RESEARCH PAPER

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Involvement of protein kinases in induction of acidic isopolyphenol oxidases in cell walls of tomato by fruit extracts of *Azadirachta indica* A. Juss

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Polyphenoloxidases have a defensive role against biotic stress. Neem fruit extract induces signaling pathway leading to defense response. The inhibitors of tyrosine protein kinases (Lavendustin A), protein serine/threonine kinases (Staurosporine), and protein kinase (K-252a) were utilized to study the role of protein kinase, as components of signaling leading to systemic acquired resistance by neem fruit extract in tomato plants. K252a-sensitive protein phosphorylation is involved in induction of signal transduction leading to appearance of a novel acidic isopolyphenoloxidases (Rf 0.71) ionically bound to cell walls of tomato plants treated with neem fruit extract. Physical pathogen barrier could be generated as a result of cross-linking of oxidized phenolics as well as due to oxidative cross-linking of cell wall proteins and enhanced lignin formation due to enhanced PPO activity. The cell wall bound acidic isopolyphenoloxidases which have a net positive charge may immobilize pathogens by interacting ionically with the negatively charged surfaces of plant pathogens.

Key words : Cell wall proteins, Isopolyphenol oxidases, K-252a, Lavendustin A, Protein kinase, Staurosporine

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INTRODUCTION

Polyphenol oxidases (PPO) have been implicated a role in the phenylpropanoid pathway (Kojima and Takeuchi, 1989) and has a defensive role since it is conspicuously appeared upon wounding, pathogen infection or insect infestation, and due to the inducibility of PPO in response to various abiotic and biotic injuries or signaling molecules (Mayer and Harel, 1979; Constabel *et al*., 1995; Thipyapong and Steffens, 1997; Maki and Morohashi, 2006). PPO acts in disease resistance by hydroxylizing monophenols to o-diphenols and oxidizing these compounds to quiñones, which are often more toxic to the microorganisms than the original phenolic compounds (Gandia-Herrero *et al*., 2005). PPO is multifunctional enzyme that can prevent biological and chemical attacks by raising physical barriers or by counterattacking a pathogen with a high production of free radicals (Passardi *et al*., 2005). In resistant varieties of grapevine, there is not only a higher basal activity of PPO, but also a rapid increase in their activity after pathogen inoculation (Kortekamp and Zyprian, 2003). The induction of a PPO isoform in tomatoes susceptible to *Pseudomonas syringae* pv. *tomato* and *Alternaría solani* suggests that this enzyme plays a role in disease resistance (Thipyapong and Steffens, 1997). Root tissue of the tolerant Goldfinger banana responds to the fungal elicitor through deposition of lignin, preceeded by the induction or activation of the enzyme activities involved in cell wall strengthening i.e. phenylalanine ammonia lyase (PAL), peroxidase (POX) and PPO (De Ascensao and Dubery, 2000). Lignin is highly resistant to attack by microorganisms, and lignified cell walls are an effective barrier to pathogen entrance and spread (Ride, 1983).

The development of an effective systemic acquired resistance (SAR) in any plant system relies, not only in their gene composition and expression, but also on an effective and rapid transduction of signal. Reversible protein phosphorylation is a key mechanism for intracellular signal transduction in eukaryotic cells. Plants harbour histidine and aspartate kinases as part of the two-component signaling system. Ser/Thr-kinases are also abundant and implicated in many signaling events, but classical Tyr-kinases are less well known in plants. It is