Plant defense-related enzymes against pathogens: A Review

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ABSTRACT

Plant disease control is mainly based on the use of fungicides, bactericides, and insecticides-chemical compounds toxic to plant invaders, causative agents, or vectors of plant diseases. However, the detrimental effect of these chemicals or their degradation products on the environment and human health strongly imposes the search for novel, harmless means of disease control. Therefore, it is essential to introduce environmentally-friendly alternative measures for management of plant diseases. Induced plant resistance is one of the promising non-chemical strategies for the effective management of diseases. The host plant mediated resistance is governed by defense response genes encoding for production of various pathogenesis-related (PR) proteins. This review chiefly explains the biochemical response of plant defense mechanism pertaining to defense-related enzymes which have been identified as PR proteins.

Keywords: *Defense-related enzymes, Induced plant resistance, Pathogenesis-related proteins*

Introduction

In nature, plants are attacked by a diverse range of biotic agents including pathogens and herbivorous insects which can have devastating effects on host plants (Ebrahim *et al*., 2011). Application of pesticides has been the chief method of controlling plant diseases (Prasannath *et al*., 2014). However, there is a growing concern in developing alternative measures aiming to minimize the harmful impacts of pesticides on the environment and human health. Inducing systemic resistance against plant pathogens is one such environmentally-friendly approach of disease management (Prasannath and De Costa, 2015).

When plants are attacked by pathogens and herbivores, these stresses can induce biochemical and physiological changes in plants, such as physical strengthening of the cell wall through lignification, suberization, and callose deposition; by producing phenolic compounds, phytoalexins and pathogenesis-related (PR) proteins which subsequently prevent various pathogen invasion (Bowles, 1990). Among these, production and accumulation of PR proteins in plants in response to invading pathogen is very important. Plants enhance defense responses by inducing activity of a broad spectrum of defense enzymes which are PR proteins, namely peroxidase, β-1,3-glucanase, chitinase, polyphenol oxidase and phenylalanine ammonia lyase which can slow an herbivore's feeding and also the rate of disease spread (Deborah *et al*., 2001; Kumari and Vengadaramana, 2017).

Host plant mediated resistance against pathogens

Interactions between plants and pathogens can lead in to successful infection (compatible response) or resistance (incompatible response). In incompatible relations, viruses, bacteria or

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fungi which infect plants will elicit a set of localized responses in and around the infected host cells. These responses consist with an oxidative burst (Lamb and Dixon, 1997), which can lead to cell death (Kombrink and Schmelzer, 2001). The pathogen may be 'trapped' in dead cells. It can lead to prevent the spreading from the site of primary infection. There are local responses in the surrounding cells which inhibit the penetration of pathogens by changing cell wall composition and synthesis of antimicrobial compounds such as PR proteins and phytoalexins (Kuc, 1995; Hammerschmidt, 1999). Also plants respond to attacks by pathogens through various defense responses. The accumulations of several factors like defense-related enzymes and inhibitors which lead to prevent infection of pathogens are several defense responses. The enzyme activities and total phenol content were increased significantly in resistant cultivars upon pathogen inoculation (Vanitha *et al*., 2009).

Plants possess a range of active defense mechanisms which respond to biotic stresses. Diseases can be reduced due to trigger of defense mechanisms in plants by a stimulus, prior to infection by a plant pathogen. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance in plant. Combination of ISR and SAR can increase defense against pathogens that are resisted through both pathways than ISR and SAR alone (Choudhary, 2007).

Induced Systemic Resistance (ISR)

When an antagonist is present at the site of exposure, an antimicrobial substance could be synthesized by the biological control agent and transported through the plant, inhibiting the pathogen directly. The induced resistance does not necessarily need to be systemic, but a local protection can be formed as a result of the induced resistance. ISR is induced by plant growth promoting rhizobacteria which are believed to produce a translocatable signal that induces protection in tissues far from the roots where the antagonist was delivered. A systemic response of the plant to an elicitor shows that induced resistance is taking place (van Loon *et al*., 1998). ISR is independent of salicylic acid, but it is mediated by jasmonic acid and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria (He *et al*., 2004). ISR is accompanied by the expression of a set of genes distinct from the PR protein genes (Pieterse *et al*., 1998).

