Recent Insights into Plant-Pathogens Interactions through Proteomics Approaches

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Abstract. Plants are vulnerable to adverse living conditions, frequently suffering from a variety of pathogens. A series of defensive events occur rapidly in plant cells, including complex biochemical and physiological responses. Several studies have been performed in plant–pathogen interactions researches. In this field of investigation, proteomics technology, has contributed to the understanding of pathogenicity strategies in pathogens, as well as the defence mechanisms in plants. Recently, the proteomics approach has drastically expanded due to some biological questions can only be addressed at the protein level. Its application is becoming a more powerful functional genomic tool and allows for a global perspective in plant–pathogen interactions. This review provides a brief overview of the current methods and progress of proteomics in the research of plant–pathogen interactions. In addition, relevant protein function and expression profiles are also reviewed.

1. Introduction

During plants’ growth and development, they may suffer from biotic stresses caused by a variety of pathogens, such as fungi, bacteria, viruses, and so on. Different types of pathogens infect plants in different ways, and the plant resistance mechanisms are slightly different. Host plants mainly have some ways to defend against pathogens. Firstly, plants have the barrier of innate immune system, consisting of morphological characteristics and secondary metabolites. Once the pathogens invade this basal protective barrier, the infection coupled with pathogen-associated signaling molecules can be sensed and recognized by plants. Then the plants differentiate to generate complex biochemical and physiological responses (Dodds et al., 2010). The signaling events occur extremely rapidly and specifically. For example, there are changes in some cell wall components, which includes the cuticle, lignin or waxy composition, as well as antifungal compounds deposition and specific emulsion secretion on the plant surface (Jones et al., 2006). These responses can help the plants defend against surrounding attacks and inhibit the spread of infection, but they are still not resistant to all pathogens.
Another defensive way is superimposed on the basal immune system, which is called induced resistance. A specific and genetically determined resistance (hypersensitive response, HR) is triggered and resistance proteins are expressed. During this process, a series of biochemical perturbation and defense reactions through various signal transduction pathways occur: such as resistance signals generation, conduction and interaction, resistance-related genes expression, metabolism changes, eventually inducing resistance. Various signaling molecules such as hormones (salicylic acid, jasmonic acid, ethylene, abscisic acid), second messengers (calcium ions, reactive oxygen species, nitric oxide), low molecular peptides (glutathione), lipids and sugars play important roles in the defense reaction process. Subsequently, the pathogens infection leads to the plant host cells and adjacent health cells (tissue) mass death (Coll et al., 2011). This rapid response enhances the plants resistance and it serves to restrict the further growth and expansion of pathogens. As a result, the plants generate local acquired resistances or systemic acquired resistances (Conrath et al., 2006).

Under these biotic stresses, many plants regulate defense-related proteins expression and produce broad-spectrum disease resistance. This process is complex filled with biochemical and physiological responses and changes, and few of the molecular mechanisms have been fully elucidated. Therefore, the study of defense-related genes or proteins may contribute to improving our understanding of plant defense mechanisms, which is of great significance for cultivating broad-spectrum disease-resistant varieties and improving crop adaptability and stability (Sergeant et al., 2010; Wang et al., 2011; Barkla et al., 2013). Recently, many significant results in the plant–pathogen interactions filed were carried out at both the nucleic and protein level (Cheng et al., 2010; Limones et al., 2010; Fernandes et al., 2014). Because of the multicellularity of complex plant organisms, each type of cells has the same genetic information, but the containing proteins vary from cell to cell under different conditions. As the main carrier of biological function, protein has its own unique activity rules. The proteins often undergo post-translational modification, transport localization, structural changes, the interactions with other biological molecules and so on. That is, one gene corresponds to not one protein and may be several or even dozens, so transcriptional changes do not reflect complex cellular signaling networks. Therefore, because of the limitation of genomics, it is necessary to study the plant–pathogen interactions at the protein level, referring to proteinic composition and function in particular.

Proteome refers to "all proteins expressed by a cell, a tissue or an individual's genome", which is a whole that changes dynamically in space and time. It is not only different in different tissues or cells of the same body, but also different in different development stages of the same body. In addition, it is also affected by various environment. Proteomics technology aims to analyze the dynamic changes of protein components, expression levels and modifications in cells, tissues or individuals from an overall perspective, understand the interactions and connections between proteins, and reveal the functions of proteins and the rules of cell life activities (Agrawal et al., 2012; Smith et al., 2013).

