Journal of Plant Physiology **164** (2007) 1071—1082

Available online at www.sciencedirect.com
ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY
www.elsevier.de/jplph

Inhibitory effect of a defensin gene from the Andean crop maca (*Lepidium meyenii*) against *Phytophthora infestans*

Julio Solis[a,b], Giuliana Medrano[a], Marc Ghislain[a,*]

[a]*International Potato Center, Applied Biotechnology Laboratory, P.O. Box 1558, Lima 12, Peru*
[b]*Universidad Nacional Mayor de San Marcos, Av. Venezuela cdra. 34, Lima 1, Peru*

Received 13 April 2006; accepted 13 June 2006

KEYWORDS
Antimicrobial peptide;
Lepidium meyenii;
Maca;
Phytophthora infestans;
Plant defensin

Summary

In this study, we report the isolation of a defensin gene, *lm-def*, isolated from the Andean crop 'maca' (*Lepidium meyenii*) with activity against the pathogen *Phytophthora infestans* responsible of late blight disease of the potato and tomato crops. The *lm-def* gene has been isolated by polymerase chain reaction (PCR) using degenerate primers corresponding to conserved regions of 13 plant defensin genes of the Brassicaceae family assuming that defensin genes are highly conserved among cruciferous species. The *lm-def* gene belongs to a small multigene family of at least 10 members possibly including pseudogenes as assessed by genomic hybridization and nucleotide sequence analyses. The deduced mature Lm-Def peptide is 51 amino acids in length and has 74–94% sequence identity with other plant defensins of the Brassicaceae family. The Lm-Def peptide was produced as a fusion protein using the pET-44a expression vector and purified using an immobilized metal ion affinity chromatography. The recombinant protein (NusA:Lm-Def) exhibited in vitro activity against *P. infestans*. The NusA:Lm-Def protein caused growth inhibition and hyphal damage at concentration not greater than 0.4 μM. In contrast, the NusA protein alone expressed and purified similarly did not show any activity against *P. infestans*. Therefore, these results indicate that the *lm-def* gene isolated from maca belong to the plant defensin family with activity against *P. infestans*. Its expression in potato, as a transgene, might help to control the late blight disease caused by *P. infestans* with the advantage of being of plant origin.

Abbreviations: Bp, base pair; CTAB, cetyl trimethyl ammonium bromide; EDTA, ethylene diamine tetra-acetic acid; *lm-def*, *Lepidium meyenii* defensin gene; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; TBE, tris borate EDTA buffer

*Corresponding author. Tel.: 51 1 349 6017; fax: 51 1 317 5326.
E-mail address: m.ghislain@cgiar.org (M. Ghislain).

0176-1617/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2006.06.002

Introduction

Phytophthora infestans, the causal agent of late blight disease, causes serious losses to potato and tomato crops worldwide and is probably the most important pathogen of both crops (William and Stephen, 1997). This disease was the cause of the Irish potato famine in the 1840s which resulted in about one million deaths and the emigration of about 1.5 million people to other parts of the world, particularly to the US. Even now, annual crop losses and fungicide costs amount to about US$4 billion throughout the world. While considerable efforts have been invested in plant breeding and genetic engineering, sources of resistance to late blight are still in great needs. Although recent successes in expressing an *R* gene from a Mexican wild potato species which confers partial and broad-spectrum resistance to late blight (Song et al., 2003; van der Vossen et al., 2003), most of genetic engineering strategies have used peptides and proteins from diverse origin. For instance, pathogenesis-related (PR) proteins such as chitinases (Nishizawa et al., 1999; Terakawa et al., 1997), 1,3-β-glucanase (Lusso and Kuc, 1996; Masoud et al., 1996), and osmotin-like proteins (Liu et al., 1994) have been used to engineer resistance to pathogens because of their capacity to degrade at least partially fungal and bacterial cell walls. Other plant proteins were shown to bear antifungal activities such as the case of the storage protein, ocatin, found in the Andean tuber crop oca *Oxalis tuberosa* (Flores et al., 2002), an isoform of patatin from potato (Sharma et al., 2004) and the sporamin storage protein from sweet potato (Yeh et al., 1997). In some cases, peptides from potato were constitutively expressed in potato and displayed antimicrobial activity such as the pseudothionin-*St*1 (Moreno et al., 1994) and snakin-1 (Segura et al., 1999).

Plant defensins comprise a family of small cationic, cysteine-rich peptides (45–54 amino acid) that are mostly found to contribute to broad-spectrum host defense against pathogens and are widely distributed among plants, including wheat, barley, spinach, pea, and several members of the Brassicaceae family (Broekaert et al., 1995, 1997; Lay and Anderson, 2005; Thomma et al., 2002). Many plant defensins can inhibit the growth of a broad range of fungi at micromolar concentrations but are non-toxic to both mammalian and plant cells (Broekaert et al., 1995; Moreno et al., 1994; Osborn et al., 1995; Terras et al., 1995). The first plant defensins that were demonstrated to possess antimicrobial activity were the two plant defensins isoforms Rs-AFP1 and Rs-AFP2 isolated from the radish seed from Brassicaceae family (Terras et al., 1992). Because at least some of the plant defensins were shown to be induced upon pathogen invasion, these are referred also as members of the family PR-12 of pathogenesis-related proteins (Van Loon and Van Strien, 1999). The use of defensin genes in genetic engineering has resulted in broad spectrum resistance against fungal pathogens (Gao et al., 2000; Kanazaki et al., 2002). Genome organization was in general reported as a small multigene family with, for example, up to 15 members in *Arabidopsis thaliana* (Thomma et al., 2002). However, a recent reassessment of defensin-like sequence in the near-complete genome sequence of *A. thaliana* revealed that 317 homologous sequences could be identified (Silverstein et al., 2005). Defensins may have evolved into such a large multigene family in order to provide non-host resistance to numerous pathogens in many different tissues and in addition seem also to be involved in non-pathogen resistance mechanisms (Silverstein et al., 2005). The precise mode of action of defensin is still under investigation but modification of plasma membrane has been reported as the most likely primary interaction, secondary activities in the cell may involve enzyme inhibition, ion channel inhibitors, and others (Lay and Anderson, 2005; Thevissen et al., 1999).

One member of the Brassicaceae family is the Andean crop maca (*Lepidium meyenii*) which is known to have good antimicrobial defense. Its cultivation is restricted today to the Departments of Junín and Cerro de Pasco of Peru at elevations above 3500 m and often reaching 4450 m in the central Andes of Peru (León, 1964; Tello et al., 1992). In this study, we report the isolation, cloning, characterization, expression and purification of a defensin gene from maca and the evaluation of its activity against *P. infestans*. Our results indicate that this defensin gene of maca is of potential use in the development of transgenic potato plants resistant to late blight disease.

Materials and methods

Plant material

Leaves of maca plants (*L. meyenii* Walp.) were obtained from greenhouse grown plants derived from seeds collected directly in the field where the crop is locally grown and consumed (central Andes of Peru, 3500 m altitude).