Differential proteomics analysis of *Frankliniella occidentalis* immune response after infection with *Tomato spotted wilt virus* (Tospovirus)

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Abstract

Tomato spotted wilt virus (TSWV) is mainly vectored by *Frankliniella occidentalis* Pergande, and it potentially activates the vector's immune response. However, molecular background of the altered immune response is not clearly understood. Therefore, using a proteomic approach, we investigated the immune pathways that are activated in *F. occidentalis* larvae after 24 hours exposure to TSWV. Two-dimensional isoelectric focusing/sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D-IEF/SDS/PAGE) combined with mass spectrometry (MS), were used to identify proteins that were differentially expressed upon viral infection. High numbers of proteins were abundantly expressed in *F. occidentalis* exposed to TSWV (73%) compared to the non-exposed (27%), with the majority functionally linked to the innate immune system such as: signaling, stress response, defense response, translation, cellular lipids and nucleotide metabolism. Key proteins included: 70 kDa heat shock proteins, Ubiquitin and Dermcidin, among others, indicative of a responsive pattern of the vector's innate immune system to viral infection.

1 Introduction

Tomato spotted wilt virus (TSWV) is the type species of the genus *Tospovirus*; family *Bunyaviridae*. It is transmitted exclusively by multiple thrips species (Whitfield et al., 2005) (Ullman et al., 1997), with *Frankliniella occidentalis* (Pergande) being the main and the most efficient vector (Riley and Pappu, 2004)(Wijkamp et al., 1995)(Whitfield et al., 2005). This specificity of the virus-vector interaction is governed by receptor based endocytosis allegedly involving surface receptor proteins reported in *F. occidentalis* and linked to the recognition of TSWV (P. Ogada et al., 2016)(de Medeiros et al., 2000)(Kikkert et al., 1998)(Bandla et al., 1998). Furthermore, evidence exists of involvement of viral glycoproteins as determinants in the recognition process in the vector's midgut (Naidu et al., 2008)(Whitfield et al., 2008).

After acquisition by *F. occidentalis*, TSWV replicates and circulates inside the host midgut, the virus is passed via cell to cell movement from the midgut lumen through the midgut cell wall into the haemocoel and finally to the salivary glands where it replicates further during thrips development ((Whitfield et al., 2005)(Nagata et al., 1999)(Nagata et al., 2002)(Ullman et al., 1993)(Moritz et al., 2004). The replication of the virus within the host suggests a likelihood for pathological (lethal) effects as well as changes in the vector's physiology which may influence the performance and fitness negatively (Stumpf and Kennedy, 2007)(Nault, 1994). Conversely, recent studies have reported that feeding of *F. occidentalis* on TSWV infected host plants resulted in improvement in fitness: in terms of improved survival and reduced development time (Stafford et al., 2011)(Shrestha et al., 2012)(Ogada et al., 2013)(Shalileh et al., 2016)(P. A. Ogada et al., 2016), assuming a triggered innate immune response to TSWV infection (de Medeiros et al., 2004). Therefore, we initiated a pilot study to investigate the immune response of *F. occidentalis* to infection with TSWV, using proteomic analysis tools to identify differentially expressed proteins. We hypothesized that defense related proteins are activated in *F. occidentalis* in response to TSWV infection.

2 Materials and Methods

2.1 TSWV isolate

TSWV isolate (TSWV-N12) was obtained from the laboratory of virology, Wageningen University, the Netherlands. The maintenance and the detection of the TSWV isolate was done according to Ogada et al. (2013). The infected plants were maintained at 28-30 °C (greenhouse conditions) and served as inoculum source for further series of mechanical inoculation, as well as virus source for acquisition by *F. occidentalis* in the experiment.

2.2 *F. occidentalis* culture

F. occidentalis (F.o. 2), was also acquired from the laboratory of virology, Wageningen University, the Netherlands. A virus free stock culture was established on bean plants

(*Phaseolus vulgaris* L.) in thrips proof cages maintained at climate chamber conditions (25±2 °C, 60-70% (RH) and 16:8h (L: D)). From the stock culture, a synchronized rearing was developed as describe in Ogada et al. (2013). Both the stock culture and the synchronized rearing were maintained in separate climate cabins completely isolated from any other sources of thrips.

