JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

REVIEW ARTICLE | DOI: 10.5584/jiomics.v5i1.179

Arabidopsis thaliana and omics approaches: a review

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Received: 12 November 2014 **Accepted:** 21 December 2014 **Available Online**: 28 January 2015

ABSTRACT

Arabidopsis thaliana is a small, flowering plant that is widely used as a model organism in plant biology, mainly because it is the first plant to have its entire genome sequenced. It has since proven to be an ideal organism for studying plant development. *Arabidopsis* is commonly used as a model plant for genomics, metabolomics and proteomics studies, and more recently it has been utilized in metallomic studies. Because of its widespread applications, many methods for *Arabidopsis* sample preparation, analytes separation and data quantification have been explored. This review briefly describes the *Arabidopsis thaliana* characteristics, the developed researches and the primary methods using this plant in different fields of OMICS. In the future, the availability of *Arabidopsis* genomic information may result in its continuous development for nanoparticles and metallomics studies.

Keywords: *Arabidopsis thaliana*; OMICS; genomics; proteomics; metabolomics; metallomics.

1. Introduction

Arabidopsis thaliana (*A. thaliana*) is a plant discovered in the sixteenth century by Johannes Thal in the Harz Mountains (Germany). It is a member of the mustard (*Brassicaceaeor Cruciferae*) family of dicotyledonous plants, which includes species such as cabbage and radish. *A. thaliana* is an annual herbaceous plant native to Europe, Central Asia and Northwest Africa [1], although it has been naturalized in many other places [2]. The location of its growth is responsible for the observed differences in *A. thaliana* life cycles that are reflective of genetic variation [3], affecting characteristics such as flowering time, natural variation, plant growth, among others [4,5]. *A. thaliana* has a rapid life cycle corresponding to approximately 6 weeks from germination to maturity.

According to Pigliucci, flowering time and seed dormancy are key traits that determine the timing and length of the *A. thaliana* natural life cycle [6]. Alvarez-Buylla *et al.* studied processes and stages of *A. thaliana* flower development using molecular genetic studies and genomic studies [7]. Based on research involving comparative and evolutionary approaches derived from *A. thaliana* studies, it is possible to establish a method for studying the molecular basis of diverse floral morphologies. When different *A. thaliana* species are grown together under similar environmental conditions, genetic variation can be observed for many traits [8].

Many different natural accessions of *Arabidopsis thaliana* have been collected, and researchers from around the world are using these to uncover complex genetic interactions, such as those underlying the plant's responses to its environment and the evolution of morphological traits. The phenotypic variation for morphological and physiological traits is abundant and enables almost every *Arabidopsis* accession to be distinguished from accessions collected at different locations. These genetically distinct variants are commonly referred to as ecotypes in the scientific literature. The distribution range of *Arabidopsis* is limited by low spring and autumn temperatures and high temperatures with low precipitation in summer [9]. Thus, this plant is an ideal model system for studying

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natural variation.

Considering the ideal characteristics of *A. thaliana* , together with the fact that it was the first plant to have its genome completely sequenced [10,11], it is easy to understand why the field of OMICS technologies uses *A. thaliana* as a model plant for biological, biochemical, physiological, toxicological and others researches. Because of the canonical relationship of gene to transcript to protein, the three OMICS platforms involving genomics, proteomics and metabolomics are inherently complementary, facilitating the detection and identification of many molecules that are expressed in different organisms [12]. Applying these integrated OMICS platforms, DNA, RNA, proteins, peptides, lipids and metabolites are currently detected and measured in different samples

The present review highlights *A. thaliana* research, specifically taking into account OMICS approaches, such as genomics, proteomics and metabolomics. Additionally, some trends regarding the application of this plant for metallomics and nanoparticles studies are briefly discussed, suggesting that both basic and applied science and all up-to-date technologies are needed to gain new insight and the most accurate information from a studied system. Fig. 1 shows a general scheme of commonly applied procedures for OMICS analysis using *A. thaliana*, of which the most important steps are as follows: 1) extraction and purification of the analyte (genes, proteins and metabolites); 2) separation of interesting species by Polymerase Chain Reaction (PCR), two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), two-dimensional difference gel electrophoresis (2-D DIGE), chromatography, etc.; and 3) identification of interesting species by mass spectrometry (MS), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), etc. The extraction and purification method as well as the analytical technique used for separation and identification are chosen according to the objective of the research.

2. Genomics studies

Arabidopsis thaliana was the first plant and the third multicellular organism after *Caenorhabditis elegans* [13] and *Drosophila melanogaster* [14] whose genome was completely sequenced [10,11]. To assist biological investigations and to define chromosomal structure, a coordinated effort to sequence the *A. thaliana* genome was initiated in late 1996, led by a consortium of researchers based mainly out of academic institutions in the USA, Europe and Japan (AGI) [11]. Separate teams within the consortium worked on different chromosomes, using distinct procedures [15]. Today, the *Arabidopsis* community is a diverse group of scientists and involves universities, research institutes and private companies.

Knowledge of the complete genomic sequence and a huge collection of gene disruptions provides a research resource that is unique for higher plants [16]. Three papers presenting the DNA sequence of the gene-rich regions on chromosomes 1, 3 and 5 of *A. thaliana* [17–19] were published first, followed by papers in which chromosomes 2 and 4 were described, altogether providing an overview on the *A. thaliana* sequence [20,21].

