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Review

A review of a promising therapeutic and agronomical alternative: Antimicrobial peptides from *Capsicum* sp.

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Pathogenic microorganisms cause great losses annually and are a constant threat to agriculture and food production. The strategies used to control pathogen microorganisms' population such as spraying of fungicides, bactericides or insecticides are becoming ineffective as pathogens have being developing resistance against many of these compounds. Today, in agriculture there are serious concerns regarding the increasing volumes of pesticides that must be applied to control plant pathogens, and the environmental contamination. The development of safer and more efficient compounds to control plant pathogens is a demand that guarantees food production with the absence of residual pesticides. An opportunity that fulfills these criteria is represented by the antimicrobial peptides (AMPs), a class of small rich cysteine peptides with biological activities to kill fungi and bacteria. Sources for AMPs have been studied in animals and plants. However, it is clear that plants are an accessible and cheaper source for this kind of compounds. Many AMPs are produced in organs that are regarded as waste after plants' fruits or seeds have been harvested. AMPs from Chili pepper (*Capsicum* sp) have been extracted from leaves and seeds. The genes encoding AMP are being expressed in heterologous systems to explore the potential of these genes to protect the host against pathogens. In the present study, we carried out a review to highlight the work related with the production and cloning of AMPs from chili pepper. We also included our findings regarding the cloning of a defensin gene from habanero pepper leaves (*Capsicum chinense* Jacq) and the antimicrobial activity of some of their AMPs isolated from seeds.

Key words: Antimicrobial peptides, *Capsicum* sp, *Capsicum chinense*, chili pepper, agronomical options, therapeutic options.

INTRODUCTION

Plants are constantly threatened by biotic and abiotic stressing factors, inducing in them a defensive state in order to implement quick and appropriated responses against the stressful condition. Currently, biotic global threats for plant health are caused by pathogens, including bacteria, fungi, viruses and oomycetes causing important losses in crop production (Makovitzki et al., 2007). To date, demand for food to support the growing population is increasing (Dey, 2010) and because of plant pathogens, it is a challenge to sustain food production.

The introduction of agrochemicals in the agriculture decades ago was with the aim to sustain and enhance plant production and crop yield, but also to protect the crops from plant pathogens (Carvalho, 2006; Dey, 2010). Unfortunately, every year higher amounts and new agrochemicals are required to protect crops because of the resistance developed by plant pathogens against this kind of compounds (Dey, 2010). In addition, increased use of agrochemicals has undesired side effects, that is, high environmental pollution, decrease in agriculturally beneficial organism populations and increase in health disorders in animals and humans (Strange and Scott, 2005; Carvalho, 2006; Dey, 2010; Valavanidis and Vlachogianni, 2011).

The agrochemicals remain as valuable tools for sup-

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porting plants' production. However, the challenge they represent to the environment and to human health have also raised the need to find new strategies that could halt the massive spread of these kinds of compounds. Genetically modified plants (GMPs) resistance to plant pathogens are an attractive alternative; however, their production is restricted to experimental areas in some countries or continents because of the concerns about their safety and use as human or animal food (Twardowski, 2010). Organic agriculture is quickly gaining support, but unfortunately, until now it is unable to sustain the massive amounts of food demanded by mankind (Carvalho, 2006; Easterling, 2007; DeFries and Rosenzweig, 2010).

One promising strategy that is being explored to limit crop losses caused by plant pathogens is the use of the plants' naturally produced compounds against pathogen infection (Bell and Gouyon, 2003), mainly constituted by plant innate protection which includes preformed defenses and the presence of small cysteine-rich peptides that exhibit strong antimicrobial properties (Barbosa-Pelegrini et al., 2011). Although some of the antimicrobial peptides (AMPs) from plants were discovered approximately 40 years ago, their antimicrobial, insecticidal and antifungal activities have only recently been characterized and described; such is the case of defensins and cyclotides (Craik et al., 1999; Barbosa-Pelegrini et al., 2011; Poth et al., 2011). AMPs from plants are composed of a variable number of amino acids, frequently from 45 to 54 amino acids (De Lucca et al., 2005; Barbosa-Pelegrini et al., 2011). AMPs from plants represent an attractive alternative to substitute current agrochemicals, as they are widely distributed in the plant kingdom, and most importantly, their source are plants which man have used for centuries, like wheat (Vinod et al., 2009), barley (Hégrová et al., 2009), corn (Duvick et al., 1992), grapes (Zamyatnin and Voronina, 2010), and chili pepper (Texeira et al., 2006; Diz et al., 2006; Brito-Argáez et al., 2009; Diz et al., 2011). Due to our interest in the medicinal and antimicrobial properties of *Capsicum* sp, we carried out this review to highlight the main findings regarding the AMPs in chili pepper that have been performed and reported by groups working on this valuable plant.

ANTIMICROBIAL PEPTIDES ARE A PROMISING ALTERNATIVE TO FIGHT PLANT DISEASES

Various classes of cysteine-rich peptides have been involved in the defensive mechanism of plants against pathogens (Broekaert et al., 1997; Maarof et al., 2011). The AMPs from plants were initially classified based on their bactericidal or fungicidal activity (Broekaert et al., 1997). However, new scientific finding have indicated that these peptides must meet additional criteria to be considered as AMPs. Those parameters include *in vitro* antimicrobial activity, gene induction and peptide accumulation *in planta* on or before infection, gene up-regulation in accordance with the severity of symptoms, and responsiveness of plant peptides to a range of virulent pathogens (García-Olmedo et al., 1998). These antimicrobial peptides are categorized into distinct families mainly on the basis of their amino acid sequence identity, number of cysteine residues and their spacing (Lay and Anderson, 2005). From the evolutionary point of view, these plant antimicrobial peptides show similar structures [cysteine-stabilized ab (CSab) motif (Fant et al., 1999; Jenssen et al., 2006; Lay and Gallo, 2009)] to several toxins from insects, scorpions, honey bees and spider venoms, and it is believed that these peptides are the arsenal for plant and animal innate immunity (Farrokhi et al., 2008).