2.3 TSWV acquisition by *F. occidentalis* and sample preparation

At least 300 of < 12 hours old *F. occidentalis* larvae (L1) were introduced on *C. annuum* leaflets that were either healthy or heavily infected with TSWV (confirmed by DAS-ELISA). The virus infected or healthy leaflets were individually placed in a 15 cm Ø petri dish layered with moistened gypsum ($CaSO_4$ and charcoal 9:1 ratio), followed by the introduction of newly hatched L1 larvae for an acquisition access period (AAP) of 24 hours. The petri dishes were carefully sealed to avoid any escape of *F. occidentalis*. After the AAP, approximately 250 L1 larvae were collected into a 2ml Eppendorf tube for each treatment using a fine paint brush, shock frozen in liquid nitrogen and then pulverized into fine powder using a bead mill pulverizer machine.

2.4 Protein isolation: Phenol extraction method

Total soluble proteins were extracted according to Colditz et al. (2004). The pulverized insect materials were homogenized in extraction buffer (700 mM sucrose, 500 mM Tris, 50 mM EDTA, 100 mM KCl, 2% (v/v) β-mercaptoethanol and 2 mM PMSF, pH adjusted to 8.0). Then saturated phenol (pH 6.6/7.9; Amresco, Solon, USA) was added. This was followed by several rounds of centrifugation before the proteins were precipitated using 0.1 mM ammonium acetate in methanol and then incubated at −20 °C overnight. The solution was again centrifuged, and then the pellet was re-suspended in 80 % acetone solution, followed by final centrifugation. The resulting pellet was air dried at room temperature and then weighed.

2.5 Two-dimensional isoelectric focusing/sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D-IEF/SDS-PAGE)

From the resulting protein pellet, 5 mg were re-suspended in rehydration buffer (8 M Urea, 2 M thiourea, 2% (w/v) CHAPS, a trace of bromophenol blue, ddH_2O, 100 mM DTT, 12 µl/ml DeStreak-reagent, 0.5% (v/v) IPG-buffer pH 3-11 NL, GE Healthcare, Freiburg, Germany) and directly loaded onto an IPG strip (18 cm, pH 3–11 non-linear (NL), GE Healthcare, Freiburg, Germany). Isoelectric focusing was performed as described in Mihr and Braun (2003). The IPG strips were equilibrated twice for 15 min each time, first by immersing them in equilibration buffer (6 M urea, 50 mM Tris–HCl (pH 8.8), 30% glycerol and 2% SDS) with DTT (1%, w/v) to reduce the cysteine residues, and then in equilibration buffer with iodoacetamide 4% (w/v), for informal alkylation of cysteine residues. For the second gel dimensions, the equilibrated strips were shortly rinsed in tricine gel buffer (3 M Tris-HCl, pH 8.45, 0.3% SDS). The IPG strips were then carefully fixed horizontally onto 12% acrylamide SDS gels. The gel run was performed for 18 h at 30 mA per gel using the Bio-Rad Protean II XL gel system (Biorad, München, Germany). The two-dimensional gel electrophoresis for TSWV exposed and non-exposed *F. occidentalis* were repeated at least three times for the 3 biological replicates.

2.6 Gel staining procedure

Fixing of all the 2D gels was done using 10% (v/v) acetic acid in 15% (v/v) ethanol for 2 hours, then stained overnight with Coomassie blue CBB G-250 (Merck, Darmstadt, Germany) as described by Neuhoff et al. (1985; 1990). The gels were then de-stained several times using ddH_2O before being scanned on a UMAX Power Look III Scanner (UMAX Technologies, Fremont, CA, U.S.A.).