The initial identification of transcriptional units in the *A. thaliana* genome sequence was carried out largely by *ab initio* gene predictions, sequence homology, sequence motif analysis, and other non-experimental methods [11,17–21]. The Institute for Genomic Research (TIGR) launched a reannotation effort [11], employing the latest annotation tools and resources and applying uniform annotation protocols across the entire genome, with the goal of improving annotation by

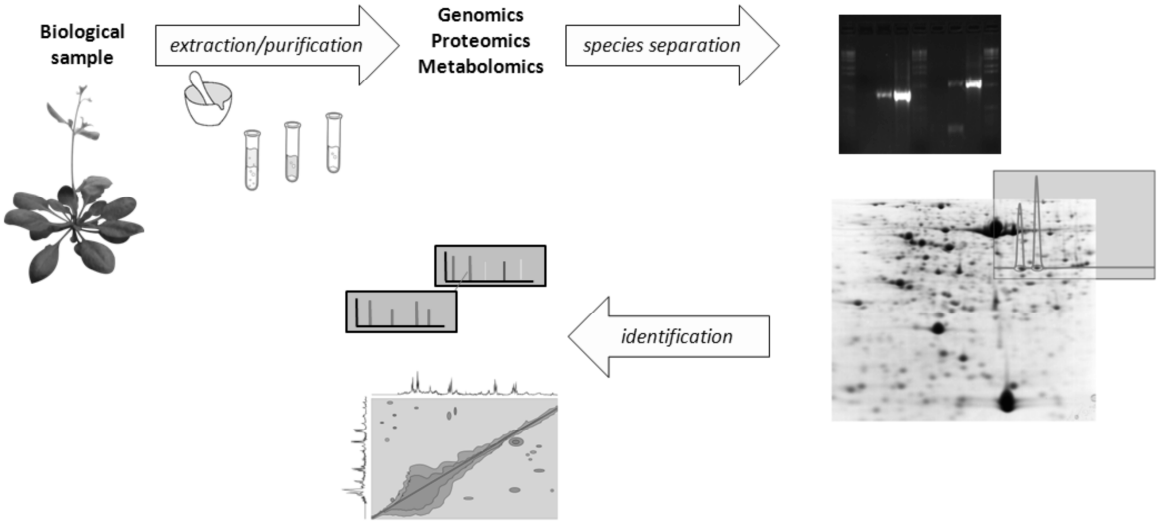

Figure 1. General scheme of common procedures applied for OMIC analysis using *A. thaliana*.

refining gene structure and gene function assignments. The final TIGR genome reannotation release contains annotations for 26,207 protein-coding genes [22]. The completed sequence of a plant nuclear genome yielded a high number of insights, particularly when comparing it with the completed genomic sequences of other species available at the time, namely *Caenorhabditis elegans* and *Drosophila melanogaster*. *A. thaliana* had many families of new proteins but also lacked several common protein families, indicating that these sets of common proteins had undergone differential expansion and contraction in the three multicellular eukaryotes [11].

Although its agronomic significance is little, *A. thaliana* has been widely used in plant biology, offering important advantages for basic research in genetics and molecular biology. It was chosen as a genetic plant model because of its short generation time, abundance of seeds, conveniently short height and solid history in genetics studies. Furthermore, this species has a small nuclear genome (114.5 Mb/125 Mb total), extensive genetic and physical maps of all 5 chromosomes, low repetitive DNA content and simple genetic transformation using *Agrobacterium tumefaciens* [23]. Sequence analysis of the 125-Mb nuclear genome of *A. thaliana* has uncovered 26,207 protein-coding genes, representing approximately 11,000 gene families. Of these genes, approximately 40% have unknown cellular roles, and an established phenotypic function has only been found in approximately 5% [10]. Thus, the wide use of *A. thaliana* in genetic and molecular studies has generated an extensive collection of point mutations, knockouts, knockdowns, over-expressers and other mutant lines.

Quantitative information for the identified proteins was used to establish correlations between transcript and protein accumulation in different plant organs. A proteome map for *A. thaliana* was assembled from high-density, organ-specific proteome catalogs generated for different organs, developmental stages, and undifferentiated cultured cells. The 86,456 unique peptides were matched to 13,029 proteins, providing the expression evidence for 57 gene models. Moreover, proteome analysis identified organ-specific biomarkers and enabled the compilation of an organ-specific set of proteotypic peptides for 4,105 proteins [24].

The flowering plant *A. thaliana* has been an important model system for identifying genes and determining their functions. Analysis of the genetic magnitude of natural variation within *A. thaliana* led to the discovery of novel functions of genes regarding a particular trait and the further characterization of previously identified genes [3]. Until recently, *Arabidopsis* was considered to have low levels of terpenoids (approximately 30 terpene synthase genes) [25], however, recent analysis has revealed the presence of sesquiterpenes in its flowers and monoterpenes in its roots [26]. Although the levels of terpenes are very low, the presence of these genes indicates that *A. thaliana* remains a suitable genetic model, especially for the study of the central pathways of terpene biosynthesis [27].

The definition of gene functions requires the phenotypic characterization of genetic variants. The availability of the *A. thaliana* genome sequence, increased use of large-scale sequencing, and improvements in the resolution of phylogenetic relationships make it an appropriate time to begin developing additional resources. The *Arabidopsis* proteome map provides information about genome activity and proteome assembly and it is available as a resource for plant systems biology [24].

In this sense, computational modeling has an important role in revealing genome-wide regulatory mechanisms. Using these programs, several-thousand new genes and pseudo -genes were added, and approximately one-third of the originally annotated gene models were significantly refined, yielding improved gene structure annotations. Additionally, each protein-coding gene was manually inspected and classified using Gene Ontology terms [22]. Complete and partial gene structures identified by this method were used to improve The Institute for Genomic Research *Arabidopsis* genome annotation (TIGR release v.4.0).

Access to the *A. thaliana* genomic sequence afforded a better understanding of the plant's developmental and environmental responses and allowed the structure and dynamics of plant genomes to be assessed [28–31]. This popular model plant is increasingly used to investigate questions in evolution and ecology; therefore, it is essential to understand patterns of natural genetic variation and to understand the dynamics of wild populations at a scale relevant to single plants [32,33]. The sequencing of complete genomes has advanced the understanding of biological systems and established a series of technologies for the analysis of gene functions, increasing information about the theoretical protein-coding capacity of organisms. The *A. thaliana* genome has been mined for clues to numerous important metabolic pathways and biological processes, many of which are documented in peer-reviewed publications, including the *Arabidopsis* Book [34]. One review [35] summarized the progress made during the past five years and speculated on the future developments in *A. thaliana* research and the implications of these developments for crop science.

3. Proteomics studies

Gene sequence information is not enough to provide significant biological knowledge regarding an organism. Proteomics, which is defined as the quantitative and exhaustive analysis of proteins expressed in a given organ, tissue, or cell, is becoming a more powerful and indispensable technology in the study of biological systems. The analysis of all expressed proteins provides complementary information about genome structure, activity, and regulation. Additionally, proteomics can provide information about post-translational protein modifications involved in developmental control and environmental responses. Thus, proteomic approaches are helpful for answering questions of protein function [36,37].