The study of proteome requires the understanding of the protein types, functions and interaction networks of each functional subgroup of proteome, and the identification of the changes of various protein molecules affected by various factors and the effects of such changes on cells, tissues or individuals. Proteomic approach is an effective way to study plant pathology. It has drastically developed in the last few years and begun to be applied to this field. The recent studies of proteome have been performed by employing the methods of two-dimensional electrophoresis (2DE), mass spectrometry (MS) and bioinformatics.
Through the study of proteomics, the qualitative and functional analysis of some key proteins as well as their response pathways to pathogens and gene regulation mechanisms have been obtained. The function of specific marker proteins and key proteins is of great significance for agricultural production under specific biological stress conditions.

2. Proteomics Techniques in Plant-Pathogens Interactions

As mentioned above, studying protein content of a cell provides us with a global perspective, and is considered as a welcome complement in the research. Together with genomics and metabolomics technology, it is better to improve our understanding of intracellular and intercellular regulatory mechanisms. There are numerous studies based on proteomic approaches, and early studies were mainly focus on identifying proteins that interacts with known proteins thorough yeast hybrid system (Quirino, et al., 2004). Although this method is also common in the current researches.

In general, preliminary studies of plant differential proteomics require the extraction of appropriate proteins from normal and control plants, followed by protein isolation and identification (Fig. 1). Reasonable sample preparation ensures high resolution and repeatability (Afroz et al., 2011). The first step in a typical proteomics studies is extracting proteins from plant cells or tissues. Most experimental protocols are based on TCA and/or acetone precipitation of proteins or on phenol extraction, in which the protein is dissolved in the phenol phase accompanied with protein denaturation and precipitation in methanol and ammonium sulfates. (Jacobs et al., 2001; Carpentier et al., 2005; Vincent et al., 2006; Gòmez-Vidal et al., 2008). The TCA method is efficient and applicable to various plant tissues proteins. Some plant cells are rich in polysaccharides, lipids and phenols, which make it difficult to extract proteins. For this recalcitrant plant proteins, phenol method can be used. In order to increase the solubility of protein solubilities, liquid release agents, surfactants, reductants and so on are often added. The isolation and extraction of nuclear proteins facilitates the study of plant proteomics. This technique relies on subcellular separation, which allows the identification of a subcellular proteome. Nuclear protein separation methods are based on protein solubility difference in buffers of different ionic strengths.
Figure 1. Flow chart and schematic representation of different approaches in proteomic studies.

With advances in technology, two-dimensional gel electrophoresis (2-DE) coupled with MS is used to identify cellular or sub-cellular proteins under different biological conditions. At present, both qualitative and quantitative results can be achieved and analyzed. For example, the samples from different time, space or treatment can be contrasted and their differently expressed proteins are analyzed using 2-D gel electrophoresis in early proteomic studies decades ago (Orrick et al., 1973, Nagabhushan et al., 1974).

Two-dimensional gel electrophoresis (2-DE) is a most common method to separate various proteins by using isoelectric points and molecular weights of proteins, combined with gel chemical properties. Isoelectric focusing (IEF) was performed on the first electrode in the high piezoelectric field, and then SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in the second electrode perpendicular to the first electrode, then matched with mass spectrometry. 2-DE is difficult to efficiently isolate extremely acidic/alkaline proteins, hydrophobic proteins, high/low molecular weight proteins and low abundance proteins. The quality of test results can be partly improved by using dyes such as Coomassie brilliant blue, silver nitrate. Novel non-gel techniques have been proposed for separating proteins instead of 2-de and tandem mass spectrometry, such as liquid chromatography (LC) and capillary electrophoresis (CE).

Gel-free protein separation method and the application of the "second generation" proteomics technology (Fig. 1), such as multidimensional protein identification technology (MudPIT), quantitative
proteomic methods, including multidimensional protein identification technology (MudPIT), quantitative proteomic approaches including isotope-coded affinity tags (ICATs), targeted mass tags (TMTs), and isobaric tags for relative and absolute quantitation (iTRAQ) have been widely used in abiotic stress adaptation of plant growth and metabolism of strategy of descriptive and comparative proteomics research (Washburn et al., 2001; Koller et al., 2002). Advances in the study of these techniques on a large number of complex plant samples have been discussed (Matros et al., 2011). This gel-free platform has also been rapidly developed and successfully applied in plant defense investigations (Pirondini et al., 2006; Irar et al., 2010).

In order to better image the intracellular low-component protein in 2-DE gel, the protein staining technique is very important. Commonly used methods of protein staining include silver staining, coomassie bright blue staining, fluorescent staining, negative staining and isotope labelling.

