Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 2, p. 192-200, 2014
http://www.innspub.net

REVIEW PAPER OPEN ACCESS

Regulatory mechanisms in the interaction between plants and pathogens - a proteomics approach

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Article published on August 09, 2014

Key words: Proteomic, pathogen, osmotion protein.

Abstract

Plants respond to stresses with inductive responses. Induction responses include chemical and structural defense responses that only activate after pathogen attack. Cellular responses greatly coordinated and with identification of pathogens and transduction pathways cause to minimize contamination. Regulatory mechanisms in the interaction between plants and pathogens are complex and dynamic. Proteomics techniques due to identification of new proteins in relation with their role are useful for understanding these regulatory networks. Proteomics is a careful method to study proteins especially expression, structure and molecular role of them. The goal of this technique is identification and description of all proteins expresses in a biological system.

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Introduction

Biological stress is created by organisms such as fungi, bacteria, viruses and insects, which affects plant growth, seed quality and yield (Wang *et al.,* 2011). Biological stress has been studied less compared with non-biological stresses, due to requirement to provide special conditions for the evaluation and quantification of pathogens. There are two approaches for studying of biological stress. The first technique involves contamination with pathogenic to create disease, while second method includes the use of chemicals such as fungal elicitors for simulating biologic stress in plant. Different techniques are used for understanding the regulatory mechanisms of plant in response to environmental agents to module the effects of biological stress on the yields. Proteomics is one of these techniques that have been applied to study plants in this field in the last decade (Agrawal and Rakwal, 2008).

Common response to environmental stress (biologic and non-biologic)

Different signal transduction pathways such as protein kinase (Beckers *et al.,* 2009), or transcription factors (Abuqamar *et al.*, 2009) need to response biological and non-biological stresses. Most of the genes that encode proteins for adaptation to non-biological stress are also induced by biological stresses. For example osmotion protein with antifungal activity (De Freitas *et al.,* 2011) which are induced by infection with pathogens, also have been detected in tobacco cell cultures in osmotic stress (Singh *et al.,* 1987). Thus set of specific genes that response to biological and non-biological stresses (Huang *et al.,* 2008) are cause of a complex network cooperation between different pathways in response to stress (Maldonado-Caldern *et al.,* 2012). Glazebrook (2005) reported amount of acetylsalicylic acid, jasmonic acid and ethylene that increasing in plants after pathogen infection. External application of mentioned substances and non-biological stresses, induce expression of genes related to defense (Schenk *et al.*, 2000; Fujita *et al.*, 2006). For example it has been shown that levels of increment in plants infected with pathogens and in water stress conditions are more or less the same (Howe and Schilmiller, 2002).

Plants roles against stress

When plants are attacked by pathogens, to inhibit the growth of pathogen, a set of defense mechanisms, is essential for these immune responses. Plants capable of identifying a set of molecules that are not specific to pathogen called elicitors, such as carbohydrates, lipids and proteins, which are structural composition of fungi and bacteria (Garcia-Brugger *et al.,* 2006). Because of few receptors have been identified for elicitors, assumed that elicitors binding to receptors in the plasma membrane of plant, activates a set of events including changes in ionic flow across the plasma membrane (Mithfer *et al.,* 2005) and formation of free oxygen (Kaku *et al.*, 2006). These events cause changes in the phosphorylation status of some proteins (Benschop *et al.*, 2007). Seems that efficiency of immune responses depend on presence and expression of new proteins which expressed after pathogen attack. The recent research indicates that degradation of proteins by ubiquitin proteasome system is one of the most important post-translational modifications which play a pivotal role in plant defense responses (Zeng *et al.*, 2006). Genes involved in stress resistance of plant cell are classified into two main groups (Thomashow, 2010). The first group of genes is encoding regulatory proteins involved in the transduction pathway or proteins involved in regulating the expression of genes related to stress such as protein kinase, phosphatase, transcription factors and adhesion proteins to RNA (Maruyama *et al.*, 2009). The second group includes proteins involved in resistance to stress such as detoxification and osmotic compatible components producing enzymes, production of water channels, anti-freeze proteins and Chapron (Tuteja, 2011).

Regulatory mechanisms in plants

Due to biological changes and complexity of the relationship between two organisms that are closely linked together, there is no alternative approach to proteomics study of plant and pathogens. These

strategies cause identification details of cascade messages during interaction between plant and pathogen (Quirino *et al.*, 2010) understanding the complexities of plant responses to environmental stresses and identification of proteins involved in plant resistance to stress. Among few proteomics studies about interaction between plants and pathogens, most of them are about interaction between plants and fungi compared with bacteria and virus. These studies have led to the identification of a large number of proteins involved in biological processes such as defense and response to stress, photosynthesis and electron transport, transduction and metabolism system. Proteomics is identified proteins that are produced or overexpressed in response to fungal infection. In some cases, these proteins with inhibition of reactive oxygen species (ROS) protect cells from oxidative damage (Afroz, 2011).