In recent years, the rapid progress in the determination,

quantification, identification and comprehension of proteins has been possible due to the use of model organisms such as *A. thaliana*. Improvements in the techniques for proteomics, including plant proteomics, based on existing platforms such as 2-DE, 2-LC and MS and some new techniques, including tandem affinity and protein chips, have been observed [38–47].

The availability of the entire genomic sequence of *A. thaliana* provides unique opportunities for the use of a post-genomic tool such as proteomics in its full capacity [11,17–22,48,49]. Research involving *A. thaliana* proteomics has made progress in the past few years, analyzing the proteome of the whole plant and at the level of organs, tissues and organelles. This progress has generated important data sets characterizing the protein-protein interactions, organelle composition, protein activity patterns and protein profiles of this plant [24,28,50–54]. Proteome analysis has proven to be an effective tool not only for analyzing the responses of plants to environmental stresses, including drought, salt, and high and low temperatures but also for allowing the analysis of differential gene expression at the protein level [38,55–57].

The number of distinct proteins that can be identified from complex samples has been increased by the use of shotgun proteomics, a gel-free liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis, compared to traditional gel-based approaches [36,46,58–60]. However, there is no single standardized procedure for the analysis of all proteins and metabolites because these are highly diverse and biochemically heterogeneous [37].

One of the major problems in analyzing a complex material such as a plant leaf sample is the dynamic range of protein abundance and the lack of similarity of the protein content in various cell types of one organism. This difference is responsible for a great diversity of cells and can occur in response to various stimuli and in different cellular compartments [61,62]. The use of multiple model organisms increases proteome research; furthermore, insight into plant proteome dynamics and cell functions are rapidly increased with the use of model plants, such as *A. thaliana* and rice (*Oryza sativa*), that have relatively small genomes. *A. thaliana* has been applied in several of the most comprehensive studies using differential-relative and absolute-quantitative strategies to enhance genome annotation, profile organelles, tissues, cells or sub-cellular proteomes, and investigate developmental processes and responses to biotic and abiotic stresses [36,37,62].

This contribution as a model organism for plants and the increasing impact of proteome research is reflected in the recent increase of proteomic studies using different proteomic techniques to accomplish the separation and evaluation of proteins from crude tissue extracts to further analyze this plant. These techniques are 2-dimensional electrophoresis (2 -DE), 2-dimensional difference gel electrophoresis (2-D DIGE) and liquid chromatography (LC), followed by the identification and characterization of the proteins by mass spectrometric techniques (MS) [38,39,41–43,63–65].

It is important to remember that the results of any experiment are dependent on the condition of the starting material. Therefore, choosing the appropriate sample preparation, based on the subsequent analytical technique and the research objectives, is crucial for obtaining significant and trustworthy results. Sample preparation is of particularly great importance in comparative proteomics because there are often only minor differences between experimental and control samples [61,66–69]. Currently, methods to simplify complex protein mixtures prior to using separation techniques have been proposed, enabling more discrete samples to be analyzed. These methods include sample fractionation and protein enrichment techniques, such as profiling isolated cell organelles and sequential extraction for the selective removal of the most abundant proteins or interfering compounds [41,66–70].

One of the most commonly applied techniques in proteomic analysis is the traditional 2-DE. This method is based on orthogonal separation of proteins according to their pI, molecular weight, solubility and relative abundance. The number, resolution and reproducibility of spots visualized on a 2-DE map depends to a great extent on the tissue sample and the protein extraction protocol [39,43,56,62,63,71,72]. For plant proteomic analysis, the presence of large amounts of non-protein components and lower protein content (compared to bacterial or animal tissues) requires customized experimental strategies for each plant to avoid compromising 2-DE separations. For this reason, simple protein extraction protocols are advisable [38,56,71,73–78].

The most universal protocol for plant tissue analysis recommends protein precipitation after tissue homogenization. TCA-acetone, TCA-phenol or TCA-methanol precipitation methods or a protocol based on a combination of TCA-acetone precipitation followed by methanol washing and phenol extraction have been used and reported [74–81]. Variants of these methods have been previously used in the analysis of the *A. thaliana* proteome. For example, Maldonado *et al.* evaluated changes in the proteome of *A. thaliana* leaves as a response to *Pseudomonas syringae* by comparing three precipitation protocols for protein extraction using 2-DE: TCA-acetone, TCA-acetone + phenol, and phenol only. The quantity and intensity of observed spots were dependent on the protocol used. The TCA-acetone + phenol protocol provided the best results in terms of reproducibility as well as the ability to focus and resolve the intensity of spots and to detect the presence of a single spot [77].

A number of modifications related to 2-DE extraction methods have been published, focusing on reproducible results and how to obtain a good extraction of proteins from plant samples, remove interferences, and preserve proteins in solution [41,62,67,68,71,73,81]. For example, the low abundance of a protein present in a plant leaf sample may be interfered with by the presence of ribulose bisphosphate carboxylase/oxygenase (RuBisCo), the most abundant plant leaf protein. The presence of this protein not only limits the dy-

namic resolution and yield but also affects the electrophoretic migration of neighboring protein species, hampering a deep analysis of the leaf proteome [41,67,68,82]. However, there are some methods for removing RuBisCo, such as those utilizing polyethylene glycol (PEG) [68,82], DTT [83], or the immunocapture of RuBisCo (RuBisCo-IgY affinity) [82], as well as Ca^{2+} plus phytate for its precipitation [66]. Kim *et al.* tested the efficiency of the protamine sulfate precipitation (PSP) method for the depletion of large and small RuBisCo subunits (LSU and SSU) in *A. thaliana*, rice, and maize leaf proteins and provided a novel method for RuBisCo depletion [67]. Espagne *et al.* described a simple mobility shift method for the large subunit of RuBisCo in the first dimension. Using a mixture of ampholine-buffer containing both 4–7 and 3–10 immobilines enabled the characterization of previously undetected protein spots [71].

