



Proteomic profile of *Piper tuberculatum* (Piperaceae)

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Abstract

Piper tuberculatum (Piperaceae) is a species that accumulates especially amides as secondary metabolites and several biological activities was previously reported. In this article, we report a proteomic study of *P. tuberculatum*. Bidimensional electrophoresis (2D SDS-PAGE) and mass spectrometry (ESI-Q-TOF) were used in this study. Over a hundred spots and various peptides were identified in this species and the putative functions of these peptides related to defense mechanism as biotic and abiotic stress were assigned. The information presented extend the range of molecular information of *P. tuberculatum*.

Keywords: Piperaceae, amidas, *Piper tuberculatum*, proteomics, 2D-SDS-PAGE.

Perfil proteômico e metabólico de *Piper tuberculatum* (Piperaceae)

Resumo

Piper tuberculatum (Piperaceae) é uma espécie que acumula especialmente amidas como metabólitos secundários e diversas atividades biológicas dessa espécie foram relatadas anteriormente. No presente artigo, relatamos um estudo proteômico dessa espécie. Eletroforese bidimensional (2D SDS-PAGE) e espectrometria de massas (ESI-Q-TOF) foram utilizadas nesse estudos. Mais de cem spots e vários peptídeos foram identificados nesta espécie e as funções putativas desses peptídeos relacionadas a mecanismo de defesa como estresse biótico e abiótico foram atribuídos. As informações apresentadas ampliam a gama de informações moleculares dessa espécie.

Palavras-chave: Piperaceae, amidas, *Piper tuberculatum*, proteômica, 2D SDS-PAGE.

1. Introduction

The field of plant proteomics has progressed significantly in recent years. With this has come a disproportionately larger growth in the amount of available information from databases when compared to that available for other organisms (Remmerie et al., 2011). That said, most of the data are derived from a small subset of economically important crops, such as rice, wheat and grapes, as well as from *Arabidopsis thaliana*. While the latter's proteome is reasonably well characterized, the number of proteins identified remains small considering over 27.000 protein-coding genes are predicted from its genome data (Baerenfaller et al., 2008; Giavalisco et al., 2005). A comprehensive study carried out

on *A. thaliana* organs resulted in a proteome map of roots, young leaves, cotyledons, seed pod, flowers, buds, flowers carpels and undifferentiated cells. So far, 13.029 proteins, which represent about 50% out of the predicted genes, have been identified. This data set has helped to isolate specific biomarkers for each organ, which in turn has allowed the correlation between the level of transcription, protein accumulation and compartmentalization of metabolic pathways (Baginsky et al., 2005; Bussell et al., 2013).

Current approaches, such as, genomics, transcriptomics, proteomics and metabolomics, that focus on systems biology are providing new data about the genes and enzymes

involved in several metabolic pathways. These global studies, however, are still scarce for tropical and wild plants, which represents 2/3 of the total of World's green plants and are one of the most important, still largely untapped, repository of chemical structures important for pharmaceutical and cosmetic applications, as well as, for example, forestry and agronomy (Balbuena et al., 2011). *Piper nigrum* (black pepper), considered the "King of Spices" because of its economic importance and its ubiquitous culinary presence, had its transcriptome data from leaves and roots recently described (Gordo et al., 2012; Joy et al., 2013).

The Piperaceae family is placed amongst primitive Angiosperms and comprises more than 4000 species in tropical and subtropical regions with five genera: *Peperomia* Ruiz and Pavon (about 1700 species), *Piper* L. (about 2000 species), *Manekia* ret. (formerly called *Sarcorhachis*), *Verhuellia* and *Zippelia* (Wanke et al., 2007).

P. tuberculatum produces especially amides as secondary metabolites (Figure 1, compounds 1-5) and several of these compounds have displayed interesting biological properties, including antifungal, insecticidal, apoptotic cancer cell death and trypanocidal activities (Cotinguiba et al., 2009; Navickiene et al., 2000; Raj et al., 2011; Silva et al., 2002).