Today, the appearance and improvement of mass spectrometry (MS) is of great significance in the biochemistry filed. The MS application can be used to identify single isolated proteins or complex mix-proteins, and MS herein is thought as an analytical technique with the component identification function (Sparkman, 2000). Mass spectrometry has high sensitivity, high throughput and automation, which makes it suitable for large-scale proteome studies. This technology employs chemical fragmentation method to ionize and separate sample molecules, and the relative molecular mass is determined according to the difference of interstitial charge (m/z). The ionization can be achieved through electrospray (ESI) or matrix-assisted laser desorption/ionization (MALDI) techniques (Siuzdak, 1996). It is very important to study the dynamic changes of protein expression and modification on the whole level for the system to understand the function of protein. In recent years, quantitative methods of proteomics based on mass spectrometry can be divided into labelled quantitative and unlabelled quantitative. The quantitative methods such as stable isotope labelling and metal element labelling are labelled quantification. As the efficiency of polypeptide ionization and signal response strength are affected by multiple factors in quantitative analysis by shotgun method, it is not possible to use the peak intensity or area of mass spectrometry to make accurate quantitative analysis. Peptides labelled with "light" and "heavy" isotopes, respectively, have the same chromatographic retention time and ionization efficiency, and can accurately reflect the proportion of peptides or proteins in the original sample, so they are used for precise quantification. Recently, the tandem mass spectrometry (MS/MS) approach greatly improves the sensitivity of mass spectrometry detection, so as to determine trace substances in mixtures (Fig. 1).

Bioinformatics is an indispensable part of proteomics research, mainly used to construct and analyse maps, search and build databases in proteomics research. Various software is used to compare samples to improve data reliability. The gel spots are removed and analysed, which are considered differentially expressed. Then the protein samples can be digested into specific peptides. The collection of these products is introduced into the MS analyser and then the mass of all the peptides can be detected. These masses can be matched with all the protein fingerprints in the database to identify an unknown protein. This high-throughput technique mentioned above is called peptide mass fingerprinting (PMF), but it is appropriate only if the protein sequence exists in the available database. (Clauser et al., 1999). For quantitative analysis, a specified software is applied to analyse and determine protein spots intensity in different gels. Based on this software, a mass of protein spots can be performed differential statistical analysis, in which we can compare expression patterns and detect the significant changes (Mathy et al., 2008).
3. Proteomic Work Involving Plant-Pathogens Interactions

Plant diseases can be divided into infectious diseases and non-infectious diseases. Infectious diseases are plant diseases caused by biological factors. Non-invasive diseases plant diseases caused by abiotic factors (inappropriate environmental conditions) cannot be transmitted to each other. Abiotic factors include climatic conditions, atmospheric environment and cultivation conditions. Non-invasive venereal diseases reduce plant disease resistance and facilitate pathogen invasion and onset. Infectious diseases weaken the plant resistance to non-infectious diseases. The two complement and influence each other.

The inducement of non-infective venereal diseases is external environmental factors, which promote the expression of corresponding anti-stress proteins. Studies of cell wall proteomics in major crops such as rice, soybean, and maize provide insights into dehydration or water stress response proteome, which includes carbohydrate metabolism, cell defense through REDOX mechanisms, cell wall modification, and cell signaling pathways (Zhu et al., 2007; Komatsu et al., 2010). Organelles proteomics research combined with large-scale genome method and determine the progress of the proteolytic activity of the enzyme in the leaf aging process were solved the complexity of the chloroplast protein hydrolysis mechanism, and according to them along the way of expression has unique physiological function is studied in different types of age-related protease (Hu et al., 2015).

Fungi are the most widely studied plant pathogens. During the interaction between fungi and plants, the secreted proteins play an important role in deciphering the plant defense mechanism and overcoming host resistance. Therefore, studying the secreted proteome of pathogenic fungi has become an important target to analyze the pathogenic molecular mechanism of pathogenic fungi. More studies have been carried out on F. graminearum secretory proteome. Fusarium gramineae is an important pathogenic fungus in wheat, maize and many cereal crops. With the completion of genome sequencing, more and more researches have been conducted on the mechanism of pathogenic mechanism of fusarium gramineae by secreting proteomics technology (Paper et al., 2017). Currently focuses on different culture medium conditions (such as different carbon source, pH, etc.), and small different kinds of (strains), gene knockout mutant and wild type, fungi of different growth stage is secreted proteomics research, as well as the secretion of in vitro plants and pathogen interactions proteomic methods, such as the establishment of the pathogenic fungi infect plants, its main purpose is to establish simulation when secreted proteome expression in real analysis method, and identify more fungi secreted protein, excavate the secretion of pathogenesis related protein, and its gene function research, to better analysis the interaction mechanism of pathogenic fungi and plant lay the foundation (Zhang et al., 2014).