Profiling isolated cell organelles is another method for simplifying complex protein mixtures before their separation by 2-DE. This strategy has been essential for understanding the biogenesis and function of these plant organelles and for learning that each compartment is enclosed by a unique complement of proteins. To achieve this profiling, it is necessary to have reliable isolation and purification techniques for the cell compartment because many proteins may be lost during these procedures [41,84,85]. Using Triton X-114 phase partitioning, Prime *et al.* characterized the presence of peripheral and integral membrane proteins in a callus culture of *A. thaliana*. A database of mitochondrial, endoplasmic reticulum, golgi/prevacuolar compartment and plasma membrane markers were generated with these results, enabling the definition of specific proteins at the *A. thaliana* callus culture plasma membrane [84].

In another study, *Arabidopsis thaliana* seedlings grown in liquid culture were used to recover proteins secreted from the whole plant. The inclusion of water-insoluble polyvinylpolypyrrolidone (PVPP) in the protocol for the purification of secreted proteins in the culture media led to the identification of a new set of apoplastic proteins, which may have been lost during classical extraction procedures. The role of PVPP was to trap phenolic compounds and to prevent their unspecific interactions with proteins [41]. Fukao *et al.* reported a method of isolating leaf peroxisomes using 2-DE for understanding the tissue-specific expression of leaf peroxisomal proteins. A protein map of leaf peroxisomes from greening cotyledons of *A. thaliana* was built from different cotyledons protein fractions obtained after the extraction procedure. The activities of catalase and cytochrome c oxidase and the content of chlorophyll (Chl) were obtained from each fraction, which were proposed as markers of leaf peroxisomes, mitochondria and chloroplasts, respectively. Additionally, leaf peroxisomes were well separated from mitochondria and chloroplasts, which were present at a high purity and concentration [85].

The high resolution of 2-DE separations makes this methodology the most-used platform for proteomic studies. However, some difficulties with this method have been reported, such as poor reproducibility, the necessity of skilled analysts, and the subjective interpretation of the data obtained through the digitized images of the spots. The lack of reproducibility is frequently attributed to the sample preparation method and natural variations of biological samples and also to the electrophoretic system itself [40,65]. In such cases, the use of 2-D DIGE increases sensitivity and repeatability compared with 2-DE. Two different samples can be run together on the same gel, minimizing the problems mentioned above. Furthermore, 2-D DIGE enables the detection of low-abundance proteins because it is based on fluorescent cyanine dyes, which have higher sensitivity compared with other dyes, such as Coomassie Brilliant Blue (CBB) and silver staining [38,40,60,65,86,87].

In recent years, an increase in 2-D DIGE application as a supporting proteomic method in expression profiling has been observed. Following the manufacturer's instructions, sample protein extraction protocols are similar to those applied in 2-DE. General applications are focusing on the discovery of biomarkers in a wide variety of situations [88] as well as assessing proteomic changes based on stress conditions, genetic modifications [38,89,90], salt [91–93], drought [94], high and low temperatures [95], and metal addition [38,96,97], among others [40,86,98,99].

Studies from Casasoli *et al.*, using 2D-DIGE separation and MS identification, showed that oligogalacturonides (OGs) induced changes in nuclear protein abundance and in the apoplastic proteins of *A. thaliana* seedlings because the plants perceived the OGs as indicators of the presence of pathogens. The nuclear proteins responding to the OG treatment were mainly involved in the protein translation machinery and translation regulation, suggesting a general reprogramming of the plant cell metabolism in response to OGs. Additionally, the differentially expressed apoplastic proteins identified, obtained by a vacuum infiltration-based protocol, included proteins involved in the recognition of OGs and proteins whose post-translational modifications (PTMs) are regulated by OGs [60,86]. Ge *et al.* proposed a model that detailed the possible mechanisms for apoplastic proteins in pollen germination and pollen tube growth of *A. thaliana* pollen grains. Through the results produced by 2-D DIGE, LC–MS/MS and bioinformatics tools, the authors observed and identified global changes of the apoplast proteome during *A. thaliana* pollen germination and pollen tube growth. Additionally, the subcellular localization of three randomly selected differentially expressed proteins was also determined [40]. Holzmeister *et al.* infected wild-type and S-nitrosoglutathione (GSNO-reductase) knock-out *A. thaliana* plants with both avirulent and virulent pathogenic strains of *Pseudomonas syringae*. The authors investigated the importance of nitric oxide (NO) in the plant defense response through a proteomic analysis of the above-mentioned system. The use of 2-D DIGE and MS enabled the identification of proteins that are differentially accumulated during the infection process and a detailed proteomic analysis of the plant defense response [99].

The use of High Performance Liquid Chromatography (HPLC) followed by protein identification and characterization by MS in plant proteomic analysis demonstrated that basic, hydrophobic and membrane-spanning proteins have a greater chance of being separated, provided that they can be obtained for analysis. HPLC separates analytes using two immiscible phases or layers, one of which is held stationary while the other moves over it [47,100]. A variety of chromatographic modes have been developed and are used depending on the analytes: protein isolation and purification using ion-exchange (AEX), hydrophobic interaction chromatography (HIC), affinity chromatography (AC), reversed-phase (RP-HPLC) and/or size exclusion chromatography (SEC) [101–103]. The AEX, HIC and RP-HPLC modes are also used for peptide analysis [59,72,101,104–107]. The chromatographic modes are based on several different mechanisms; RP-HPLC is used for the separation of neutral species on the basis of hydrophobicity, AEX is used for the separation of ionic solutes on the basis of charge, SEC is used for the separation of molecules on the basis of differences in molecular size, and AC is used for the separation of biomolecules on the basis of the lock-and-key mechanism prevalent in biological systems [100].

Zolla *et al*. proved that the use of intact mass measurements (IMMs), performed by coupling RP-HPLC on-line with electrospray ionization mass spectrometry (ESI-MS), is an attractive alternative for monitoring the subtle changes that often accompany physiological adaptations of plants in terms of the concentration of components, measured by the integration of the chromatographic peak. This study reported the relative molecular mass (Mr) for all photosystem I (PSI) proteins in ten plant species, including *A. thaliana*, separated by RP-HPLC and identified by either in-solution trypsin digestion with peptide fragment fingerprinting or the close correspondence between the actual IMMs and those predicted from the DNA sequence [103].