Systemically Acquired Resistance (SAR)

Plants can acquire resistance against the initiating of diseases through various biological agents including necrotizing pathogens, non-pathogens and soil borne rhizosphere bacteria and fungi. SAR is a mechanism of induced defense responses (Gajanayaka *et al*., 2014). In SAR a mobile signal is generated in the site of induction and translocated in the plant, bringing about an induced state in tissues, far from the site of exposure to the elicitor (van Loon *et al*., 1998). It provides long-lasting protection against a broad spectrum of microorganisms. SAR requires the signal molecule salicylic acid and it is associated with accumulation of PR proteins, which are believed to contribute to resistance (He *et al*., 2004). The development of SAR is associated with various cellular defense responses, such as synthesis of PR proteins and phytoalexins, rapid changes in cell wall, and enhanced activity of various defense-related enzymes (Durrant and Dong, 2004). SAR is induced systemically after inoculation with necrotizing pathogens or application of some chemicals such as salicylic acid (Pieterse *et al*., 1998; Prasannath *et al*., 2014). Certain plant growth promoting microorganisms could stimulate defense activity and enhance plant resistance against soil borne pathogens (Whipps *et al*., 2001).

Pathogenesis-related (PR) proteins

PR proteins are a structurally diverse group of plant proteins that are considered to play important roles in plant disease resistance (Mahendranathan *et al*., 2016). They are widely distributed in plants in trace amounts, but are produced in much greater concentration following pathogen attack or stress. PR proteins exist in plant cells intracellularly and also in the intercellular spaces, particularly in the cell walls of different tissues (Agrios, 2005). The several groups of PR proteins have been classified according to their function, serological relationship, amino acid sequence, molecular weight, and some other properties. Currently PR proteins are categorized into 17 families according to their properties and functions, including β-1,3-glucanases, chitinases, thaumatin-like proteins, peroxidases, ribosome-inactivating proteins, defensins, thionins, nonspecific lipid transfer proteins, oxalate oxidase, and oxalate-oxidase-like proteins (van Loon and van Strien, 1999). PR proteins are either extremely acidic or extremely basic and therefore are highly soluble and reactive (Legrand *et al*., 1987). The signal compounds responsible for induction of PR proteins include salicylic acid, ethylene, xylanase, polypeptide systemin, jasmonic acid and probably others (Agrios, 2005).

Defense-related enzymes

Defense enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, chitinase and β-1,3-glucanase are related to resistance inducement in plants (Prasannath and De Costa, 2015; Gajanayaka *et al*., 2014; Seneviratne *et al*., 2014). Peroxidases have been implicated in a range of defense-related processes, including the hypersensitive response, lignification, cross-linking of phenolics and glycoproteins, suberization and phytoalexin production (Nicholson & Hammerschmidt, 1992; Wojtaszek, 1997). Polyphenol oxidase catalyzes the phenolic compounds to quinones, thus decreasing of nutritional quality of food and reducing protein digestibility (Felton and Duffey, 1990; Felton *et al*., 1994). The intensification of production of phenolic compounds, known as defense molecules of plants against pathogens and insects, is indicated by an increase in phenylalanine ammonia lyase activity in wounded plant tissues (Bi and Felton, 1995). Chitinase and β-1,3-glucanase are responsible for the hydrolysis of cell wall components in sequence such as chitin and β-1,3-glucans (Ebrahim *et al*., 2011).

Peroxidases

Peroxidases are a distinguished class of PR proteins and induced in host plant tissues by pathogen infection. They belong to PR protein 9 subfamily and are expressed to limit cellular spreading of infection through establishment of structural barriers or generation of highly toxic environments by massively producing reactive oxygen species (Passardi *et al*., 2005). Peroxidase activity or peroxidase gene expression in higher plants is, indeed, induced by fungi (Sasaki *et al*., 2004), bacteria (Lavania *et al*., 2006), viruses (Diaz-Vivancos *et al*., 2006), and viroids (Vera *et al*., 1993). Cross-linking of the phenolic monomers in oxidative coupling of lignin subunits has been associated with peroxidase using H_2O_2 as oxidant. One significant event in plant defense reactions is oxidative burst, a general early response of host plant cells to pathogen infection and elicitor treatment (Almagro *et al*., 2009). Peroxidase also participates in the production of ethylene the concentration of which increases frequently in pathogenesis process (Tudzynski, 1997).