Protein fractions from leaves and fruits of *P. tuberculatum* were analyzed it aimed to supplement the existing proteomics information about tropical and non-model species. We characterized part of the proteome of both fruits and leaves of by 2D-SDS-PAGE and mass spectrometry (ESI-Q-TOF).

2. Methods

2.1. Plant material

Tissue samples (leaves and fruits) were harvested from adult specimens of *P. tuberculatum* in September, 2009. The plants were growing in field (21°48' 26.62" S 48°11' 33.51" W; 651 m) at the Institute of Chemistry at Araraquara (Universidade Estadual Paulista, UNESP). The specie was identified by Dr. Guillermo E. D. Paredes (Universidad Pedro Ruiz Gallo, Lambayeque, Peru) and

the voucher (Kato-163) were deposited at the Herbarium of the Institute of Biosciences, USP, São Paulo-SP, Brazil.

2.2. Protein extraction

The protein fractions were obtained according to Durst et al. (1996), with few modifications. The tissues (20 g) were ground to a fine powder in liquid nitrogen, and homogenized in 100 mL of extraction buffer (250 mM sucrose, 1 mM EDTA, 1 mM DTT, 40 mM sodium ascorbate, 10% glycerol and 4 g polyvinylpyrrolidone in 0.1 M PBS, pH 7.4), by shaking at 4 °C for 10 minutes. After centrifugation (10,000 × g for 15 minutes at 4 °C) the supernatant was separated and a solution 1 M of MgCl₂ was added to give a final concentration of 30 mM. This solution was stirred for 20 minutes at 4 °C and centrifuged at 40,000 × g. The pellet was dried and resuspended in 1 mL of 0.1 M PBS. This sample and the supernatant were used as protein sources after determination of protein concentration employing Bradford method (Bradford, 1976).

2.3. 2-DE

The 2-DE with immobilized pH gradients (IPG-Dalt) was carried out according to the supplier information (GE Healthcare®). The IPG strips (13 cm) were rehydrated for 16 hours with sample of proteins (5 µg of protein/µL) previously treated with the 2D Clean-Up Kit (GE Healthcare®) in an Immobiline DryStrip Reswelling Tray. The first dimension isoelectric focusing (IEF) was carried out in an Ettan IPGphor II Manifold applying a manufacturer's standard protocol with some modifications (strips were pre-focused at 500 V for 1 hour, 1,000 V for 1 hour and 8,000 V for 7 hours). The second dimension was performed on a vertical 12% polyacrylamide SDS gel (14 × 16 cm), in a SE 600 Ruby System, 45 mA/gel and 600 V. Proteins were stained using Coomassie Brilliant Blue G-250 after fixation with a 10% (v/v) acetic acid and 40% ethanol for 1 hour. The gels were scanned and analyzed with Image Master 5.0 software (Amersham Bioscience), in which the isoelectric point (pI) and an apparent molecular mass (M_r) for spots were assigned. Each spot was extracted with a scalpel, cut into segments of approximately 1 mm³

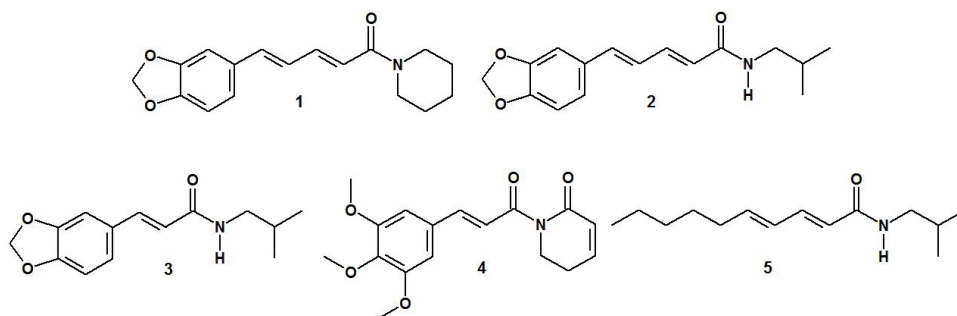


Figure 1. Antifungal metabolites isolated from *Piper tuberculatum*: piperine (1), piperlonguminine (2), fagaramide (3), (*E*)-piplartine (4) and pellitorine (5).

