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Cloning and characterization of cDNA encoding an elicitor of *Phytophthora colocasiae* ☆

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Summary

The rapid and effective activation of disease resistance responses is essential for plant defense against pathogen attack. These responses are initiated when pathogen-derived molecules (elicitors) are recognized by the host. A cDNA encoding elicitor, the major secreted extracellular glycoprotein of *Phytophthora colocasiae*, a pathogen of taro (*Colocasia esculenta*) plants, was isolated, sequenced and characterized. The expression of the corresponding elicitor gene during the disease cycle of *P. colocasiae* was analyzed. Elicitor was shown to be expressed in mycelium grown in culture media, whereas it was not expressed in sporangiospores and zoospores. *In planta*, during infection of taro, particularly during the biotrophic stage, expression of elicitor was down-regulated compared to *in vitro*. The highest levels of expression of elicitor were observed in *in vitro* grown mycelium and in late stages of infection when profuse sporulation and leaf necrosis occur. The elicitation of the suspension-cultured taro cells was effective in the induction of the enzyme activity of L-phenylalanine-ammonia lyase, peroxidase and lipoxygenase as well as the expression of defense-related endochitinase gene. All these biological activities were exerted within a low concentration range. The glycoprotein represents a powerful tool to investigate further the signals and their transduction pathways involved in induced disease resistance. It may also be useful to engineer broad disease protection in taro plant against *Phytophthora* leaf blight.

Introduction

As a result of host—pathogen coevolution, plants have developed sophisticated mechanisms to protect themselves from diseases. Pathogen specialization results when a complex set of preformed and

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induced mechanisms is put into motion to defend a plant against invading pathogens. In some interactions, preformed physical barriers and antimicrobial compounds in the plant help to ward off pathogens (Osbourn, 1996). In other interactions, perception by the plant of signal molecules namely elicitors produced by the virulent pathogen leads to the induction of effective defense responses including a programmed cell death response termed the hypersensitive response (HR), that limits the spread of microorganisms (Lam et al., 2001). In addition, the distal uninfected parts of the plants usually develop systemic acquired resistance (SAR), which leads to a broad range of resistance against diverse pathogens (Wang et al., 2003). Both the HR and SAR are regulated by a complex network of signaling molecules, including AOS, salicylic acid, nitric oxide and jasmonic acid (Wendehenne et al., 2001).

Different classes of elicitor protein have been reported from various species of *Phytophthora* fungi: acidic-elicitins (capsicein and parasiticein) from *Phytophthora capsici* and *Phytophthora parasitica* respectively, and basic-elicitins (cryptogein and cinnamomin) from *Phytophthora cryptogea* and *Phytophthora cinnamomi*, respectively. These various types of elicitor molecules induce biochemical changes as part of the resistance response. Electrolyte leakage, oxidative burst, production of phytoalexins and PR proteins, and increased biosynthesis of ethylene have been described in leaf tissue treated with non-specific elicitors (Peever and Higgins, 1989) and with specific elicitors (Hammond-Kosack et al., 1996). Many biochemical and physiological aspects of the defense response were studied in suspension-cultured plant cells. A 32 kDa glycoprotein purified from *Phytophthora megasperma* f.sp. *glycinea* was shown to stimulate various defense responses in cultured parsley cell suspensions including ion fluxes, oxidative burst, expression of defense-related genes and phytoalexin accumulation (Nürnberger et al., 1994). A glycoprotein of molecular mass 46 kDa isolated from *Phytophthora nicotianae* was shown to elicit phytoalexin accumulation in tobacco callus (Farmer and Helgeson, 1987). Treatment of suspension-cultured tobacco cells with elicitins from *P. megasperma* leads to rapid protein phosphorylation, Ca^{2+} influx, extracellular and transient H_2O_2 production, alkylinization of the extracellular medium, acidification of the cytosol, lipid peroxidation, gene expression, disruption of microtubular cytoskeleton, and cell wall modifications (Sasabe et al., 2000; Binet et al., 2001).

Phytophthora colocasiae, a hemibiotrophic oomycete plant pathogen, causes leaf blight, an economically devastating disease of taro (Raciborski, 1900). The life cycle and infection process of *P. colocasiae* are well known (Misra et al., 2008). The molecular basis of host specificity of *P. colocasiae* is poorly understood. To date, no race-specific avirulence gene of *P. colocasiae* has been isolated. In the course of our studies in *P. colocasiae*–taro interactions, we got the evidence of glycoprotein of 15 kDa in the 7 days old culture filtrate of *P. colocasiae*. It activates a qualitatively similar spectrum of defense responses as the well-characterized oligopeptide elicitor of molecular mass 14.9 kDa derived from *P. megasperma* (Baillieul et al., 2003). The present work describes the cloning of a cDNA encoding the protein moiety of the molecule. This elicitor can be used as a potential tool to engineer disease resistance against a broad spectrum of pathogens by manipulating the HR in transgenic taro plants.

Material and methods

Isolation of elicitor and N-terminal amino acid peptide sequencing

The elicitor protein was purified from the mycelium of *P. colocasiae* race 98–111 by anion exchange chromatography and gel filtration chromatography. Protein concentration was measured by the method of Bradford (1976) with bovine serum albumin (BSA) as standard protein. In order to sequence the N terminus of the protein moiety, the purified glycoprotein (2.8 μg) was subjected to SDS-PAGE before being transferred onto a PVDF Immobilon membrane (Bio-Rad), in 50 mM Tris base, 50 mM boric acid at 15 V overnight. After staining with a solution of 0.1% Amidoblack in 45% methanol, 1% acetic acid, the protein band was cut out and the protein was sequenced directly on the membrane. Sequencing of the amino terminus was performed by automated Edman degradation (Edman and Begg, 1967) at the Rajiv Gandhi Biotechnology Centre (Trivandrum, India). The sequenced peptide was reverse translated and for primer designing highest frequency codons were selected by using *S. cerevisiae* codon and standard genetic code table.

Nucleic acid extraction

Total RNA from *P. colocasiae* and from infected taro leaves was isolated using the guanidine hydrochloride extraction method (Logemann et al., 1987). Poly $(A)^+$ mRNA was purified from