Typically, the most common application of HPLC is high-throughput peptide analysis, due to its coupling with MS/MS. In this case, the protein content of a biological mixture is digested prior to separation and analysis. The MS/MS spectra obtained are searched against a protein database to identify peptides in the sample. Shotgun proteomics performed on a subcellular organelle enables the definition of the organelle proteome and can lead to novel insights into intracellular protein trafficking and sorting [59,69,104,105]. For example, Mitra *et al.* developed an effective chloroform extraction method to improve plasma-membrane protein identification. Rather than traditional solid-phase extraction (SPE), the authors used chloroform extraction prior to off-line AXC and RP-HPLC tandem LC/MS/MS analysis, facilitating the removal of chlorophyll a and b and trypsin used in the digestion and increasing the number of unique peptides for plasma-membrane protein identification [69].

Multidimensional separations are emerging methods designed to increase the resolution power of protein separation, which use off-line or on-line systems, each with specific advantages and limitations [104,108]. For example, a method combining sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel separation with RP-HPLC-MS/MS has been used. In this method, proteins are first separated by size on standard polyacrylamide gels or by isoelectric point on IPG strips. After separation, the gel slice is treated similarly to spots excised from 2D gels, and the peptides are separated on an RP column coupled with MS/MS. Variants of these methods have been used in the analysis of the *A. thaliana* proteome [59,106,109]. Batailler *et al.* carried out a proteomic survey of the phloem exudates of *A. thaliana*, collected by the ethylenediaminetetra-acetic acid (EDTA)-facilitated method. Phloem sap proteins of *A. thaliana* were separated by SDS–PAGE. The gel was stained, and bands distributed along the entire length of the lane were excised and subjected to manual in-gel digestion. After separation, the extracts were analyzed by HPLC-MS/MS [109]. To gain insight into the systemic responses of plants to local viral infection or wounding, Niehl *et al*. performed protein profiling of distal, virus-free leaves four and five days after local inoculation of *A. thaliana* plants with either oilseed rape mosaic virus or inoculation buffer alone. Using the system described above, they revealed biomarkers for systemic signaling in response to wounding and viral infection in *A. thaliana* [106].

There has recently been increased attention paid to plant proteomics, exemplified by the above-mentioned efforts concerning plant-specific tissues and organelles. Additionally, the search for possible biomarkers includes evaluating the responses to various biotic and abiotic factors in biological systems, using genetic-model plants such as *A. thaliana*. For this task, proteomics requires three key steps: high-quality extraction, separation, and visualization of complex protein mixtures from crude extracts; identification and characterization of the separated proteins by MS; and database searches. In conclusion, the use of proteomic techniques is critical for plant studies and helps elucidate several key aspects of the metabolic regulation of essential processes. The generation of plant proteome maps, including the identification of low-abundance proteins, requires effort in the most critical proteomic steps, protein extraction and sample preparation, as well as the integration of data obtained through the technologies developed for high-resolution protein separation and rapid, automated protein identification.

4. Metabolomics studies

The metabolome represents the collection of all metabolites in a cell, tissue, organ or organism, which are considered the end products of cellular processes [110]. Thus, metabolomics is the study of chemical processes involving metabolites, intermediates and products of metabolism. The large-scale analysis of metabolites in biological samples (metabolomics) has received increased attention in recent years as a complement to the large-scale analysis of gene transcription and proteins. The usual aim of metabolomic

studies is to quantify the entire metabolome in biological samples; because metabolomic correlations complement the information about changes in metabolite levels, these data may help elucidate the organization of metabolically functional modules.

Plant metabolomics is the study of predominantly low-molecular weight metabolites within cells, tissues or organisms, and it is a widely applied approach for the elucidation of gene function in a wide range of plant species [111]. In plant-based metabolomics, it is common to refer to "primary" and "secondary" metabolites. Primary metabolites are directly involved in the normal growth, development, and reproduction of the plant and are extremely essential to live. Unlike primary metabolites, secondary metabolites are not directly involved in those processes, and their absence does not result in a sudden death; however, secondary metabolites can influence the long-term survivability of the organism, impair fecundity, or affect aesthetics [112].

Plants produce and accumulate a wide variety of secondary metabolites via processes in which precursor structures are modified through biochemical steps driven by different classes of enzymes. Small fluctuations in the metabolome across independent plants may provide information regarding the build-up of a metabolic network [113–116].

Deciphering the metabolome is essential for a better understanding of cellular metabolism as a system. Metabolomics has been utilized not only to investigate plant metabolism but also to identify unknown gene functions by comparing the profiles of wild-type and genetically altered plants and of plants during various developmental stages [113,114,117–119]. Metabolomics studies have demonstrated their robustness in metabolic engineering, process engineering, biomarker discovery, and the functional characterization of novel genes. Furthermore, metabolomics represents one of the most powerful tools to probe the overall effects of gene down-regulation and knockout in transgenic plants at all stages of growth and development.

In plant species, 50,000 metabolites have been characterized, and *Medicago truncatula* and *A. thaliana* are the main models regarding metabolomics projects [113,116,120–122]. The physical and chemical properties of metabolites are highly variable because metabolites include many different types of compounds, such as amino acids, fatty acids, carbohydrates, and organic acids.

In metabolomics studies, efficient and reproducible protocols for the extraction and analysis of metabolites are applied to maximize the number and amounts of metabolites extracted and minimize analytical variations. These well-developed protocols have led to the acquisition of large amounts of information on the composition of *A. thaliana* metabolites [123]. The most common method used for the extraction of metabolites in this plant is one based on shaking the sample at low or high temperatures in organic solvents or in mixtures of solvents [6–8]. For polar metabolites, methanol, ethanol, and water are often used, while chloroform is the most commonly applied solvent for lipophilic compounds.