The idea of using AMPs from plants as therapeutic compounds or natural agrochemicals was postulated in the late 1990s (Zasloff, 2002; Zucca and Savoia, 2010). However, after 20 years no AMPs has reached into the market (Bommarius and Kalman, 2009; Brito-Argáez et al., 2009). AMPs from plants represent intriguing natural compounds as in some cases have been described as an alternative form of nitrogen storage (de Souza-Candido et al., 2011), but most frequently as a promising therapeutic option, because of their role as elements of the innate immunity and because they are the first defensive weapons to handle plant pathogenic microorganisms. Interestingly, when they are directly applied to animal cells, plant AMPs have shown angiogenic, immuno-modulating, and anti-inflammatory properties, two required characteristics in immunosuppressant and chemotherapeutic compounds (Steinstraesser et al., 2009). On the basis of their electrical charge, plant AMPs can be divided into anionic (AAMPs) and cationic peptides (CAMPs) (Hancock and Lehrer, 1998; Brogden, 2005; Texeira et al., 2006; Barbosa-Pelegrini et al., 2011). CAMPs can be divided into four classes according to their molecular and conformational structure as:

Cysteine-rich β-sheet structures with one or more disulphide bonds, such as defensins (Wang and Wang, 2004; Barbosa-Pelegrini et al., 2011),
Linear α-helical peptides without disulphide bonds (Barbosa-Pelegrini et al., 2008),
ii) Loop structured peptides, such as cyclotides (Craik et al., 1999) and,
Extended trypthophan-rich peptides (Oyston et al., 2009).

The exact mechanism of action of CAMPs is yet to be established, but according to the most accepted model, the initial phase is common to anionic and cationic plant peptides and involves an electrostatic interaction with the surface of the target cell. After this, a rapid dis-organization of plasma membrane occurs within seconds to minutes, where the binding of the AMPs to intracellular targets takes from 3 to 5 h (Barbosa-Pelegrini et al.,

2011).

The AMP defensins, cecropins and magainins kill microorganisms by membrane permeabilization with a detergent-like effect accompanied by pore formation (Hancock and Rozek, 2002; Barbosa-Pelegrini et al., 2011). This mechanism is fast, concentration dependent and most importantly, it does not need the interaction with a specific receptor, thus avoiding the induction of resistance. The CAMPs mechanism of action depends upon the differences in the composition and physico-chemical properties of germ and host cell membranes. For example, magainin induces pore formation in bacterial membranes rich in anionic phospholipids, but not in animal cell membranes rich in neutral phospholipids and cholesterol (Lay and Gallo, 2009). In this regard, the cationic peptides are attracted to negatively charged molecules such as anionic phospholipids and lipopolysaccharides (LPS) present in gram-negative and techoic acid in gram positive bacteria, which are located asymmetrically in the membrane architecture (Lay and Gallo, 2009). The positively charged residues can also interact with membrane lipids through specific receptors at the surface of the cell (Zasloff, 2002; Barbosa-Pelegrini et al., 2011). Peptide binding to the membrane can disturb integrity and activate pathways that cause cell death (Barbosa-Pelegrini et al., 2011).

Since AMPs are active between the nanomolar and micromolar range against a broad spectrum of bacteria, fungi and protozoa, the possibility to use them as therapeutic compounds have been intensively explored (Korbila and Falagas, 2008; Zhang and Falla, 2009; Brinch et al., 2009). However, such application has been hindered by different (and not yet solved) problems, such as the technical challenge of producing them at the scale and purity required for a pharmaceutical product, their ability to stimulate the immune system, and their unknown direct toxicity on mammalian cell membranes. Currently only the Novozyme Company has developed a bactericidal product based on the defensin plectasin, a peptide produced by the fungus *Pseudoplectania nigrella* that shows good activity against a broad spectrum of gram-positive bacteria and low cytotoxicity against mammalian cells. The mechanism of action of plectasin involves the binding to the bacterial cell wall precursor lipid II (Brinch et al., 2009). Natural CAMPs or synthetically developed peptides have been tested in topic treatments during clinical studies (Zhang and Falla, 2009). Pexiganan, a magainin II homologue was the first that reached phase III trials after it was evaluated as an antibiotic cream for foot ulcers; unfortunately, its use was not approved in 1999 by the US Food and Drug Agency (FDA) because of their questionable efficacy (Oyston et al., 2009). The protegrin I homologue (Isoganan) was tested against oral mucositis but also fail the efficacy tests. Bloodstream infections arising from multidrug-resistant strains (MRS) are an increasingly alarming threat, especially in immunocompromised patients. Four promising peptides are available for intravenous administration only and are currently under investigation. Dalbavancin, a novel semisynthetic lipoglycopeptide that inhibits cell wall synthesis is especially active against MRS, and is undergoing phase III clinical trials for skin and soft tissue and catheter-related bloodstream infections (Bayley and Summers, 2008). In addition, telavancin and oritavancin, both closely related with dalbavancin and human lactoferrin I-II, are being tested in patients infected with *Candida* sp. (Korbila and Falagas, 2008).