2.7 Quantitative gel analysis of the differentially expressed proteins

The gel scans were evaluated using Delta2D software, version 4.3 (Decodon, Greifswald, Germany) as described in Berth et al. (2007) and Lorenz et al. (2014). Three replicates per treatment (*F. occidentalis* exposed or non-exposed to TSWV) were used (Supplementary data, Fig. 1A). Spot detection was done automatically with minor manual corrections of obvious gel disturbances. Delta2D software was used for in-gel normalization of the overlays of three replicate gels per treatment (Supplementary data, Fig. 1B). Significant variation in spot volumes between the exposed and the non-exposed *F. occidentalis* gels was determined using a Student's t.-test (p-value ≤ 0.05) based on the normalized relative spot volume. Spots differing in volume were color coded according to their respective treatment (Supplementary data, Fig. 1C). A true difference in protein abundance was considered only if the variation in spot volumes between the compared treatments had a factor ≥ 1.5. Protein spots with significant variation in volume were subsequently identified by mass spectrometry.

2.8 Protein identification by mass spectrometry

Analyses of the differentially expressed proteins between treatments were based on proteins identified by mass spectrometry (MS). Tryptic digestion and MS analysis were performed according to Klodmann et al. (2010) using the EASY-nLC System (Proxeon) coupled to a MicroTOF-Q II mass spectrometer (Bruker Daltonics, Bremen, Germany). Protein identification was carried out using the MASCOT search algorithm (www.matrixscience.com) against the (i) Ensembl Metazoa (www.metazoa.ensembl.org), (ii) Flybase (www.flybase.org), (iii) SwissProt (www.uniprot.org) and (iv) NCBInr (www.ncbi.nig.gov) databases.

A schematic of the experimental workflow is shown in Supplementary data, Fig. 2.

3 Results and Discussion

Evaluation of Delta2D for changes in the *F. occidentalis* L1 proteome after 24-hour exposure to TSWV revealed 901 differentially expressed spots, but only 30 showed significant variations in volume according to a *p*-value of ≤ 0.05 based on the normalized relative spot volume (Fig. 1). Out of the 30 spots, 22 increased in volume in response to TSWV while 8 decreased in volume (Supplementary data, Table 1 and 2). Proteins in spots that significantly changed in volume (Fig. 1) were analysed by mass spectrometry. However, this experimental approach did not allow the quantification of the changes in abundance for individual enzymes because most spots on the 2D gels included more than one protein. Nevertheless, in some cases, quantitative changes were visible for individual proteins, and in several others tendencies could be seen. The names and physiological background of the identified proteins are given in Table 1. Furthermore, an interactive gel-map is available online (www.gelmap.de/1290 password: FoProt) which summarizes Fig. 1 and Table 1.

Due to the absence of *F. occidentalis* immune related protein sequence databases, most proteins were identified via protein sequences of other insects which included: *Drosophila* spp (64%), *Anopheles gambiae* (5%), *Ceratitis capitata* (9%), *Lysiphlebus testaceipes* (5%), *Sarcophaga peregrine* (5%), *Bactrocera dorsalis* (9%) and *Vespula vulgaris* (5%), as well as other metazoan sequences (Supplementary data, Fig. 3). Furthermore, majority of studies on the invertebrate innate immune system have been on *Drosophila* as the model organism, making it the main reference point with regards to the available information and sequence databases (Barillas-Mury et al., 2000)(Hoffmann et al., 1999).

During virus recognition and infection process, key proteins are involved which have to be considered when discussing the vector's innate immune response to viral infections. In this study, we classified the identified proteins according to their functional groups (Figure 1, Table 1). Of the proteins that were found to be significantly increased in abundance in response to the virus; 26% were identified to be involved in translation, 15% in insect defense

response, 13% in signaling pathways, another 13% in anatomical development, 9% in cellular lipid metabolic processes ($NADP^+$), 8% were structural proteins, another 8% were linked to stress response, 5% involved in nucleotide metabolism and 3% were actin binding proteins (Supplementary data, Fig. 4). On the other hand, 63% of the proteins that were significantly decreased in abundance were structural proteins, 17% are involved in energy metabolism, 8% in amino acid metabolism, 4% in protein processing, another 4% in sugar metabolism and another 4% involved in cell cycle (Supplementary data, Fig. 5).