The virus uses host proteins to help infect the host (Di Carli et al., 2012). Brizard et al. firstly isolated Rice yellow mottle virus and Rice host protein complex by using an effective method to isolate the virus-host protein complex, and identified the host protein by mass spectrometry. They found that virus recruitment and binding of different host proteins at different stages of infection, which are involved in different pathways of plant defense response, metabolism, translation, protein synthesis and transport, are critical to the life cycle of the virus and optimize the efficiency of virus translation. This analysis method is helpful for us to find new antiviral target proteins (Brizard et al., 2006).

The ability of most plant pathogenic bacteria to induce hypersensitivity and susceptibility to disease is controlled by hypersensitivity and pathogenicity (Hrp) genes. There are two key transcriptional activators that regulate Hrp, one of which is the arac-type transcriptional activator, such as HrpB. Kang et al. analyzed
proteins regulated by HrpB in Burkholderia glumae by proteomics, and most of the proteins whose expressions changed were secretory proteins. The secretory function of HrpB protein is mainly mediated by type II protein secretory system (T2SS). Pathogenicity analysis showed that both T2SS and Hrp type III protein secretion system (T3SS) played an important role in the pathogenicity of B. glumae (Kang et al., 2008). Proteomics is also gradually applied to the study of root nodule symbiosis.

The protein secreted by plant parasitic nematodes is the key factor to complete the parasitic life of nematodes. Proteins secreted by the epidermis, lateral organs and oesophageal glands of nematodes are necessary for parasitic life of nematodes. It is possible to study the control of plant nematodes with drugs or novel biological control methods based on the known parasitic related genes. Mapping the precise three-dimensional structure of proteins and revealing key parts of their structure, such as the sites that bind to drugs and determine their activity during disease research, could provide guidance for drug development.

4. Conclusion

Plant pathology is a discipline that studies the causes of plant diseases, the rules of disease occurrence and development, the interaction mechanism between plants and pests, as well as disease prediction and prevention. When plants are subjected to persistent stress from pathogenic organisms or adverse environmental conditions and their interference intensity exceeds the tolerance level, the normal physiological functions of the plants will be seriously affected, showing abnormalities in physiology and appearance, and the plant that deviates from the normal state will be affected by diseases. Stress signals regulate the change of proteome in cells, tissues or individuals through signal transduction, and then adjust their physiological state or shape to improve their tolerance to abiotic adversity. Therefore, studying the dynamic changes of plant proteome under stress signals can understand the nature of physiological and pathological processes of plant diseases from the molecular level.

Stress signals can lead to significant changes in plant protein expression through signal pathways. The comparison of a stress-induced protein profile with a normal protein profile determinable proteomics technique reveals the nature of physiological and pathological processes. Proteomics studies have helped to shed light on almost every aspect of cell function in plant response to stress and to reveal the possible relationship between protein abundance and/or modification and plant stress tolerance. More and more studies are discussing the role of proteomics in the molecular mechanisms of plant response to stress and signaling pathways that link changes in protein expression to cellular metabolic events, such as studies on model plants such as Arabidopsis, rice and sorghum, protein databases of major monocotyledon grains and dicotyledon legumes (such as corn, wheat, barley, soybean, etc.) have been widely used to study quantitative changes in protein abundance of various species under different abiotic stresses (Hu et al., 2015).

The proteomic technology, as an emerging more powerful analytical approach, can help us to study protein structure and function and improve the comprehension of the mechanisms involved in plant–pathogen interactions, which is crucial to develop novel plant resistance strategies.

Although proteomics is not mature enough, especially in plant biology investigation. The potential of proteomics has not been fully exploited in the field of plant research. For example, labelling technology is considered as the second generation of proteomics, but this method is used less widely due to its low reproducibility and fancy price. Isotopic labelling of proteins such as metabolic labelling
is not suitable for the plant system, because this method needs cell culture. Tag-based protein-labelling (ICAT, LOPIT, iTRAQ) methodologies are not often applied in plant systems. There are challenges in PTM and interaction omics, which contribute to making a better understanding of molecular phenotypes interpretation. In this respect, the powerful platforms, protein microarrays and gel-based microarrays (biochips) for large-scale analysis of proteins will attract more attention (Popescu et al., 2007; Rubina et al., 2008). Therefore, additional experiments such as genetics, biochemistry, metabolites can be complementary. The growing sequenced genome and advances in technology have a impact in plant protein identification. It is believed that with its further development, proteomics will make breakthrough in revealing mechanisms and patterns of plant–pathogen interactome. To put our studies into practice, we can clone and transform corresponding genes or proteins for the purpose of broad-spectrum resistant varieties. Combined with the genetic engineering application, some key proteins can be found and are useful in the design of plant protection strategies.

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