To date, most clinical trials have focused on the topical use of peptides, as the oral and intravenous administration routes that pose two orders of challenges; the limited stability of the molecules inside the host (where are exposed to degradation by proteases), and the still unknown toxicity. Possible side effects could manifest as direct and immediate cellular damage or as a delayed effect on the immune response. These issues, currently under extensive investigation, are the main causes of the delay of the AMP availability for clinical use, in addition to the possibility that pathogens may develop resistance against AMPs concerns (Bell and Gouyon, 2003).

ANTIMICROBIAL PEPTIDES IN CHILI PEPPER

Chili peppers are important ingredients in cuisine as spices, but have clearly nutritional and medicinal properties as well. The ancient amerindians recognized these properties and used them therapeutically. Ethnobotanical data suggest that *Capsicum* species harbor many potentially economically significant compounds yet to be discovered (Cichewicz and Thorpe, 1996). It remains unclear exactly which properties led ancient mesoamericans to include *Capsicum* species in their pharmacopeia and keep them in use by traditional cultures (Alcorn, 1984; Cichewicz and Thorpe, 1996), but it may have been in response to their therapeutic properties as antimicrobial and anti-hemolytic agents. The presence of the secondary metabolite capsaicin in these species has long been associated with strong analgesic properties (Cordell and Araujo, 1993), alterations in the pH of gastrointestinal tract epithelial cells, prevention of microbial infections (Tellez et al., 1993) and possible anticarcinogenic effects (Surh and Lee, 1995). However, recent studies have shown that chili species also contain peptides with strong antimicrobial activity and that these peptides are encoded in the chili genome.

Plant diseases caused by bacteria such as *Ralstonia solanacearum*, *Clavibacter michiganensis, Xanthomonas campestris* pv vesicatoria, as well as fungal pathogens, represent important agricultural constraints in tropical and subtropical regions (that is, pepper wilting caused by *Phytophthora capsici*; *Fusarium oxysporum* or *Pythium*;

and bacterial spot, caused by *X. campestris* pv *vesicatoria*) for some solanaceous species, such as bell peppers, do not represent serious agricultural concerns to chili peppers as the species posses natural antimicrobial compounds to prevent infections by these pathogens (Kimati et al., 1997). Bio-prospection of antimicrobial and antifungal activity in chili pepper plants have shown that some of the activity is associated with the presence of cationic and anionic peptides contained in leaves, fruits and seeds. Texeira et al. (2006) described the antimicrobial activity of pepper peptide-enriched fractions against the plant pathogenic bacteria *R. solanacearum* and *C. michiganensis* sp. *michiganensis*. Such fractions were isolated from pepper leaves and contained cationic and anionic peptides. Pepper leaves represent a good source of cationic and anionic peptides to potentially develop plant protection agents; these organs are generally discarded after fruits are harvested. Recently, it has been shown that in chili pepper AMPs are expressed in several of their organs (that is, seeds, roots, fruits and flowers), therefore chili plants may be integrally exploited.

Diz and collaborators (2006) isolated three peptide fractions from pepper seeds, coded F1, F2, and F3. Further characterization showed that F1 fraction was mainly composed of three peptides ranging from 6 to 10 kDa. N-terminal amino acid sequencing of the 10 kDa peptide showed high homology to lipid transfer proteins (LTPs). Fraction F1 also showed fungicidal activity against *Candida albicans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and promoted morphological changes to *C. albicans*, which includes formation of pseudohyphae. F1 fraction caused the permeabilization of yeast plasma membrane to the dye SYTOX Green. In a further study, the same group purified an LTP protein from fraction F1, called *Ca*-LTP$_1$, and demonstrated that it was responsible for the strong antifungal activity through plasma membrane permeabilization in *Candida tropicalis* and *Colletotrichum lindemunthianum*. *Ca*-LTP1 has a dual localization inside seed cells; it is located within dense vesicles, but it may also be a secreted protein when seeds are involved in the imbibitions and germination events (Diz et al., 2011).

In another study carried out by Brito-Argáez et al. (2009), from Habanero chili pepper seeds they purified one peptidic fraction nominated G10P1.7.57 which was composed of several peptides, but the most abundant were of 5.6 and 7.5 kDa. The fraction showed a strong activity against several human and plant pathogenic bacteria, using *in vitro* assays (Figure 1). N-terminal sequencing of the 5.6 and 7.5 kDa peptides did not show a clear association with previously described AMPs; however, they were related with NAC and WRKY transcription factors, both involved in direct regulation of the plant defense response against pathogen attack. In addition, the 7.5 kDa peptide showed low homology with a 3-oxo-acyl1-carrier protein synthase from *Capsicum chinense* Jacq (Brito-Argáez et al., 2009). Although both peptides did not show a relationship with the previously described AMPs, here we showed that these two peptides have strong antimicrobial activity (Brito-Argáez et al., 2009).

The present results confirm the potential of chilies as a source of AMPs, but highlight the importance of the habanero chili pepper seeds as a source of AMPs, which may have pharmaceutical and agricultural applications. Furthermore, experimental findings clearly showed that habanero pepper seeds could be used in alternative processes beside plant production.