Proteomics

Gene sequencing and biology systems science are revolution in biology sciences and proteomic is as a basic technique for this new research. Proteomics complements other Omics techniques such as transcriptomics and metabolomics (Wienkoopa *et al.*, 2010). The purpose of biology systems is outline of all regulatory processes and response of systems biology (phenotypic plasticity) to environmental disturbances. The accuracy of these processes is increasing with data of protein metabolites and transcriptomics (Weckwerth, 2008). Understanding the cell biology of an organism is requires an understanding of all the proteins that expressed by the genome of a cell, tissue or organ in a certain time. So that proteomics identify actual state of a cell or an organism in the particular environmental conditions. Proteomics is as a bridge between transcriptomics and metabolomics (Vitamvas *et al.*, 2007). In addition, identification of proteins to amino acid sequencing or mass spectrometry needs to existent databases that have genome sequence of living organisms. Thus we can compare the proteomics data with mentioned databases to identify the proteins and peptides in the sample (Quirino *et al.*, 2010). Proteomics provide the study of all sets of existence proteins in a unit biological, simultaneously. Aspect of this technique involve: descriptive proteomics that includes the list of all proteins, population proteomics involves the expression changes of genotype-dependent, comparative proteomics involves expression changes in response to environmental effects, quantitative proteomics involves the determination abundance of protein, interaction proteomics that is about post-translational modifications and interaction with other proteins and biomolecules. In summary purpose of proteomics, state this entry that how, where, when and how several thousand special proteins are produced by a living organism and how interact with other proteins and biomolecules. So that molecular cooperation is the cause of suitable construction of cell, growth, development and adaptation of plants to biological and non-biological changes (Jorrin Novo *et al.*, 2009).

For nearly two decades proteomics research involves in the identification of proteins, determination of the expression levels and changes in various physiological conditions in a variety of cells and tissues. Thus it is expected that this information make better understanding of biological performance and comparison of the molecular mechanisms in tissues under two situations control and infected with pathogen (Zhang *et al.*, 2010). The ultimate goal of proteomics is determination of total proteins expressed in a proteome. Key point for understanding biological processes understands the structure and role of proteins and interaction with other molecules, such as other proteins, DNA, metabolites and complex molecules. Identification and quantification of proteins are two major steps for complete characterization of a proteome pattern (Zhang *et al.*, 2010).

Application of proteomics in environmental stresses

Beneficial of proteomics technique is to describe the functional status of protein expression in different

tissues, cells in developmental stages under different biological or non-biological stresses (Rossignol *et al.,* 2006). Proteomics is applied for analysis plant proteins in responses to biological and non-biological stresses (Kim *et al*., 2003; Salekdeh *et al*., 2002). Through quick advances in extraction, separation and identification of proteins, proteomics is applied to study changes in protein profiles. In this manner, use as a suitable technique for studying the effects of biological and non-biological stresses in gene expression, identification of regulatory proteins that respond to environmental stresses and understanding plant defense pathways mechanisms (Agrawal *et al*., 2009; Wang *et al*., 2011). Use of proteomics approach for studies of plant pathology is consisting of common techniques such as two-dimensional electrophoresis and mass spectrometry. Today this approach can be used for determination agent of virulent intra-or extracellular due to pathogens and in order to investigate the changes in protein levels in host plants under conditions of pathogenesis (Kav *et al*., 2007)

Applications of proteomics in interactions between plants and pathogens for biotechnology purposes

Proteomics studies lead to understand the complexity of plant responses to various environmental stresses. Also this technique identifies the proteins that produced or increased expression in response to fungal infection, that are generally involved in resistance to stress. (Afroz *et al*., 2011). So that based on these information acquired one can select genes under stress conditions to improve plant production (Srivastava, 2006) and use them to produce the cultivars which are resistant to stress. (Afroz *et al*., 2011).

Importance of PR proteins (pathogen related protein) in the interaction between plant and pathogen

The first step of plant defense responses against infection with pathogens often begins by plant resistance genes. Genome of plants encodes several classes of resistance genes and products of these genes are classified as PR proteins. The activity of PR proteins is related to resistance to disease (Meyers *et al*., 2005). Protein related pathogen (PR) is broad term for all plant induced protein by microbes which are usually present in plant tissue and only overexpressed during the infections (Ryals *et al*., 1996). General role of these proteins is adaptation plant to biological stress (Sticher *et al*., 1997).