Metabolome analysis has already been reported using nuclear magnetic resonance (NMR) [5,6], Fourier transform infrared spectroscopy [7], pyrolysis/electron impact-mass spectrometry (pyrolysis/ EI-MS) [8], gas chromatography/ electron impact-mass spectrometry [9], electrospray mass spectrometry (ESI-MS) [12] and ESI-MS coupled with liquid chromatography (LC/MS) [10,11,13]. Analysis by GC-TOF/MS (gas chromatography time-of-flight mass spectrometry) and GC-EI/MS (gas chromatography electron ionization mass spectrometry) are the most applied techniques in *A. thaliana* metabolic studies, and a reliable protocol for analysis has been generated with a relatively limited number of experiments [68,69,70]. These approaches are invaluable for the study of metabolomics in *Arabidopsis*, due to their high reproducibility and the short, constant time between sample preparation and analysis, and have led to the identification of many metabolites.

The application of analytical methods using *A. thaliana* as a model plant is mainly focused on genetic studies [114,124,125], gene function elucidation [111,126], and, in most applications, understanding the expansion of metabolite correlation to gene-expression correlation and studying mediated defenses against biotic and abiotic stresses [114,119–121,126-128].

The metabolomics-based screening method is useful for the rapid characterization of novel genes in both *A. thaliana* and rice [129]. Screening *A. thaliana* lines over-expressing rice full-length (FL) cDNAs (rice FOX *A. thaliana* lines) with gas chromatography was carried out to identify rice genes that caused metabolic changes. Using this technique, it was discovered that the function of LBD37/ASL39 is likely conserved between the dicot and monocot model plant species (*A. thaliana* and rice). For more details on gene expression, a review discussing the study of gene-function relations using the over-expression of *Saccharomyces cerevisiae* and *Escherichia coligenes* cDNAs in *A. thaliana* is suggested [130].

Using *A. thaliana* as a model plant, statistical methods have been performed on metabolomics data. A large amount of microarray data is available, making it easier to build gene coexpression databases [131] and to survey the organization of the transcriptome [132–134]. For example, similarities and dissimilarities in metabolomics correlations were investigated by GCTOF/MS in the aerial parts of 3 *A. thaliana* genotypes: Col-0 wild type (WT), methionine-over accumulation 1 (mto1) and transparent testa 4 (tt4). Multivariate statistical analyzes showed the distinct metabolomes of these plants, provided complementary information on metabolomic correlations about changes in the main metabolite levels, and helped elucidate the organization of metabolically functional modules [120,135]. Regarding the transcript levels, microarray data have collaborated in the evolution of metabolomic studies. The application of MANOVA (Multivariate Analysis of Variance) has allowed researchers to handle multifactorial experimental designs and has revealed clear trends of biological interest. For example,

MANOVA has been applied to analyze *A. thaliana* metabolomic data from factorially designed experiments. This application was demonstrated by a metabolomic investigation using two different factorial designs, *A. thaliana* ethylene signaling mutants and their wild-type counterparts [136]. In this work, the putative *A. thaliana* FLS gene family was studied using a combination of genetic and metabolic analyzes. Although several of the FLS gene family members were expressed, only FLS1 appeared to influence flavonoid biosynthesis in this plant species.

Flavonol synthase (FLS) was the first flavonoid enzyme identified that may be encoded by a gene family in *A. thaliana* plants [137]. In addition to the characterized gene FLS1 (At5g08640), five putative FLS genes (FLS2–FLS6) have been identified in the *Arabidopsis* genome [128]. Studies based on the putative *A. thaliana* FLS gene family revealed that although several of the FLS gene family members were expressed, only flavonol synthase 1 (FLS1) influenced flavonoid biosynthesis. Seedlings of an *A. thaliana* FLS1 null mutant (FLS1-2) showed enhanced anthocyanin levels, a drastic reduction in flavonol glycoside content, and concomitant accumulation of glycosylated forms of dihydroflavonols (a substrate of the FLS reaction). Using a leucoanthocyanidin dioxygenase (LDOX) FLS1-2 double mutant, it was found that the remaining flavonol glycosides found in the FLS1-2 mutant are synthesized in the plant by the FLS-like side-activity of the LDOX enzyme [126]. The results revealed that the *A. thaliana* genome contains at least 24 flavin-containing monooxygenase genes, 272 cytochrome P450 genes, and more than 20 *S*-adenosylmethionine-dependent methyltransferase genes [133].

In most applications involving metabolomic studies and during the development of analytical approaches, *A. thaliana* has been used as a generic plant model to understand mediated defenses against biotic and abiotic stress [114,119–121,127,128]. For example, determining the responses to toxic heavy metals at the level of metabolomics was carried out using *A. thaliana* seedlings as a bio-indicator of Cd pollution [138]. The purpose of these studies is an understanding of the metabolic answer and the adaptation of plants towards heavy metal exposure. The study of Cd impact on the plant metabolome using multivariate statistical analyzes was carried out to compare the metabolic fingerprints and to isolate and identify some discriminating metabolites. *A. thaliana* cell suspensions were treated with different Cd concentrations at different time intervals, and then metabolites present in *A. thaliana* cells grown on Murashige and Skoog media were extracted and injected into the chromatographic system coupled to MS. Three types of data, pretreatment, multivariate statistical analysis (PCA, PLS and PLS-DA) and the PLS methods, proved to be appropriate for the classification of samples and for the extraction of discriminating variables. Additionally, an OSC-PLS2 approach enabled researchers to visualize time-induced and Cd dose-induced changes on the metabolism of *A. thaliana* cells [120].

Isolated *A. thaliana* cells were also used to study the intracellular localization and the biochemical effects of Cs in plant cells [43]. The incorporation and localization of ^{133}Cs in a plant cellular model and the induced metabolic response were analyzed as a function of external K concentration using a multidisciplinary approach. The cellular response to the Cs stress was also analyzed using proteomic and metabolic profiling.

A study involving cultures of *A. thaliana* subjected to high CO_2 stress was carried out to validate a systems biology methodological framework for the analysis of stress-induced molecular interaction networks in the context of plant primary metabolism [139]. An enhanced gas chromatography-mass spectrometry (GC-MS) metabolomic data correction strategy and a new algorithm for the significance analysis of time-series OMICs data were used to extract information about the transcriptional and metabolic plant response. The framework involved the application of time-series integrated full-genome transcriptomic and polar metabolic analyzes on liquid plant cultures. The treatment indicated changes in both transcriptional and metabolic activity, and the identified pathways through which these activities changed revealed insights regarding regulatory processes.