THE ANTIMICROBIAL PEPTIDES ARE ENCODED IN THE PLANT GENOME

It is well accepted that AMPs are encoded in the genome of plant cells and several databases containing the cDNA and DNA sequences of AMPs are available (Hammami et al., 2008; Wang et al., 2011). Moreover, a database has been developed to predict AMPs from genomes based on sequence alignment and feature selection methods (Wang et al., 2011). In the case of chili pepper, Meyer and collaborators (1996) described the cloning of a defensin from *Capsicum annuum*. In our group, using the primers (DEF1 5´-gatatgatggcgaggcaaag-3´ and DEF2 5´-agagttaattaagcacagggcttc-3´), reported by Meyer et al. (1996), and testing its amplification by DNA isolated from *C. chinense* leaves by using PCR, we were able to amplified a 176 pb DNA fragment, which after being cloned and sequenced showed a high homology with defensins, where the highest similarity was of 99% with the defensin previously cloned from *C. annuum* (accession number NCBI X95730) and 93% with the gamma thionin (accession number NCBI X 95363; Figure 2B; Table 1). The *C. chinense* defensin was named Def-fito1 and kept on deposit in the NCBI (accession number EU 2399549). Comparison of the *in silico* amino acid deduced sequence of Def-fito1 with amino acid sequences described for defensins from different plants showed the highest similarity (37%) with defensins from *Triticum aestivum* (NCBI AB089942) and 35% with *Arachis diogoi* (NCBI AY288448). The lowest similarity (6.9%) was with defensin from *Nicotiana benthamiana* (NCBI EU076714.1; Figure 3). Southern blotting used as probe for the Digoxigenin-labelled-defensin clone in our group showed that it hybridizes with at least two DNA fragments in *C. chinense* (Figure 4). Comparison of DEF-Fito1 with a defensin previously cloned from placental tissues of *C. chinense* fruit (NCBI 128239) showed that they were different (Figure 5), thus suggesting that in the genome of *C. chinense* there are at least two genes encoding defensin proteins.

The defensin cloned from placental tissues of *C. chinense* (Aluru et al., 1999), was heterologously expressed in bovine cells increasing the resistance of bovine

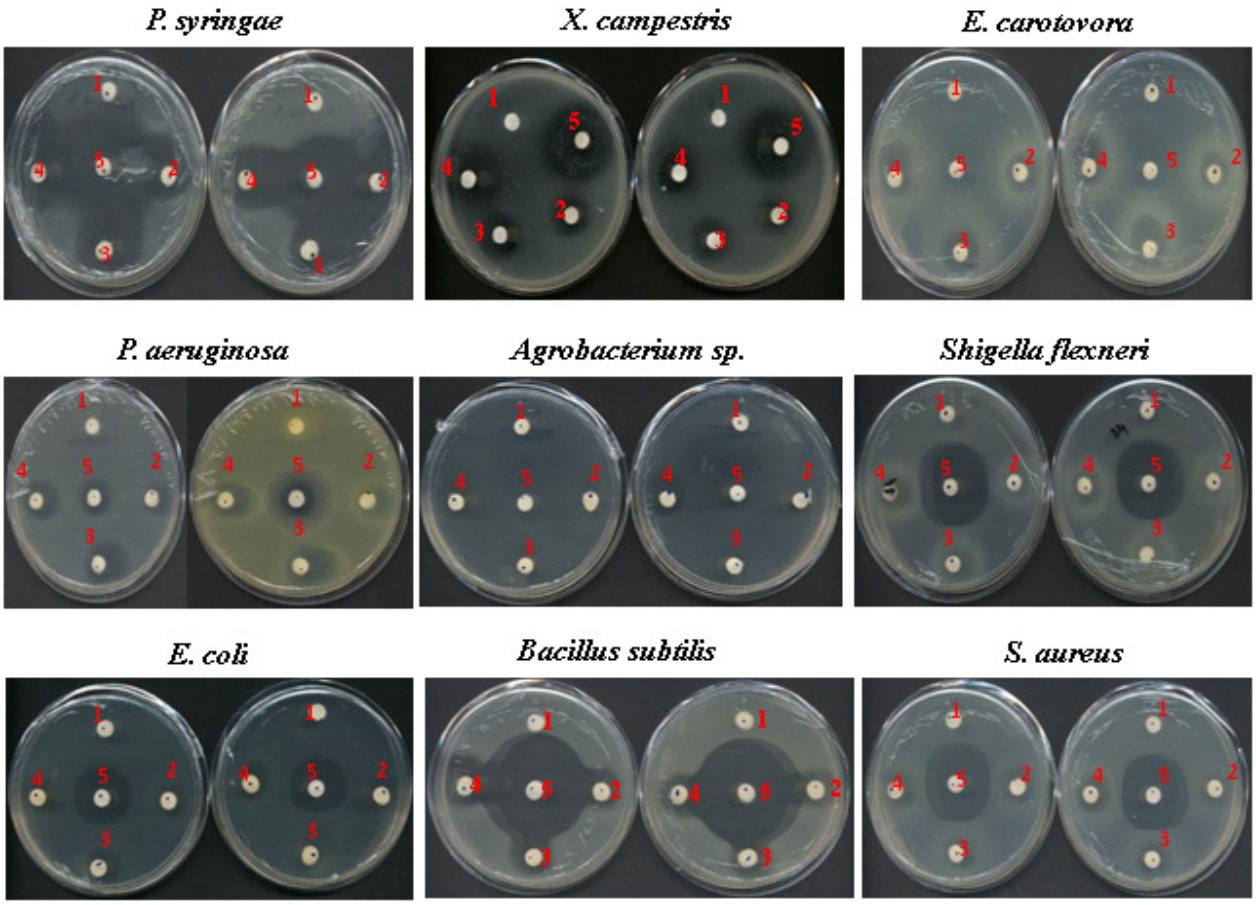

Figure 1. Antibacterial effect of protein fraction from seeds of *Capsicum chinense* Jacq. Protein (12.5 µg/µL) from the G10P1.7.57 fraction inhibited the *in vitro* growth of *P. syringae*, *X. campestris*, *E. carotovora*, *P. aeruginosa*, *Agrobacterium* sp., *Shigella flexneri*, *Escherichia coli*, *Bacillus subtilis* and *Staphyllococcus aureus*. Buffer of protein resuspention, as negative control of inhibition,1. Different concentrations of *C. chinense* G10P1.7.57; 5 µg; 2, 10 µg; 3, 20 µg; 4, 40 µg of ampicillin; 5, positive control.

