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Enzymes in Fungal Plant Pathogenesis

By

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K e y w o r d s : Plant defense, constitutive and active mechanisms, hypersensitivity, oxidative burst, non-protein and protein substances.

S u m m a r y

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In fungal plant pathogenesis, enzymes are playing a crucial role and they are involved in the external and internal interactions. To restrict the development of fungal pathogens, the plants formed many defense mechanisms. They built mechanical barriers from lignin, suberin, callose and produced a lot of antimicrobial compounds with low molecular weight like phenols, chinons, alkaloids and others. Enhanced production of some enzymes and their activity increasing is one of the most important processes in plant defense. These enzymes occur frequently in many isoforms and are involved in synthesis of defense substances or have a direct antimicrobial activity.

I n t r o d u c t i o n

Research of different aspects of interaction specificity and defense mechanisms of plants against potential fungal pathogens has received great attention in the last few years (DANGL & HOLUB 1997, HAMMOND-KOSACK & JONES 1997, VIDHYASEKARAN 1997). Plant defense mechanisms can be divided into constitutive (structural) and active (biochemical) mechanisms according to their function. Structural compounds restrict the developing pathogen by the construction of mechanical barriers or by preformed chemical substances; induced and/or biochemical processes participate in active defense reactions of plants against pathogen.

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In this review we elucidate the relationships between the plants and plant parasitic fungi at the physiological and biochemical level, with special emphasise on the role of enzymes in the process of pathogenesis and plant defense.

Biochemical (active) defenses

Active plant defense mechanisms are very complex and complicated and they include various responses like death of the plant cells (HR), the induction of the phenylpropanoid pathway and synthesis of lignin (lignification), accumulation of phytoalexins, synthesis of hydroxyprolinerich glycoproteins (HRGP) and enzymes degrading the fungal walls (chitinases, glucanases) and elicitation of the production of volatile compounds (e.g. ethylene).

Hypersensitive reaction and the oxidative burst

Defense by hypersensitive reaction (HR) occur frequently in specific incompatible host plant-fungus interactions and is mostly challenged by avirulent isolate (HAMMOND-KOSACK & JONES 1996). However, some HR was also recorded in compatible specific interactions (LEBEDA & REININK 1994). Enzymatic processes of HR are not well known (GOODMAN & NOVACKY 1994). Currently it is considered that the generation of reactive oxygen species (the oxidative burst) and hydrogen peroxide are substantially responsible for plant cell death (BOLWELL & WOJTASZEK 1997). The oxidants may function directly, in cell wall cross linking or as part of signalling mechanisms (KUC 1997).

Non-protein substances in plant defense

Non-protein substances are generally characterized by their low molecular weight. These active compounds produced in plant tissue can be divided according to the rise before (alkaloids, saponins, stilbens and others) (GOMES & XAVIER-FILHO 1994) or after the infection. After infection the synthesis of some compounds, for example dihydroxyphenols, tanins, chinons and aromatic aminoacids is enhanced. Damage of tissue allows also a contact between precursors of defense substances and enzymes activating them. New products synthesized only as a response to pathogen infection are known as phytoalexins (KUC 1997). The presence of elicitors stimulates the plant to produce great amounts of phytoalexins. The phenolic compounds, terpenoids and isoflavonoids have received the greatest attention. For example in *Cochliobolus gloeosporoides* both pectin-degrading enzymes are inhibited by epicatechin, a phenolic compound present in damaged peel of avocado fruits (MENDGEN & al. 1996).

Proteins and enzymes in plant defense

Depending on their function during the defense response, proteins can be grouped into three classes. The structural proteins in the first class participate in strengthening and repairing of the cell wall or modification of the properties of the extracellular matrix. The second class of proteins exhibit direct antimicrobial

activities or catalyse the synthesis of antimicrobial compounds. The third class comprises of proteins, which function in plant defense is not well known (SCHOELTENS-TOMA & al. 1991).

Extensins are structural proteins rich in hydroxyproline (HPRGs). They have the central role in organization of the primary cell wall of plants. All extensins are basic, highly glycosidated proteins. Extensins polypeptides are synthesized inside cells, secreted as soluble monomers to the apoplast and incorporated into the cell wall. Another group of structural proteins are GRPs, rich in glycine. The expression of genes for HPRGs and GRPs is regulated by signals produced during physiological or environmental stress including wounding and pathogen attack (GOMES & XAVIER-FILHO 1994). Currently, the research is also aimed on leucine-rich repeat proteins (LRR proteins), which have a significant role in plant defenses. They may be involved either as resistance proteins or as proteins required for resistance proteins to function. However, LRR proteins may also function as PR proteins (JONES & JONES 1997).

Proteins participating in defense mechanisms after pathogen attack are generaly called pathogenesis related proteins (PR-proteins). They are divided to five classes according to their solubility, Mr, biochemical activity and other chemical properties. PR-proteins with enzymatic activity are β-1.3 glucanases and chitinases (DAUGROIS & al. 1990). They occur in healthy plants mainly in apoplast. β-1.3-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). Together with chitinases they release components from cell walls of plant pathogenic fungi (DE LORENZO & al. 1997). Glucans and N-acetyl-glucosamines (parts of chitin) serve as elicitors for the synthesis of these defense substances (REPKA 1993). Enhanced production of them was noted in more cases of plant-pathogen interaction, in connection with both hypersensitive reaction and systemic acquired resistance.

