as disease detection and health in general, studies have already started in fields such as evolution, chemotaxonomy, agriculture, ecology and food quality control, among others.

Nowadays, there are many databases related to chemical compounds, their identification and their structural elucidation, and probably over the years these databases will increase significantly due to the increasing number of studies in this area; therefore, we will have access to a huge number of metabolites, their properties, their possible health benefits and other properties.

Certainly, with the passing of the years, the number of studies related to the functionality of what is already sequenced will increase, since in this field there are still some delays. This is the main challenge of metabolomics for the next years, and it will be conquered by integrating the study of several fields focused on the same objective, or by integrating the latter.

The complete understanding of the function of the cell system and the deciphering of gene function will arrive with time, since metabolomics is integrated with genomics, transcriptomics and proteomics; thus, an integral work will give the result that everyone wants in this branch of knowledge, which is the complete understanding of cell function [81].

Chemical studies in plants, from their origins, have been based on their traditional use and knowledge, since people used them to get some benefit, but without really knowing what caused such effect. Therefore, something that must be addressed is to make the composition and the beneficial effects of the compounds available to the general public in an easy-to-understand way.

Metabolomic studies in plants related to natural products will increase simply because they are everywhere, they can be used for everything, and every day new applications are found for them; most of these applications will benefit humans.

It has mentioned that metabolomics is not a goal in itself, but a tool to improve our understanding about the metabolism and biochemistry of the organisms [6]. Therefore, among all, this must be the most important future perspective: to know and completely understand metabolism and cell function.

8. Conclusion

Metabolomics is a relatively new field. During the last years, this discipline has been growing because diverse applications have been found for it, and different analytical techniques have been developed and improved; this has allowed an easier interpretation and analysis of the results. The inclusion of a wide variety of crops in this type of studies is paramount, because it is necessary to know the qualities that they have, and take from them the most important traits with the aim of developing an application that benefits food, health or industry.

The field of study of agriculture, with respect to metabolomic aspects, will keep growing, because in the next years, there will be challenges to ensure world food sovereignty. Currently, most crops and their diversity are at risk; therefore, it is necessary to carry out actions focused on their conservation, rescue and rational exploitation.

The range of analytical techniques implemented in metabolomics allows us to be a step ahead in the analysis of extracts or chemical compounds, through which new uses or applications for the plant species studied can be found, or strengthen those already existing, in addition to the development of improvement programmes based on distinctive chemical traits.

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