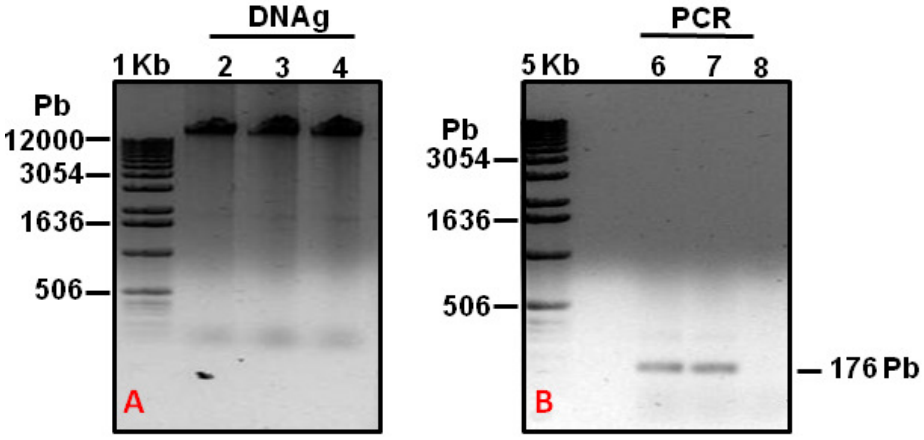

Figure 2. High molecular DNAg extracted from *Capsicum chinense* leaves and PCR amplification of the Def-fito1 defensin. Genomic DNA (15 µg) was electrophoresed in 1% agarose gels and then ethidium bromide stained to check the DNA integrity. A, Genomic DNA (25 pg) was used in PCR reaction with oligonucleotides DEF1gatatgatggcggaggcaaag and DEF2 agagttaattaagcacagggcttc to amplify the defensin Def-fito1 which has a size of 176 pb. 1Kb DNA ladder; Lane 1, DNA from *C. chinense* leaves; Lanes 2, 3 and 4; B, product with expected size for Def-fito1; Lanes 6 and 7, PCR reaction that lacks of DNA template was used as negtive control, 8.

Table 1. List of accession numbers.

Species	Nucleotide sequence	*In silico* amino acid translation	Size (bp)	Similarity (%)	NCBI accession number
Capsicum annuum defensin gene	Not shown	Not shown	5461	99	X95730
Capsicum annuum gamma thionin gene	Not shown	Not shown	1390	93	X95363
Capsicum chinense Jacq. Defensin gene, this study	5-CCTTAGTTA ATTAAGCACAGGGCTTCCT GCAGAAGCATTTAAGAGG AAATCCGCGGCAAACGCC ACTGGTAAATCCCTCTCTA CGGCAAACATTACCACAAT CGCGGCTACTAAGGCACA ACCCCTTGAAGTTGCCGCT CAACGCCTCGCAGATCTTT GCTCCGCCATCATATCA-3	5-L S N L S T G L P A E A F K R K S A A N A T G K S L S T A N I T T I A A T K A Q P L E V A A Q R L A D L C L R H H I-3	176	99 and 93% with *C. annuum* defensin and gamma thionin genes, respectively	EU239954

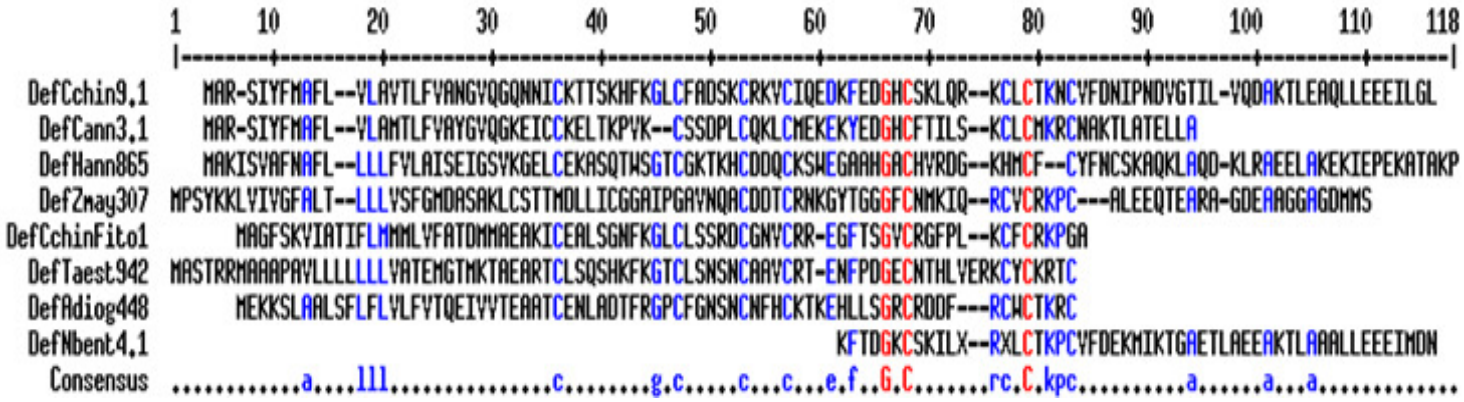

Figure 3. Deduced amino acid sequence of DefFito1 and its alignment and comparison with defensin amino acid sequences deposited on the data bank of National Center for Biotechnology (NCBI). Sequences were aligned using the bioinformatic tool "Multiple sequence alignment with hierarchical clustering". http://multalin.toulouse.inra.fr/multalin/multalin.html.