Deposition of callose in response to pathogen attack or mechanical injury is involved in structural defenses (SMART 1991). The enzyme callose-synthetase catalyse the formation of β-glucans which are components of extracellular matrix. In this case it would be interesting to study the regulation of these processes - resolution and synthesis of compounds composed of β-glucans.

Peroxidases are frequently associated with plant defense against pathogens. Peroxidases catalyse the oxidation of substrates like phenol and its derivates, by hydrogen peroxide. They are responsible for the radical dehydrogenation of sinapilalcohol and koniferylalcohol during the lignin synthesis. Peroxidases participate in the synthesis of flavons, stilbens and other phenolic secondary metabolites. They are represented by many of the isoenzymes. Peroxidase polymorphism could be also used as a biochemical marker related to the different levels of field resistance (LEBEDA & al. 1999). Peroxidase participate in processes which occur in the extracellular matrix (BUONARIO & MONTALBINI 1993). Their association with the cell wall was confirmed. Peroxidases remove the toxic hydrogen peroxide from tissues, participate in synthesis of phenolic compounds and in the building of intermolecular bonds during the organisation of

the cell wall at the sites of infection by pathogens (REPKA & SLOVÁKOVÁ 1994). The production of phenolic compounds include synthesis of chinons, tanins, melanins and also the polymerization of lignin and suberin composing monomers. Tanins and melanins are dihydroxyphenol and chinon oligomers, these are toxic for pathogens. Lignin and suberin are involved to structural defenses. Peroxidase also participates in the synthesis of ethylene the concentration of which increases frequently in pathogenesis process (TUDZYNSKI 1997). Generally peroxidases enhance their activity after a pathogen attack, because they participate in defensive lignification and synthesis of phenolic compounds effective against pathogens (NICHOLSON & HAMMERSCHMIDT 1992).

Catalase is included in defense plant reactions. This enzyme occurs in peroxizomes and decompose the hydrogen peroxide to water and oxygen. There is only a few results related to the role of catalase in plant defense processes. This enzyme is the competitor of peroxidase, because they use the same substrate. Enhancement of their activity after inoculation of tobacco plants by *Erysiphe cichoracearum* was reported. However, the considerable decreasing of catalase activity was noted in correlation with very high peroxidase activity (BUONARIO & MONTALBINI 1993).

Studies on the role of amine oxidases in plant defense are currently in progress. Plant amine oxidases are predominantly localized in the exocellular matrix (BOLWELL & WOJTASZEK 1997). Amine oxidases catalyse the oxidative deamination of biogenic amines which are produced during degradation of aminoacids. Some cyclic metabolites like Δ^1-pyrrolin and Δ^1-piperidein formed from aminoaldehydes are precursors for plant alkaloides (PEČ & FRÉBORT 1990). Enhanced diamine oxidase activity during stress processes was reported (ZAJONCOVÁ & al. 1990) and also a positive correlation between activities of diamine oxidase and peroxidase was found (ANGELINI & al. 1990). It is under consideration the possibility that hydrogen peroxide, as a secondary product of the diamine oxidase catalysed reaction, represents the substrate for peroxidases.

In plant defense reactions is also involved superoxid dismutase which synthesize the hydrogen peroxide from very reactive and toxic compounds superoxide anion, hydroperoxyl radical and proton. Initial compounds of this reaction are more often produced in incompatible interactions, for example between potato and *Phytophthora infestans* (HAMMOND-KOSACK & JONES 1996). The enhancement of superoxid dismutase activity in infected tobacco plants by PVY^N was observed and correlated with enhanced activity of peroxidases (BUONARIO & MONTALBINI 1993). Higher generation of hydrogen peroxid in plant tissues increase the resistance. At the concentration known to be produced in plants, it could be directly toxic to pathogens. At a higher level of H_2O_2, hydroxyproline and proline-rich cell wall glycoproteins are rapidly oxidatively cross-linked in cell walls after fungal elicitor treatment (HAMMOND-KOSACK & JONES 1996).

Lipoxygenase form free radicals, and after pathogen attack, its activity rapidly increase (MACCARONE & al. 1997). Reactions of free radicals can result in the production of toxic volatile and nonvolatile fatty acid-derived secondary

metabolites that could directly attack invading pathogens. Alternatively, lipoxygenase can cause irreversible membrane damage and cell death (HAMMOND-KOSACK & JONES 1996).

Some proteins form a complex with fungi and insects proteases and inhibit their activities. Salicylic acid, abscisic acid, systemin, methyl jasmonate and ethylene act as endogenous signals that induce the synthesis of these inhibitors. The regulation of their synthesis involves substances produced by insects and pathogens like oligouronides. They interact with receptors in cells and initiate the signalization process of this defense mechanism through systemic acquired resistance (STICHER & al. 1997).

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