and stored in 1% acetic acid at 4 °C. The gel segments were washed thrice in a solution containing 50% (v/v) acetonitrile and 25 mM ammonium bicarbonate (AMBIC) for 30 minutes until the gel became colorless. Then, the samples were dehydrated twice with 100% acetonitrile for 10 minutes. The acetonitrile was removed and the remaining residue was evaporated from the gel at room temperature. The gel fragments were rehydrated and reduced by dithiothreitol (DTT) (20 mM DTT/AMBIC 50 mM) at 56 °C and alkylated for 40 minutes with iodoacetamide (IAA) (55 mM IAA/AMBIC 50 mM) in darkness for 30 minutes. The iodoacetamide was removed and the fragments were washed with 25 mM AMBIC and then dehydrated in 100% acetonitrile. The acetonitrile was discarded and the residue remaining in the gel was allowed to evaporate at room temperature. Gel fragments were rehydrated with 15 µL solution containing 150 ng of trypsin in 25 mM AMBIC. After 15 minutes, 25 mM AMBIC was added to cover spots and was maintained at 37 °C for 14 hours. The reaction was stopped by adding 15 µL of blocking solution (50% v/v acetonitrile and 5% v/v formic acid). Peptides were eluted from acrylamide with three washes of 15 minutes with eluting solution (50% v/v acetonitrile, and 1% v/v formic acid) and two washes with 100% acetonitrile at 40 °C in an ultrasound bath. The solution containing the peptides was dried in a Speed Vac concentrator type (Thermo Scientific). After drying the peptides were resuspended in 12 µL of formic acid 1% (v/v) for further storage. Prior to MS/MS analysis, the mixtures were purified using ZipTips mini-columns (C₁₈) eluted with acetonitrile (mixed with TFA 0.1% in ultrapure water), 0.1% TFA in ultrapure water and methanol 5% (0.1% TFA in ultrapure water) (Celedon et al., 2007).

2.4. Mass spectrometry analysis

Peptide mixtures were analyzed by liquid chromatography using a Cap-LC coupled to a mass spectrometer Q-TOF Ultima API (Waters, England). Ten microliters of each sample were submitted to a pre-column (C₁₈, 5 µm, 5 X 30 mm, Waters®) for pre-concentration and desalting. Then the peptides were separated in a column (Symmetry C₁₈, 5 µm, 32 ×

150 mm, Waters®) and eluted using a linear gradient of 10-45% solvent B (95% acetonitrile, 0.1% FA in water) in Solvent A (5% acetonitrile and 0.1% FA in water) at a flow rate of 5 mL/minute for the first 15 minutes, changing to 2 mL/minute for the next 25 minutes, and finally to 5 mL/minute during the last 5 minutes. All MS analyzes were performed using the positive ion mode (voltage of 3 kV) between *m/z* 300 and 2000, and the spectra of MS/MS were acquired for the most intense peaks, with at least 15 points (Celedon et al., 2007).

2.5. Data processing

MS/MS-ion database searches were carried out by Mascot on line in the web. Peptides were searched in the Viridiplanteae ESTs from the NCBI (tblastn) and the resulting nucleotide sequences were annotated using the