epithelial cells against *C. albicans* infection (Anaya-López et al., 2006), showing that defensin confers resistance against pathogens that classically affects bovine. Heterologous expression of plant defensin could be used as a strategy to confer protection to the receptor organisms against some common infectious agents. In the case of *C. annuum* defensin CADEF1, it is well established that defensin transcript is up-

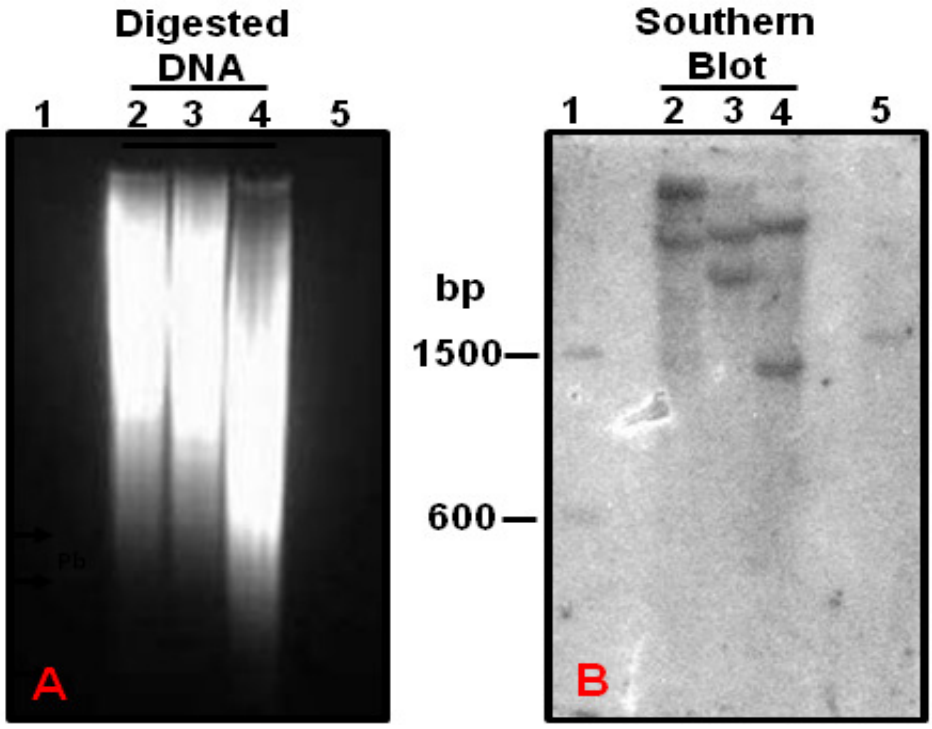

Figure 4. Enzymatic digestion of DNA from *Capsicum chinense* leaves and Southern blot with probe DIG-labelled Def-fito1. DNA of *C. chinense* (50 µg on each lane) digested with Hind III; 2, Xba I; 3, and a mixture of Hind III/ Xba I; 4, and revealed with ethidium bromide on 0.8% agarose gel. A or hibridyzed with the Dig-UTP-Def-fito1 and revealed with CDPstar chemiluminicent reagent B. 100 bp size marker (1), 50 ng of DNA from plasmid containing the 176 pb Def-fito1 (5).

regulated upon bacterial attack, abiotic elicitors and environmental stresses; unfortunately, the complete transcript of the native protein have been elusive to be cloned or purified from chili pepper (Li and Li, 2009). In a recent study using a cDNA library constructed from ripening *C. annuum* fruits, it was described that the isolation of full length cDNA of 534 bp that encodes a defensin denominated *CDef1.* Such defensin is expressed just in the fruit mesocarp at the onset of fruit ripening and continues thereafter (Maarof et al., 2011). In this study, the expression of *CDef1* and presence of CDef1 in ripening fruits was associated with a defensive antimicrobial role because as fruits ripe, their structural macromolecules (sugars, proteins, lipids) are hydrolyzed, thus being more vulnerable to pathogen or microbe infection. It is therefore postulated that the increase in defensin content counteract pathogen or microbial attack and make *C. annuum* fleshy fruits attractive for consumers that assist in seed dispersal (Maarof et al., 2011).

TRANSGENIC PLANTS EXPRESSING CHILI PEPPER ANTIMICROBIAL PEPTIDES AS AN ALTERNATIVE TO FIGHT PLANT PATHOGENS

Transgenic expression of AMPs seems to be an adequate strategy to protect their recipient hosts (animals or plants). In the case of animals, strong evidence supporting this hypothesis comes from the expression of *C. chinense* defensin in the epithelial tissues of bovine and whose expression protected the tissues against *C. albicans* (Anaya-López et al., 2006). Li and Li (2009) also obtained additional evidence with transcript of defensine CDef1 from *C. annuum*. The full length cDNA of *CDef1* was expressed in *Escherichia coli* by using the expression vector pET28a. Western blot of the recombinant CDef1 revealed a 5.6 kDa protein. Total amount of recombinant protein accounted for 15% of total bacterial protein. The recombinant protein showed antifungal activity against *Verticilium dahliae* on *in vitro* assays, showing an inhibition average of 68%. The result