Peroxidase is a key enzyme in the biosynthesis of lignin and suberin. Peroxidases have been associated with a

number of physiological functions that may contribute to resistance, through hypersensitive responses, oxidation of hydroxyl cinnamyl alcohol into free radical intermediates, phenol oxidation, polysaccharide cross linking, cross linking of extension monomers, and the deposition of phenolic material in plant cell walls during resistance reactions (Thakker *et al*., 2013). When peroxidase level increases due to the induced systemic resistance (Prasannath *et al*., 2014), quick synthesis of reactive oxygen derivatives by oxidative burst leads to cell death and inhibits pathogenic activities (Halfeld-Vieira *et al*., 2006). Peroxidase oxidizes phenolics to quinines and generates hydrogen peroxide. It is antimicrobial and also releases highly reactive free radicals and further increases the rate of polymerization of phenolic compounds into lignin-like substances. These substances are then deposited in cell walls and papillae and hinder the further growth and development of the pathogen (Agrios, 2005).

β-1,3-glucanases

They have been classified as PR-2 proteins which are β-glucanases (glucan endo-1,3-β-glucosidases) able to catalyze endo-type hydrolytic cleavage of the 1,3-β-D-glucosidic linkages in β-1,3-glucans. β-1,3-Glucans are the major components of the cell walls of oomycetes, a group of fungi that do not contain chitin (Wessels *et al*., 1981). The induction of β-glucanase as part of the hypersensitive reaction is a stereotypic response; the pattern of induction is similar for viral, bacterial, and fungal pathogens. It creates resistance against various fungi such as *Aspergillus parasiticus*, A. *flavs, Blumeria graminis, Colletotrichum lagenarium, Fusarium culmorum, Fusarium oxysporum, fusarium udum, Macrophomina phaseolina* and *Treptomyces sioyaensis* (Rezzonico, 1998; Wu and Bradford, 2003; Hong and Meng, 2004; Wrobel-Kwiatkowska *et al*., 2004, Liang *et al*., 2005; Roy-Barman *et al*., 2006). β-glucanases participate in the decomposition of glucans like callose which occurs in plant tissues as one of the components of wall modifications involved in resistance responses (Smart, 1991). Even though antifungal β-glucanase I appear to be tailored for defense against fungi, other studies of β-glucanase I-deficient mutants generated by antisense transformation suggest that these enzymes also play a vital role in viral pathogenesis (Beffa *et al*., 1996). The endo-type β-1,3-glucanase enzyme seems to be most important for the degradation of the callosic walls, while the exotype β-1,3-glucanase is involved in the further hydrolysis of released oligosaccharides. It has been proposed that these glucanohydrolases perform in at least two different ways: directly, by degrading the cell walls of the pathogen and indirectly, by promoting the release of cell wall-derived materials that can act as elicitors of defense reactions (Bowles *et al*., 1990).

Chitinases

Chitinases are large and diverse group of enzymes and also one of the important plant pathogenesis related (PR) protein that degrades chitin, it improves plant defence against chitin containing plant pathogens (Jalil *et al*., 2015). β-1,3-glucan and chitin, polymer of N-acetylglucosamine are major cell wall components of many fungi. Since β-1,3-glucanase and chitinases have been shown to be capable of attacking cell wall of fungal pathogens, these enzymes have been proposed as direct defense enzymes of plants (Abeles *et al*., 1970). In addition, Mauch *et al.* (1988) reported that in combination, chitinase and β-1,3-glucanase act synergistically to inhibit fungal growth. The mode of action of chitinase is relatively simple. They degrade the cell wall chitin polymers *in situ*, resulting in a weakened cell wall and rendering fungal cells osmotically sensitive (Jach *et al*., 1995). These

chitinases have significant antifungal activities against plant pathogenic fungi like *Alternaria* spp. for rice grain discoloration, *Rhizoctonia solani* for rice sheath blight, *Bipolaris oryzae* for rice brown spot, *Botrytis cinerea* for tobacco blight, *Curvularia lunata* for clover leaf spot, *Fusarium oxysporum*, *F. udum*, *Mycosphaerella arachidicola* and *Pestalotia theae* for tea leaf spot (Chu and Ng, 2005; Saikia *et al*., 2005; Kirubakaran and Sakthivel, 2006). The level of protection observed in the plants is variable and may be influenced by the specific activity of the enzyme, its localization and concentration within the cell, the characteristics of the fungal pathogen, and the nature of the host-pathogen interaction (Punja and Zhang, 1993).