NCBI data base (blastx). Search parameters used were: Database: NCBI; Enzyme: Trypsin; Allow up to: 1 missed cleavage; Quantification: none; Taxonomy: Viridiplanteae (Green plants); Fixed Modifications: Carbamidomethyl (C); Variable Modifications: Oxidation (M); Peptide Tolerance: 50 ppm; MS/MS tolerance: 0.6 Da; Peptide charge: +2 +3 +4; Data format: PKL; Instrument: ESI-Q-TOF. For each combination of peptides, the Mascot provides an index of protein, termed protein score, $[-10 * \log(P)]$, where P is the probability that the set of identified peptides is observed in a random event. Protein score values above 60 were considered significant ($p < 0.05$) and the higher these values, were considered as reliable. The values of relative molecular mass (M_r) and isoelectric points (pI) were also provided and these values were compared to those obtained experimentally. If the amount of protein for identity score protein is highly reliable, but the masses are not equal, it can be resulting from a post-translational modification or cleavage event. The total measured ion score provides information on how well the spectrum MS/MS provides the identity of the combination of peptides. The confidence limit for total ion score is the same as for the protein score. The value indicates the probability that the observed set between MS/MS spectra and sequence peptide results from the same protein/peptide. The identity of the peptide (ID) number is given by *gi* (genInfo identifier) which is a unique number that identifies a full and particular exact sequence. The coverage (coverage protein) indicates the percentage of residues in each protein sequence that was identified when compared to the peptide bank (Table 1). Peptides with “no identity” are those with no similarity to any other peptide sequences in the NCBI database.

3. Results

The 2-DE gels from *P. tuberculatum* proteins yielded more than 300 proteins spots. It was possible to remove from the gel only some of them. These spots were analyzed by MS (ESI-Q-TOF) and several proteins/enzymes involved in primary and secondary metabolism and other events were characterized (Table 1). Both gels were visually inspected, and spots in the range of 14-90 kDa were observed. The amount of spots in the leaf extract for both species was higher than in the fruits, accounted by the high concentration of enzymes involved in basal metabolism as fixation of carbon dioxide in leaves.

The proteins characterized in each organ of each species were classified into six main biological processes, as described by Rison et al. (2000) and modified by Celedon et al. (2007). The analysis reveals that in *P. tuberculatum* leaves, the *Metabolism and Energy* category were the most abundant (39%) follow by *Cellular Processes* (21%). The 50% remaining are involved in *Protein Transport and Signaling* (3%), *Structure and Organization* (11%), *Information Pathway* (11%), *Miscellaneous* (4%) and *No Identity* when confronted to the database (7%). Regarding *P. tuberculatum* fruits, the proteins were characterized as belonging to *Metabolic Processes* (27%); *Cellular Processes*

Table 1. Peptides sequences of *P. tuberculatum*: number of the spots, protein score, total ion score, peptide sequence, number of entry in the database (NCBI), the identity of the protein, species, relative molecular mass (Mr, kDa) and isoelectric point (pI), (literature), relative molecular mass (Mr, kDa) and isoelectric point (pI) (experimental), coverage (%), access (UniProt) and Biological Process.