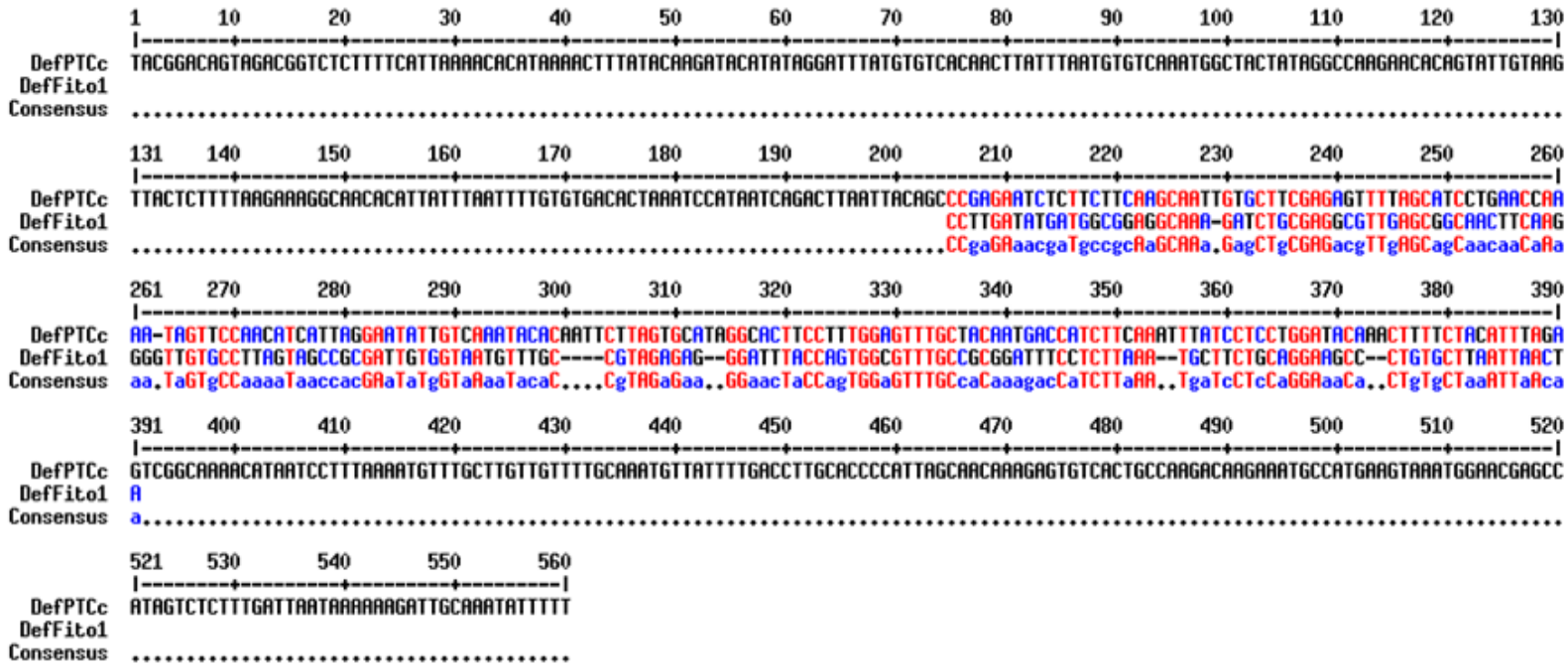

Figure 5. Alignment of nucleotides of defensins cloned from *Capsicum chinense* Jacq. Sequence from placental tissues (DefPTCc; Access No. AFI128239.1) or leaves (DefFito1; Access No. EU2399549).

suggests that activity of CDef1 might benefit by preventing fungus-related diseases in agricultural production (Li and Li, 2009). In an additional study, Zainal et al. (2009) generated transgenic tomatoes carrying *C. annuum* defensin gene *Cdef1* which encodes a 5 kDa peptide. These transgenic plants were more resistant against *Fusarium* sp. and *Phytophthora infestans* infections than the untransformed plants. Immunocytological analysis revealed that total protein extracts from the transgenic plants contained the peptide corresponding to *C. annuum* defensin and such extracts were able to inhibit *in vitro* the growth of *Collectotrichum gloesporioides*, *P. infestans*, *Fusarium* sp. and *Curvularia* sp. Interestingly, the *Cdef1* showed a mendelian inheri-tance (3:1) after transgenic plants were self-fertilized and seeds of the subsequent generation (segregants) analyzed and selected with genta-mycin. Analyses of the seed-obtained transgenic tomatoes acquire higher resistance against *P. infestans* and *Fusarium* sp than wild plants (Zainal et al., 2009).

A novel antimicrobial protein gene, CaAMP1 from *C. annuum*, was isolated from pepper (*C. annuum*) leaves infected with *X. campestris* pv *vesicatoria*. Expression of the recombinant CaAMP1:smGFP fusion protein in *Allium cepa* showed that it was localized mainly in the external and intercellular regions of onion epidermal cells. Over expression of CaAMP1 in *Arabidopsis thaliana* conferred broad-spectrum resistance to hemibiotrophic bacterial pathogen *Pseudomonas syringae* pv tomato, biotrophic oomycete *Hyaloperonospora parasitica* and fungal necrotrophic pathogens *Fusarium oxysporum* f. sp. *matthiolae* and *Alternaria brassicicola*. CaAMP1 over expression induced the salicylic acid pathway-dependent genes PR1 and PR5, showing that the antimicrobial peptide CaAMP1 is involved in a broad-spectrum resis-tance from bacterial to fungal pathogen infection (Lee et al., 2008). Taken together, these results show that plant transformation with vectors carrying genes encoding AMPs from chili pepper has potential to

protect different crops against the important phyto-pathogens. Such strategy might increase their resistance against common bacterial or fungal infections.