Phenylalanine ammonia lyase (PAL)

PAL is the key enzyme that is responsible for linking primary metabolism of aromatic amino acids with secondary metabolic products (MacDonald and Dcunha, 2007). PAL catalyzes the non-oxidative deamination of phenylalanine in to *trans*-cinnamic acid and ammonia which is the initial step in the biosynthesis of phenolic compounds. PAL is a reliable treatment for the genetic condition phenylketonuria, due to the natural ability of the enzyme to breakdown L-phenylalanine (MacDonald and Dcunha, 2007). PAL is one of the most extensively studied enzymes in plants due to synthesis of various phenolic compounds as well as anthocyanin which are responsible for the resistance of plant pathogens (Dixon and Paiva, 1995). Changes in PAL activity can take place during pathological events (Seneviratne *et al*., 2014). PAL activity can be induced by the plant hormone ethylene and plant signal molecules including salicylic acid and jasmonic acid (Campos-Vargas and Saltveit, 2002; Kim *et al*., 2007), and also it can be induced by various biotic and abiotic stresses such as pathogen invasion, wounding, chilling and ozone (Lafuente *et al*., 2003). When treated strawberry plants with abscisic acid, anthocyanin and PAL activity are increased (Jiang and Joyce, 2003). All phenylpropanoids compounds are derived from cinnamic acid, which is formed from phenylalanine by the activity of PAL. These phenylpropanoids are accountable for disease resistance, crop development and mechanical support (Barber and Mitchell, 1997; Chen *et al*., 2007; Harakava, 2005) as well as insect pest damages (War *et al*., 2012). PAL activity may be regulated by feedback inhibition by the pathway product, cinnamic acid, which may modify the expression of the PAL gene (Christensen *et al*., 2001; Del Rio *et al*., 2004).

Polyphenol oxidase (PPO)

PPOs are a group of copper containing enzymes that catalyze oxidation of hydroxy phenols to their quinone derivatives, which have antimicrobial activity (Chunhua *et al*., 2001). Because of its reaction products and wound inducibility, PPO plays a role in defense against plant pathogens (Mayer and Harel, 1979). Plants immediately respond to pathogens so there is an immediate rise in PPO indicating immediate synthesis of antimicrobials to ward off the pathogens. Pathogen-induced PPO activity continues to be reported for various plant taxa, including monocots and dicots (Chen *et al*., 2000; Deborah *et al*., 2001). Increase of PPO activity was reported in banana roots treated with *Fusarium oxysporum* derived elicitor by Thakker *et al.* (2007). A striking increase of PPO activity was observed in banana roots treated with *Psuedomonas fluorescens* against fusarium wilt (Sarvanan *et al*., 2004). Similarly, studies showing correlations of high PPO levels in cultivars or lines with high pathogen resistance continue to provide support for a pathogen defense role of PPO (Raj *et al*., 2006). Several groups have also attempted to correlate the protective effects of rhizosphere bacteria with an induction of defense enzymes including PPO, with

mixed success (Chen *et al*., 2000; Ramamoorthy *et al*., 2002).

Li and Steffens (2002) suggested several possibilities, including general toxicity of PPO-generated quinones to pathogens and plant cells, accelerating cell death, alkylation and reduced bioavailability of cellular proteins to the pathogen, cross-linking of quinones with protein or other phenolics, forming a physical barrier to pathogens in the cell wall, and quinone redox cycling leading to H_2O_2 and other reactive oxygen species (Jiang and Miles, 1993). While reactive oxygen species are known to be important factors in plant pathogen interactions and defense signaling, PPO is implicated in the formation of melanin-like polymers in potato black spot lesions (Stevens *et al*., 1998). However, none of these hypotheses of how PPO might affect pathogens has been tested rigorously so far.

Conclusion

Plants protect themselves against biotic factors by physical strengthening of the cell wall through lignification, suberization, and producing various PR proteins including defense-related enzymes such as peroxidase, β-1,3-glucanase, chitinase, phenylalanine ammonia lyase and polyphenol oxidase in response to pathogen infection. These defense enzymes are also induced in plants through application of exogenous substances so that more studies are needed to investigate the defense responses that are triggered by these elicitor treatments. Thus, the knowledge on plant defense-related enzymes can definitely be beneficial for the development of new control strategies.

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