Spot	Protein Score	Total Ion Score	Peptide Sequences	ID (NCBI)	Proteins	Species	Mr (kDa) and pI (lit.)	Mr (kDa) and pI (exp.)	Coverage (%)	Access	Biological Process
<i>P. tuberculatum (leaves)</i>											
6	63	63	GGLDFTKDDENVNSQPMPR	gi 15148424	RuBisCO	<i>Cardiandra alternifolia</i>	52416/6.08	71480/6.32	6	Q00NY7	1.1.1.2
7	58	58	DTDILAAFR	gi 8096541	RuBisCO (large subunit)	<i>Solanum lycopersicum</i>	53398/6.55	54825/3.89	6	Q00NY7	1.1.1.2
8	177	117	DITLGFVLLRDDDFEQDR	gi 169563	RuBisCO (large subunit)	<i>Solanum tuberosum</i>	53371/6.19	51615/4.97	11	Q00NY7	1.1.1.2
11	111	79	VALEACVKAR	gi 169563	RuBisCO (large subunit)	<i>Solanum tuberosum</i>	53371/6.19	52677/6.65	11	Q00NY7	1.1.1.2
12B	141	78	VALEACVKAR	gi 349144	RuBisCO (large subunit)	<i>Solanum lycopersicum</i>	53398/6.55	52744/6.92	12	Q00NY7	1.1.1.2
13	55	55	VALEACVKAR	gi 1352794	RuBisCO (large subunit)	<i>Harpagophytum grandifolium</i>	52139/6.23	46612/5.33	6	Q00NY7	1.1.1.2
17	279	96	IVDSFPGQSIDFFGALR	gi 10720247	RuBisCO (large subunit)	<i>Oryza sativa</i>	50897/8.61	43219/5.74	12	Q00NY7	1.1.1.2
18	131	105	IVDSFPGQSIDFFGALR	gi 10720247	RuBisCO (large subunit)	<i>Oryza sativa</i>	48055/5.85	43226/5.91	6	Q00NY7	1.1.1.2
9	341	124	IFNVLGEVPVDNLGPVDTR	gi 52082860	ATPase (subunit β)	<i>Piper hispidum</i>	50620/5.04	51980/5.26	20	P11574	1.1.3
30	193	124	VAVLGAAGGIGQPLALLMIK	gi 126896	malate dehydrogenase	<i>Citrullus lanatus</i>	36406/8.88	36711/7.40	10	P17606	1.1.3
29	60	60	ILFIGGTGYIGK	gi 1708422	isoflavone reductase	<i>Solanum tuberosum</i>	33831/6.16	32566/5.92	3	Q00016	1.2.8
14	191	135	AAQDIALAELAPTHPIR	gi 1345588	Protein 14-3-3-like	<i>Saccharum (hybrid cultivar CP65-357)</i>	28978/4.79	46447/5.52	22	Q96299	2.1.2
37	185	135	AAQDIALAELAPTHPIR	gi 1345588	Protein 14-3-3-like	<i>Sugar cane</i>	28978/4.79	28619/5.81	16	Q96299	2.1.2
38	50	50	ADVDNKESVGK	gi 1345588	RNA polymerase IV (second large unit)	<i>Rhododendron macrophyllum</i>	116144/8.98	27455/5.71	1	Q9LK40	2.1.2
27	39	39	ELGIGIVPYSPIGR	gi 78708648	Protein induction auxin	<i>Oryza sativa</i>	37905/5.81	37698/5.76	4	Q9ZTX8	2.2.3
28	152	72	SGPLQPGVDIIEGPKV	gi 132270	Rubber elongation factor	<i>Hevea brasiliensis</i>	14713/5.04	36441/6.10	42	P15252	2.2.3

Table 1. Continued...