A diversity of metabolites was found by studying the response of *A. thaliana* [124,140–142] to varying light and temperature conditions. The culture was exposed to different environmental conditions in light intensity and/or temperature, and the resulting data sets were subjected to a number of statistical analyzes [143]. In similar studies, metabolome exploration by GC-MS of contrast ecotypes of *A. thaliana* showed that the highest natural variation for plant tolerance existed at lower temperatures than for acclimatory processes [144]. Finally, the resistance of *A. thaliana* plants to the damaging effects of ultraviolet (UV) radiation was investigated through *in vivo* biochemical changes using integrated physiological and metabolic responses. The ability to metabolize xenobiotic compounds was investigated over the entire life cycle of the plant. The results of this metabolic profiling showed that changes in the phenyl propanoid pathway was the key mechanism in both acclimation and plant development [142].

5. Trends: metallomics and nanoparticles

To complement areas such as genomics, proteomics and metabolomics, studies involving metals and metalloids (sometimes linked to the structure of proteins, sometimes free in equilibrium) became important. Metallomics characterizes the metal species present in metalloproteins and tries to elucidate their functions in living organisms [145–147]. Metallomic studies can be classified according to whether they are ionomic and/or metalloproteomic [148]. The first aims to determine free or elemental species in tissue samples, and the second aims to selectively define the metals/metalloids associated with different proteins, protein conformations and protein functions.

Recently, metallomic and proteomic studies performed on

A. thaliana leaves showed the effects of transgenesis and the effects promoted by addition of excess of selenium (Se) [37]. The detection of differentially expressed proteins was carried out by 2-D DIGE. Images of the distribution of Se and sulfur (S) in the leaves were obtained by laser ablation imaging inductively coupled plasma mass spectrometry [LA(i)-ICP-MS]. As a result, 68 species of differentially expressed proteins were detected, of which 27 were identified by ESI-Q-TOF MS/MS. The main biological events modified by these proteins were the glycolysis pathway, photosystems I and II, and the Calvin cycle. The images obtained by LA(i)-ICP-MS showed that added Se was translocated to the leaves and that transgenic plants absorbed higher amounts of Se compared with non-transgenic plants. The results indicated that genetic modification did not influence the production of differential protein species, but it did confer some resistance to the plant regarding abiotic oxidative stress induced by the presence of Se.

Nanoparticles (NPs) are classified as particles sized between 1 and 100 nm that can be dispersed in gaseous, liquid or solid media [149]. Because of the variety of potential applications in biomedical, manufacturing and materials, environmental, energy, optical and electronic fields, the production and characterization of these materials have been widely reported in the literature [150]. Some examples of the applications of NPs are as follows: the use of cerium oxide nanoparticles act as an antioxidant to remove oxygen free radicals that are present in a patient's bloodstream following a traumatic injury, a synthetic skin manufactured with nickel NPs and polymer used in prosthetics, the use of iron nanoparticles (Fe-NPs) to clean up carbon tetrachloride pollution in ground water, and silicon nanoparticles (Si-NPs) coating anodes of Li-ion batteries to increase battery power and reduce recharge time [149].

The scientific literature also contains studies involving NPs and plants. In general, these studies are focused on the effects of NPs on plant germination and growth, aimed at their potential use in agricultural fields [151]. In recent years, some researchers have produced interesting results; Lodeiro *et al.* [152] showed the use of NPs as chemosensors, including a revision about the ability of NP devices to detect metal ions.

In terms of constitution, there are different types of NPs and nanomaterials that have been used in plant science [153], and there is agreement that the effects produced by NPs are dependent on this type, along with the plant species and substrate (*i.e.,* soil, hydroponics, culture medium). Stress response to NPs is a field that appears in an extensive number of studies on metal response in plants. An increasing number of publications have recently considered the interactions of NPs with plants, and most of these studies are focused on the phytotoxicity, uptake and accumulation of NPs in plants [154–156].

Studies involving *A. thaliana* have evaluated the exposure of this plant to some NPs in different categories, such as metal oxides (nAl_2O_3, $nSiO_2$, nFe_3O_4 and nZnO) [157]. Seedlings of this plant were used for reporting the phytotoxicity of silver nanoparticles (Ag-NPs) [158], which was observed at low concentrations. Another study evaluated the impact of citrate-stabilized AgNPs on *A. thaliana* at three levels—physiological phytotoxicity, cellular accumulation and subcellular transport [159]. The phytotoxic effects of AgNPs could not be fully explained by the release of silver ions. Plants exposed to AgNP suspensions bioaccumulated a higher silver content than plants exposed to $AgNO_3$ solutions (Ag^+ representative), indicating AgNP uptake by plants. At three levels, the impacts of AgNPs differed from equivalent dosages of $AgNO_3$. In summary, the studies cited show that phytotoxicity is dependent upon the concentration and particle size of the NPs.

Changes in *A. thaliana* phenotype, at both the cellular and macroscopic level, were also observed. These changes were dependent on the distribution of NPs in the tissue, thereby revealing their bioaccumulative effect. Based on these findings, the researchers stressed that the exact mechanisms remained unclear and required elucidation, as was also observed in another group's research paper. Recently, changes in gene expression in *A.* thaliana exposed to polyvinylpyrrolidone-coated AgNPs and silver ions were evaluated by Kaveh *et al.* [160]. Many genes differentially expressed by AgNPs and Ag^+ were found to be involved in the response of plants to various stresses, providing insights into the molecular mechanisms of the response of plants to AgNPs and Ag^+. Exposure to gold nanoparticles (GNPs) significantly improved the seed germination rate, vegetative growth and antioxidant potential of *A. thaliana*. This was the first report showing GNPs as a promising tool to enhance the seed yield of plants [161].

The characterization of NPs is essential to obtain more information about their properties as well as their applications when focusing on toxicological studies. Responses to NPs would also be a key element in identifying mechanisms involved in stress tolerance and NP toxicity. Many subjects, studies and challenges involving the biological effects of NPs are still unresolved, and their interactions with plant-soil-microorganisms systems still need to be investigated.

In Table 1, the OMICS studies using *A. thaliana* cited in this review are summarized, including the target study, comments and reference number.

