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Original article

Molecular cloning of a potato leaf cDNA encoding an aspartic protease (*St*Asp) and its expression after *P. infestans* infection

María G. Guevara [a,*], Carla Almeida [b], Julieta R. Mendieta [a], Carlos J. Faro [b], Paula Veríssimo [b], Euclides Vieyras Pires [b], Gustavo R. Daleo [a]

[a] *Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CC 1245, 7600 Mar del Plata, Argentina*
[b] *Departamento de Bioquímica, Faculdade de Ciências e Tecnologia and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3004-517 Coimbra, Portugal*

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Abstract

Aspartic proteinases (EC 3.4.23) are widely distributed in the plant kingdom, and a number of cDNAs have been isolated from different plants. Here we report the isolation an expression analysis of a cDNA from *Solanum tuberosum* L. (cv. Pampeana) named *St*Asp. The *St*Asp cDNA clone was obtained using a reverse transcriptase-polymerase chain reaction (RT-PCR) and degenerated primers encoding to plant aspartic proteinases conserved domains. The coding region of the gene is 1494 bp long encoding 497 amino acids of a predicted 54 kDa molecular mass and with a p*I* of 5.5. The gene shares a high homology with an aspartic proteinase cDNA of tomato, 97% and 94% homology on the level of DNA and protein, respectively. The deduced amino acid sequence contains the conserved features of plant aspartic proteinases, including the plant specific insert. Northern blot analysis indicated that *St*Aps transcripts are differentially accumulated in potato leaves after *Phytophthora infestans* infection in two potato cultivars with different degree of field resistance to this pathogen. In the resistant cultivar (Pampeana), induction was higher and more durable than in the susceptible cultivar (Bintje), suggesting that the *St*Asp level expression are associated with the resistance degree of potato cultivars to *P. infestans*. Results obtained previously about the induction of *St*AP proteins in stress conditions and these results suggest that potato aspartic proteinases are components of the plant defense response.

Keywords: Aspartic proteinases; *Solanum tuberosum*; *Phytophthora infestans*; Antimicrobial proteins; Saposin-like proteins

1. Introduction

Aspartic proteases (APs) (aspartic endopeptidases, EC 3.4.23) are one of the four main classes of proteinases, the others being serine, cysteine, and metallo proteinases [4] and are a widely distributed class of proteases present in animals, microbes, viruses, and plants [10,27,39,32]. Plant APs have characteristics common with aspartic proteinase A1 family, are active at acidic pH, are specifically inhibited by pepstatin and have two aspartic acids residues responsible for the catalytic activity [11,21].

APs have been found in seeds, tubers, flowers, and petals of many species and in the pitchers of several insectivorous species [15,16,27]. A number of aspartic proteinases cDNAs have been isolated from different plants including *Arabidopsis*, barley, *Brassica*, cardoon, pumpkin, rice, and tomato

Abbreviations: APs, aspartic proteases; cv., cultivar; PSI, plant specific insert; SAPLIP, saposin-like protein; *St*AP, *Solanum tuberosum* aspartic protease.

* Corresponding author.
E-mail address: gguevara@mdp.edu.ar (M.G. Guevara).

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[2,5,8,9,12,22,35,38,46]. The typical plant AP sequences predict preproproteins which are similar to the animal and fungal aspartic proteinases with a signal peptide and a proregion at the amino-terminus of the mature protein. In contrast, plant AP genes, except to nucellin, barley AP [7] and AP encoded by the *cdr*-1 gene from *Arabidopsis* [46], have an extra region of approximately 100 amino acids called as "plant specific insert" (PSI). This sequence has homology to the precursor of mammalian saposins, lysosomal sphingolipid-activating proteins [43,19].

Biological functions of plant APs are not as well assigned or characterized as those of their mammalian, microbial or viral counterparts that were shown to perform many different and diverse functions [27,10,32]. Plant APs have been implicated in protein processing and/or degradation in different plant organs, as well as in plant senescence, stress responses, programmed cell death and reproduction [39]. As respect to plant APs involved in plant stress response, Rodrigo et al. [34] have reported the constitutive expression of APs that degrades pathogenesis-related proteins (PR proteins) in the intercellular fluid of tobacco and tomato plants and suggest that these proteinases might be involved in the turnover of PR proteins as well as in the pathogenesis process itself. There are other examples as an AP mRNA (*Le*AspP) induced when tomato leaves are wounded [38], APs induced when cauliflowers seeds are treated with polyethylene glycol [13] and recently, an AP from *Arabidopsis*, induced in response to pathogen attack [46].