Spot	Protein Score	Total Ion Score	Peptide Sequences	ID (NCBI)	Proteins	Species	Mr (kDa) and pI (lit.)	Mr (kDa) and pI (exp.)	Coverage (%)	Access	Biological Process
46	82	58	SGPLQPGVDIIIEGPKV	gi 132270	Rubber elongation factor	<i>Hevea brasiliensis</i>	14713/5.04	18613/	19	P15252	2.2.3
4	41	41	VIDEIRER	gi 3763925	Ligation protein/clathrin	<i>Arabidopsis thaliana</i>	30781/9.30	60320/5.1	2	Q8Y07	3.3
3	224	111	SLDIERPTYTNLR	gi 6094431	α -3-tubulin	<i>Hordeum vulgare</i>	50193/4.95	62256/4.90	11	P08841	4.3
45	54	54	IWHHTFYNELR	gi 3219759	Actin	<i>Glycine max</i>	41796/5.23	21742/5.32	2	Q96292	4.3
44	45	45	LSGEVDANKAEVK	gi 159487461	Protein 2 - chromosome structural maintenance	<i>Chlamydomonas reinhardtii</i>	128165/6.66	23389/4.92	1	Q9C5Y4	4.5
47	222	119	LPTDETLVAQIK	gi 162458009	Factor 5A - Initiation of eukaryotic translation	<i>Zea mays</i>	17714/5.61	19100/4.63	34	Q76E23	5.3.3
1	97	45	AVVTVPAYFENDSQR	gi 24638460	heat shock protein 70	<i>Cucumis sativus</i>	75480/5.15	76250/4.81	4	P22953	5.3.5
5	61	61	GVEELADAVR		GroEL-like chaperone	<i>Vitis vinifera</i>	61673/5.85	61293/5.52	1	Q8L7B5	5.3.5
10	441	130	KIEDAVDSAISWLDNSQLAEVDEFEDK	gi 242054931	Hypothetical protein SORBIDRAFT_03g039360	<i>Sorghum bicolor</i>	71261/5.10	52456/6.15	27		6.2
<i>P. tuberculatum</i> (fruits)											
S7	137	83	ELAAQPDVDGFLVGGASLKPFIIDINAATVK	gi 195605636	Triose phosphate isomerase	<i>Zea mays</i>	27278/5.53	35125/5.32	28%	P48491	1.1.2
S2	190	91	TVLIMELINNIK	gi 6687280	ATP synthase (β subunit)	<i>Codonopsis pilosula</i>	53628/5.07	57186/5.10	15%	P11574	1.1.3
S4	46	46	LKLELAQFAELQAFASALDK	gi 51556908	ATP synthase (α subunit)	<i>Elymus sibiricus</i>	55549/6.03	55566/6.12	4%	P11574	1.1.3
S6	404	148	DNLTLTWTSIDSEDPAAEIR	gi 195635799	Protein 14-3-3-like (GF14-6)	<i>Zea mays</i>	30985/4.84	42255/7.82	20%	Q96299	2.1.2
S9	205	114	SSITPGTVLILLAGR	gi 195605226	60S ribosomal protein L6	<i>Zea mays</i>	24273/10.14	30158/5.42	11%	Q9C9C5	4.4.2
S10	127	127	AMVPVAEYNVPVVGHHAGITILPLFSQATPK	gi 224061310	Not identified	<i>Populus trichocarpa</i>	35704/8.53	35732/7.38	9%	-	6.3

(9%); *Structure and Organization* (9%) and *Miscellaneous* (9%), remaining 46% with no matches, classified as *No Identity*. Proteins from fruits gave no information about “information pathway” and “transport and signaling”.

4. Discussion

Proteins identified from *P. tuberculatum* were mostly related to the primary metabolism. The enzymes involved in the cycle of carbon fixation, such as carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) were detected only in leaves fractions but not in fruits in spite of the presence of photosynthetic pigments. RuBisCO, identified in the leaves, is an enzyme involved in the Calvin cycle which catalyzes the first major atmospheric carbon fixation steps. This multimeric enzyme is also the most abundant protein in the leaves and the most abundant protein on earth (Kim et al., 2013). Given its importance in the biosphere, there are currently efforts to genetically engineer crop plants with more efficient RuBisCO. In plants, algae, cyanobacteria and phototrophic proteobacteria and chemoautotrophic organisms this enzyme normally consists of eight repetitions of two types of protein subunits, called large subunit (L, about 55 kDa) and small subunit (S, about 13 kDa) (Dhingra et al., 2004; Portis Júnior and Parry, 2007).

Isoflavone reductase-like (IFR) (spot 29 – *P. tuberculatum* leaves) is an oxidoreductase enzyme involved in the biosynthesis of isoflavones and catalyzes the reduction of NADPH-dependent. In rice (*Oryza sativa*), for example, it is suggested that isoflavone reductase (OsIRL) contribute as tolerance factor with damage caused by reactive oxygen species and also in grapefruit induced pathogen resistance (Yun et al., 2013).

The proteins 14-3-3 (spots 14 and 37 - leaves of *P. tuberculatum*; spot S6 - fruits of *P. tuberculatum*) were classified in the *Cellular Process* category. This is a large family of proteins which exist primarily as homo- and heterodimers in all eukaryotic cells. There is a high similarity of conservation and the sequence identity in all isoforms. 14-3-3 proteins are considered master regulators of many signal transduction cascades in eukaryotes and plants while their role is related to the processes of nutrients assimilation. Recent studies indicated that these proteins act on the metabolism of nitrogen, sulfur and phosphorus (Diaz et al., 2011; Shin et al., 2011).