PR proteins first time was identified in tobacco leaves treated with tobacco mosaic virus then various proteins belonging to this group were identified in other families (Van Loon and Van Kammen 1970). Recently more than 17 different PR proteins have been identified based on the characterization of structure and function in monocot, and dicotyledonous plants. Most of these proteins due to hydrolysis have antimicrobial activity and because of that participate in the defense mechanism. Often PR proteins may be involved in inhibition of growth and proliferation of pathogens spread and causing resistance to pathogens (Ryals *et al*., 1996). Several isoforms of PR proteins were identified in rice, which were the kind of inducible protein depending on the type of stress and tissue examined. For example Jwa *et al* (2006) reported, induction of PR proteins alkaline in leaves of the rice inoculated with blast fungus and jasmonic acid. It has been reported that PR_5 protein expressed after infection of rice leaves with blast fungus, although the role of enzymatic PR_5 protein is still unknown but the defense role of some members of this protein family and induction of several PR proteins in cell suspension cultures of rice infection with blast fungus have been reported (Kim *et al*., 2003). Also induction of PR_{10} protein have been observed in leaves and cell suspension cultures of rice in interaction with the blast fungus and elicitor (Kim *et al*., 2004). Many of PR_{10} proteins activate in plants upon pathogen attack or after treatment with elicitor. The role of PR_{10} proteins is unknown, although recommended that these proteins have ribonuclease activities (Bantignies *et al*., 2000). Velazhahan *et al* (1998) reported that ^{sheat} blight fungus, will induce PR proteins. Rakwal and Komatsu (2000) after treatment rice seedlings with jasmonic acid, identified

induction of several PR proteins family, which indicative role of jasmonic acid in rice plant defense system.

Role of Beta-1, 3 glucanase in the interaction between plants and pathogens

Glucanase protein is one of defense proteins family PR and is ahydrolytic enzyme typically in plants and breaks down link beta-1,3glucan which one of the major components of fungal cell wall (Yamaguchi *et al.*, 2002). Since many fungal pathogens have beta-1,3glucan in their cell walls, the main role of this enzyme in plant is defense response against fungal pathogens (Yanisch-perronet *et al.*, 1985). Indirect role of this protein due to degradation of polysaccharides to oligocharides (thesecompoundscanbe usedin theplant's defense system) is defense responses (Boller, 1988). Finally this enzyme has main role in many biological pathways such as breakdown of polysaccharides, storage and building and cell signal (Bhatia *et al.*, 2002).

Plant upon interaction with pathogen such as bacteria, fungi and viruses secrete a set of hydrolysis enzymes in defense systems, and beta-1,3glucanase is one of these enzymes (Bowles, 1990). Since these proteins are secreted in extracellular and this location becomes first place for cellular communication, these proteins has important role in plant defense upon pathogens attack (Jones and Dangl, 2006). So secretory proteins in plants may be have important role in early diagnosis pathogen and induction of defense responses against pathogen invasion (Kim *et al.*, 2009).

Variations of glucanase protein expression in response to interactions between plant and pathogen

Protein beta-1, 3glucanase is induced in many plants at different developmental stages and in response to different pathogens (Hennig *et al.*, 1993; Van Loon and Van Strien, 1999). In addition treatment with biotic stress like a disease, beta-1,3glucanase gene can be induced by treatment with acetyl salicylic acid, methyl jasmonate and ethylene (Linthorst *et al.*, 1990). Also beta-1,3glucanase expressed by environmental stress, mechanical damage and plant hormones in growth period of plants (Akiyama and Pillia, 2001). Lee *et al* (2006) reported up-regulated of glucanase observed only in resistant cultivars of rice compared with the susceptible after infection with Raisoctoniasolani. Also Bera and Purkayastha (1997) reported increased expression of beta-1, 3 glucanase after infection with Raisoctoniasolani fungus. Kim *et al* (2004) reported induction of this protein in leaves and cell suspension cultures of rice inoculation with the rice blast fungus and elicitor. They also reported presence of this enzyme in response to fungal blast, antifungal activity. Comprehensive studies were performed about beta-1,3glucanase gene family, of 27 studies beta-1, 3 glucanase in rice plant in 22 studies increased expression of these proteins in response to infection with rice blast fungus was reported. Scientists suggested that beta-1,3glucanase is related to defense mechanisms against blast fungus (Hwang *et al.*, 2007). Transgenic plants carriers of beta-1,3glucanase show more resistance in response to rice blast disease (Nishizawa *et al.*, 2003). Beta-1, 3glucanase expression associate with chitinase causes inhibition of fungal growth. In transgenic rice coordinate expression of these two proteins cause increase of plant resistance against fungus Raisoctoniasolani (Kim *et al.*, 2003b). Kimet *et al* (2004) reported that expression of defense proteins in resistant cultivars more and faster than of susceptible varieties and accumulation of these proteins causes resistance.

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