We have been studying the relationship between the expression patterns of potato aspartic proteinases with the field resistance levels of potato cultivars to *P. infestans.* Two forms of genetic resistance to *P. infestans* in potato species have been described, either race-specific (vertical resistance) or race-nonspecific (horizontal or field resistance) [41]. Race-specific resistance is characterized by interactions between products of dominant R genes in the host and corresponding avirulence (avr) genes in the pathogen. In contrast, race-nonspecific resistance or field resistance is assumed to be multiple genes based. This type of resistance is durable and thus commercially more attractive than race-specific resistance [6,42,5]. In the latter, new virulent races evolve rapidly, rendering the R genes ineffective [44]. The importance of studying the expression patterns of these enzymes in potato horizontal or field resistance to *P. infestans* is to identify the resistance proteins involved in partial resistance to economically important oomycetes, such as *P. infestans*.

Initially we have identified three potato aspartic proteases, one from tubers (***St*AP1**) [15] and two from leaves (***St*AP2** and ***St*AP3**) [16]. Two of these isoforms, ***St*AP1** and ***St*AP3** have been purified and characterized. Both proteins have extracellular localization; antimicrobial activity and are induced by both abiotic and biotic stress [15,18] in resistant potato cultivars. Here, we have isolated and characterized a cDNA encoding an aspartic proteinase from potato leaves *St*Asp. Northern and western blots analyses suggest that a differential ***St*APs** expression is associated with the potato cultivar resistance levels to *Phytophthora infestans*.

2. Results and discussion

2.1. Molecular cloning of StAsp cDNA and sequence analysis

In the first stage, RT-PCR reactions were performed as described in Section 4 using potato leaf cDNA. Degenerated primers encoding two conserved domains of the deduced amino acid sequences of Phytepsin, from barley (accession number X56136), LeAspP from tomato (accession number: L46681.1), an AP from *Brassica napus* (U55032) and an AP from *Arabidopsis thaliana* (accession number: AY088657.1) were used in the PCR amplification. Only one cDNA fragment of 500 bp PCR-amplified was isolated of the agarose gel band corresponding to an internal segment of the *St*Asp cDNA. In homology study using BLAST program database search [1] showed that the sequence of the cDNA fragment has a high homology of 97%, 94% and 90% with cDNA coding to tomato, *B. napus* and *A. thaliana* aspartic proteinase, respectively. Based upon this cDNA sequence a primer was designed to perform RACE-PCR of 3´ end to amplify a full-length cDNA. Only one cDNA fragment PCR-amplified was isolated of the agarose gel band corresponding to 1700 bp approximately, as estimated in agarose gels. We had analyzed 10 sequences of different blue colonies, the result obtained of the alignment of these sequences shown that they are 100% homologous. Analysis of this cDNA sequences obtained using Blast algorithm shows the highest sequence identity (97%) with a tomato AP cDNA (accession number: L46681.1); 95% with a *Oryza sativa* AP cDNA (accession number: XM_475576.1); 94% with *B. napus* AP cDNA (accession number: U55032), 86% with *Glycine max* AP cDNA (accession number: AB070857.2) and 80% with *A. thaliana* AP cDNA (accession number: AY056403.1). We named the new cDNA *Solanum tuberosum* aspartic protease (*St*Asp) and its complete nucleotide sequence is under GenBank accession number **AY672651**. The *St*Asp was 1680 bp long containing an open reading frame (ORF) of 1494 bp (Fig. 1) This ORF encodes a 497 amino acids polypeptide with a calculated molecular mass of 54 kDa with a theoretical isoelectric point (p*I*) of 5.5.

Comparison of the *St*Asp deduced amino acid sequence with GenBank and EMBL databases reveals a high degree of conservation with other plant aspartic proteinases (Fig. 2). The highest score (94% identity) was found with the sequence of LeAspP from tomato (accession number: AAB18280.1). Lesser identities were detected with APs sequences from other species: Oryzasin from *O. sativa* (accession number: Q42456) (62%) and phytepsin from *Hordeum vulgare* (accession number: X56136) (62%) (Fig. 2).

The predicted *St*Asp amino acid sequence contains a hydrophobic N-terminal signal sequence, followed by a prosegment and a N-terminal domain and a C-terminal domain separated by an insertion common to all plant aspartic proteinases named as PSI [39], except those of barley nucellin [7], an AP-like protein from tobacco chloroplast [28] and the prod-