In addition to 14-3-3 proteins, rubber elongation factor protein (REF) (spots 28 and 46 from leaves of *P. tuberculatum*) is an enzyme engaged in the rubber biosynthetic machinery. Natural rubber, an important macromolecule with many industrial uses, is produced by more than 2000 plant species distributed in four of the six dicotyledonous super-orders. Several experimental evidences show that plants produce latex to protect themselves against predators attack. The rubber obtained from *Hevea brasiliensis* consists of long chains of *cis*-polyisoprene which are synthesized via the mevalonate pathway from acetyl CoA molecules derived from glycolysis. The polymerization of isopentenyl

pyrophosphate (IPP) is assisted by REF which leads to long chains of *cis*-polyisoprene which form aggregates of rubber particles surrounded by a membrane lipoprotein (Priya et al., 2007). Despite of no isolation of rubber from *Piper* genus, several meroterpenes substituted with mono- or sesquiterpene chains were isolated. Prenyltransferases and REF share IPP and DMAPP as substrates in their enzymatic reactions, suggesting some analogy in their active sites.

Clathrin (spot 4 from leaves of *P. tuberculatum*) was classified in the *Transport and Signaling* category. It is best known for its role in membrane trafficking. Clathrin-coated vesicles are formed at the plasma membrane and the trans-Golgi network where they are involved in endocytosis and transport of secretory cargo, respectively. Recently was better defined the role of this protein in the function of the mitotic spindle (Royle, 2012).

Chaperones (spot 5 from leaves of *P. tuberculatum*) are included in the *Structure and Organization* category. They are proteins that act in signaling/transport of other proteins with impaired performance for the proteasome (protein breakdown) and assist the newly synthesized proteins to act properly. In addition to promoting the proper functioning of proteins, chaperones are also important to protect cells against the effects of high temperatures, especially under denaturing conditions (Santoro, 2000).

Heat shock proteins (HSPs) (spot 1 from leaves of *P. tuberculatum*) were classified in the *Information Pathway* category. HSPs are found in virtually all living organisms, from bacteria to humans (Wu, 1995). This family of proteins is named according to their molecular mass, and the HSP60, HSP70 and HSP90 refer to families of proteins with approximately 60, 70 and 90 kDa, respectively (Srivastava, 2002). The increase concentration of HSPs can be triggered by exposure to different environmental stress conditions, such as infection, inflammation, exposure to toxins (metals, ultraviolet light, among others), nitrogen deficiency or water deprivation. Some HSPs proteins are expressed in low to moderate levels in all organisms, owing to their essential role in the maintenance of protein structure, or can be expressed in non-stressful conditions, simply for monitoring other cellular proteins. Several studies have demonstrated the role of HSPs in conferring tolerance stress in hybrid plants for agriculture (Vinocur and Altman, 2005).

The identification of all peptides had some complexity. Some were classified in the *Miscellaneous* category, because the MASCOT presented results as “unknown” or “not identified”, ie, there are similar peptides sequences already reported in other plants, but with no functions as a protein assigned yet. Other analyzed peptides showed no similarity to any sequence in the database and were categorized as “no identity”.

5. Conclusions

P. tuberculatum species is a non model plants, as well as *Piper* genus and Piperaceae family; therefore efforts devoted to add information about protein expression have great value. In this study proteins were characterized from

leaves and fruits samples. The information obtained here certainly should contribute to the plant proteome database, which is still quite poor when compared to databases of other organisms. Some proteins/enzymes were identified as rubber elongation factor, isoflavone reductase, 14-3-3, catalase among others related to the processes of the organism's defense against biotic or abiotic processes. These data are the first information about the proteome of both species and will contribute to a better comprehension of the protein expression pattern in the Piperaceae family.

Our studies intend to contribute to expanding the range of biological information, enabling better understanding of the fundamental aspects of plant biology and may contribute in the future to agronomic practices using these species as model.

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