PERSPECTIVES

In most parts of the world, the basic role of agriculture is associated with the production of food and the main goal of this human activity is resumed by the simple phrase: produce more and produce it cheaper. However, in some places the scenario is a little more complicated from a political and economical point of view. The world can be divided in two geographic areas, the Northern hemisphere [mostly Organisation for Economic Co-operation and Development (OECD) countries], where the price of food is not a critical factor, but the quality is very important and the Southern part of the world, where quantity and price are the most important factors and they are interconnected (Twardowski, 2010). Today, the future of agriculture is clearly connected with the production of food using traditionally implemented methodologies or produced by biotechnologically manipulated organisms. Obviously the last way brings up the subject of genetically modified organisms (GMOs), goods which are not familiar today. Thus, we have to decide, if we need GMOs and if we need it for direct consumption as food or to feed animals that we can further eat. It seems true that there is no way to avoid genetic engineering to produce foods as it seems to be the key to support the demand of enough foods in the future (Twardowski, 2010).

Before we decide to use GMOs for food production, there are some alternatives that we must explore intensively. Coordinated efforts are needed to increase the production of food, but with a view to enhanced food quality and safety as well as to control residues of persistent pesticides in the environment (Dey, 2010). Natural compounds that are a rich source of defense agents against plant pathogens can be used. Many of them present low toxicity to humans and animals, low environmental impact, low residues in food and compatibility with integrated pest management programs (Castro and Fontes, 2005). AMPs are important components of innate defense of insects, amphibians, plants and mammals (Hancock and Lehrer, 1998). Biotechnologically, application of peptides produced by plants offers exciting pharmaceutical possibilities as topical antimicrobial agents for animals and humans. They differ structurally from conventional antibiotics produced by micro-organisms and acts mainly in the pathogen membrane, both characteristics makes that AMPs have a very low risk to induce resistance in pathogens (Heinemann et al., 2000). In agribusiness, research with AMPs are intended to develop plant protection compounds as they generally exhibit a broad range of activity against bacteria, fungi and protozoa, with the disruption of the membrane integrity, at low concentrations (Hancock and Lehrer, 1998; Castro and Fontes, 2005; Brito-Argáez et al., 2009).

Chili peppers are highly demanded and consumed around the world; their secondary metabolites (capsaicinoids) have been largely associated with hot taste but also with antimicrobial and antifungal properties. The former property seems widely exploited in the earlier times by man. It is though that man consumed chilies to prevent food intoxications by food-contaminant pathogenic microorganisms (Tewksbury et al., 2008). However, in recent decades it was demonstrated that chili plant also contains antimicrobial agents that were unrelated to capsaicinoids. These new compounds are encoded in chili pepper genome and show strong antimicrobial activity *in vivo* and *in vitro*.

Present studies regarding the antifungal activities of chili defensin is consistent with previous suggestions that chili plant defensin participates in host defense responses against pathogens, since as after CaDEF1 defensin was recovered from recombinant *E. coli*, the CaDEF1 defensin inhibited *in vitro* growth of *Verticilium dahliae* (Terras et al., 1995; Gao et al., 2000; Li and Li, 2009), in transgenic tomato plants expressing the chili defensin it acquired a better protection against some fungal pathogens. Additionally, Meyer et al. (1996) also showed that purified chilli fruit-specific defensin was effective in suppressing growth of the fungi *Fusarium oxysporum* and *Botrytis cinerea*. Thus antimicrobial chili pepper peptides have the potential to save the constant losses caused by pathogens in foods.

Finally, conclusive evidence for the involvement of chili defensins in the plant defense response could be obtained by inactivating these genes or expressing different transgenes under a single promoter sequence (Lee et al., 2008; Zainal et al., 2009; Maarof et al., 2011). Certainly, recent developments of effective genetic transformation methods and the tools implemented to transform plants is now feasible to test the importance of these genes through antisense or gene targeting strategies (Narin et al., 2011). On the contrary, over expression by up-regulation of endogenous antimicrobial peptides encoding genes may be a strategy to help plants to fight their natural pathogens. As regards the recently described *C. chinense* Jacq., defensin genes supports the previously described presence of AMPs in chili species and also supports our suggestion to use the species as a natural source of AMPs (Anaya-Lopez et al., 2006). The suggestion is because of the all plant biomass in which only the fruits are consumed, but it represents ~0.01% of the total biomass of chili plant, thus the rest of the plant tissues are discarded as garbage, because no alternative application for these tissues has been found, yet. It will be interesting to scale the AMPs extraction from discarded leaves and tissues in order to evaluate the antimicrobial yield.

In conclusion, the use of *C. chinense* seeds as a

source of antimicrobial is supported by the positive growth inhibition of the common plant and human pathogenic bacteria in the G10P1 fraction. The peptides in the G10P1 fraction may also have potential economic advantages over commercial synthetic agrochemicals and medicines since its extractions from pepper seeds is far less expensive than chemical synthesis usually required to manufacture artificial compounds (Zasloff, 2002).

The results also underline the potential commercial applications of *C. chinense* pepper peptides, as plant defense against two bacterial groups that differ in the cell coat composition; as AMPs probably develop their activity in the microbial cell surface. These compounds could represent a new strategy in biotechnology development, when peptides might be used as natural molecules able to neutralize and or inhibit plant pathogens without inducing resistance (Heinemann et al., 2000). Synthetic peptides could be produced or defensives could still be formulated by using the crude-enriched peptide extracts for application in agribusiness.

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