6. Conclusions and final remarks

Today, *A. thaliana* remains the standard reference plant for all of biology and it is an efficient tool for the analysis of plant functioning, combining classical genetics with molecular biology. The continuous advancement of *A. thaliana* knowledge enhances its value for plant biology. This plant offers important advantages for OMICS research; it was the first plant to have its entire genome sequenced, making it an ideal model system and a powerful tool for the development in this field. This review highlighted advances in OMICS studies, particularly genomics, proteomics and metabolom-

Table 1. Summary of the OMICS studies cited in the present review using *A. thaliana* as a model plant.

GENOMICS		
Target study	**Comments**	**Ref.**
General characteristics	Classification, flowering time, plant growth, diverse floral morphologies and seed dormancy. Studies of variation observed in life cycles due to genetic variation	[1–10,24]
Genome sequence	Definition of the chromosome structure, effort to sequence the complete genome	[11,15,17–22]
Studies based on genomic sequencing	Genetic transformation using *Agrobacterium tumefaciens*	[23]
	Suitable model, especially, for study of the central pathways of terpene biosynthesis	[25–27]
	Use of computational modeling for revealing genome-wide regulatory mechanisms.	[22]
	Assessment of the structure and dynamics of plant genomes, enabling a better understanding of plant development and environmental responses	[28–31]
	Investigation of evolution and ecology, for understanding patterns of natural genetic variation and the dynamics of wild populations	[32–34]
PROTEOMICS		
Target study	**Comments**	**Ref.**
Methodologies to simplify complex protein mixtures prior to the use of 2-DE	Evaluation of changes in the proteome of *A. thaliana* leaves in response to *Pseudomonas syringae* by comparing three precipitation protocols	[77]
	The selective removal of RuBisCo using protamine sulfate precipitation (PSP) and the mobility shift method	[67,71]
	Profiling of isolated cell organelles; characterized the presence of peripheral and integral membrane proteins in callus culture, the secreted proteins in culture media (apoplastic proteins), and leaf peroxisomes	[41,84,85]
Comparative proteomics studies using 2D-DIGE	Evaluation of proteomic changes based on different stress conditions, genetic modifications, salt, drought, high and low temperatures, and metal addition, among others	[38,40,60,86,91,93, 95,96,98,99]
The use of HPLC in plant proteomic analysis, followed by protein identification and characterization by MS, facilitates greater chance of the separation of basic, hydrophobic and membrane-spanning proteins	Monitoring the subtle changes in the proteome, produced by physiological adaptations of the plants, performed by intact mass measurements (IMMs), using RP-HPLC -ESI-MS.	[103]
	Shotgun proteomics on a subcellular organelle for the definition of the organelle proteome. The protein content of a subcellular organelle is digested prior to separation and analysis.	[59,69,104]
	Use of multidimensional separations, such as combining SDS-PAGE with HPLC-MS/MS, for the analysis of the phloem sap proteins and to gain insight into systemic responses to local virus infection or wounding. The proteins are digested after SDS-	[106,109]
METABOLOMICS		
Target study	**Comments**	**Ref.**
Metabolite profiling	*A. thaliana* is used as plant model for metabolomic projects	[113,116,117]
Effects on metabolites caused by biotic and abiotic stress	Investigation into the effects on *A. thaliana* metabolites following exposure to metals, pathogens, light and temperature changes	[43,120,122, 124,127,138, 139,142–144]
Genes studies, gene expression, functions and annotation	Demonstrating the robustness of metabolomics studies in the functional characterization of novel genes	[111,114,121,126,128–131]
Methods for extraction and derivation for metabolomic analysis	Investigations of extraction and derivation protocols for metabolomic studies	[6–8,123]

Analytical methods more commons in metabolomic studies	Nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy, pyrolysis/electron impact-mass spectrometry (pyrolysis/ EI-MS), gas chromatog-electron impact-mass spectrometry, electrospray mass spectrometry (ESI-MS) and ESI-MS coupled with liquid chromatography (LC/MS)	[5–13,68–70,113] [125]
Statistical methods performed in metabolomics	*A. thaliana* is used as model plant for development of statistical methods in the area of metabolomics. Evaluation of data pretreatment methods for further statistical analyzes	[120,131–136,139]

METALLOMICS AND NANOPARTICLES

Target study	**Comments**	**Ref.**
Metallomic studies	Study of the distribution of Se and S in the leaves through LA(i)-ICP-MS	[38]
Nanoparticles studies	Evaluation of the exposure of plants to NPs in different categories, and AgNPs phytotoxicity studies	[157–159]
	Study of the bioaccumulation effect of AgNPs and changes in gene expression due to exposure with GNPs	[160,161]

ics, focusing on the use of *A. thaliana* as a versatile plant model. These applications are responsible for the development of numerous methods for *Arabidopsis thaliana* analysis in different OMICS fields.

Considering genomics, *A. thaliana* has been an important model system primarily for identifying genes and determining their functions, thus providing information about genome activity. Having the complete *A. thaliana* genome sequence allowed further understanding of the structure and dynamics of plant genomes. The proteomics approach is helpful for answering questions regarding the functional analysis of proteins. The rapid progress in the determination, quantification, identification and comprehension of proteins has been possible due to the use of model organisms such as *A. thaliana* and their role in improving the existing techniques for proteomics. The proteome map of *A. thaliana* provides information about proteome assembly and is available as a resource for plant systems biology. The contribution of this plant as a model organism for plants increases the impact of proteome research and is reflected in the increase of proteomics studies. With regard to the metabolomic field, *Arabidopsis* has been utilized not only to investigate plant metabolism but also to identify unknown gene functions by comparing the metabolic profiles of nontransgenic and transgenic species.

As a final remark, because there are already defined genomic and proteomic databases available for this plant, *A. thaliana* is useful as a model plant for metallomic studies aiming to elucidate the physiological and biological functions related to the bioactive metallomes of proteins. Regarding the prospects of NPs, studies involving *A. thaliana* proteomics, genomics and metabolomics will be helpful for those researchers who decide to better understand the mechanisms involved in the interactions of NPs with plants, and, in future studies, this plant may become a great tool to clarify the phenomena of phytotoxicity, uptake and bioaccumulation.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil), the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil).

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