The primary immune components in invertebrates are mainly found within the hemolymph and are involved in virus recognition as well as initiation of defense responses (Christensen et al., 1989)(Paskewitz and Christensen, 1996). Some of the most abundantly increased proteins in *F. occidentalis* after TSWV infection included: **Fibrinogen alpha chain protein,** which is a ligand for the Toll-like receptor 4 (TLR4), which recognizes pathogen-associated molecular patterns (PAMPs) expressed by the virus and mediates the production of cytokines necessary for the development of effective immunity, by initiating signal transduction events induced by the pathogen, leading to blood coagulation and melanization reported in vertebrates (Cerenius et al., 2010)(Lavine and Strand, 2002)(Hoffmann and Reichhart, 2002)(Waltenbaugh et al., 2008). This reaction is well studied in mammals, however, whether or not a comparable coagulation process in insect hemolymph is involved in host defense is not yet confirmed (Cerenius et al., 2010)(Lavine and Strand, 2002)(Hoffmann and Reichhart, 2002). Studies on *Helicoverpa zea* larvae infected with *Autographa californica polyhedrovirus*, indicated that such circulating haemocytes encapsulate and kill the invading pathogen (Washburn et al., 1996). Also, inhibition of virus survival in *F. occidentalis* hemolymph has been reported and linked to the potential barriers to virus replication within the hemolymph. Thus, the mechanism of TSWV movement within the vector's body remains unknown (Nagata et al., 1999)(Whitfield et al., 2005).

Heat shock cognate protein 70kDA (Hsp70); a ligand for the Toll-like receptor 2 (TLR2) (whose function is similar to the TLR4), was abundantly increased, and is associated with an antiviral autophagy dependent on the Toll signaling pathways in *F. occidentalis*, in response to stress as a result of exposure to TSWV. Autophagy: the natural catabolic process dependent on the cytosolic lysosome, is constitutively competent to destroy infectious viruses and essential viral components that link viral detection to the signaling of the antiviral innate immunity (Richetta et al., 2013)(Mizushima, 2007). It is also reported to be crucial for antiviral defense in insects (Shelly et al., 2009). The three types of autophagy pathways include: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA), all taking part in the immune response (Mizushima, 2007). CMA is very specific and complex, involving recognition by the 70kDA heat shock cognate protein (Hsp70)-containing complex (Česena et al., 2012)(Bandyopadhyay et al., 2008). The pathway is triggered upon viral recognition independent of viral replication. In *Drosophila*, it is triggered upon recognition of the VSV-G (Nakamoto et al., 2012). The Hsp 70 act as chaperones which enable the cell to cope with the harmful aggregation of denatured proteins due to stress. They are associated with the CMA which is reported to be active at all times in different tissues in mammals and almost all cell types in culture studies, but highly triggered in response to stresses and variation in nutritional status of the cell (Cuervo et al., 1995).

TLR4 and TLR2 have been reported to detect several viral glycoproteins (Akira et al., 2006). For instance, in mammals, since the TLRs are known pattern recognition receptors (PRRs), *Vesicular stomatitis virus* glycoprotein (VSV-G) was previously reported to induce antiviral response dependent on TLR4 signaling (ligands include several viral proteins) (Georgel et al., 2007) which activates the immune system. Additionally, the envelope protein of *Mouse mammary tumor virus* (MMTV) activates TLR4 (Burzyn et al., 2004). Viruses or viral components such as *Human cytomegalovirus* (HCMV), *Herpes simplex virus* 1 (HSV-1) and *Measles virus* (MV) hemagglutinin protein have been reported to activate TLR2 (Bieback

et al., 2002)(Compton et al., 2003). Therefore, we can conclude that the stimulation of proteins which function as ligands for the TLR2 (Hsp 70) and TLR4 (Fibrinogen alpha chain protein) in *F. occidentalis* in response to infection by TSWV, which is enveloped with glycoproteins, is an antiviral response in the host innate immune system.

Additionally, TLR 2 and TLR 4 mediate the production of cytokines which are expressed abundantly in the peripheral haemocytes, and are responsible for the mediation of the host response to pathogen intrusion via stimulation of **nuclear factor Kappa-light-chain-enhancer of activated B cells (NF-κB)** (Hayden et al., 2006)(Doyle and Neill, 2006). NF-κB is a protein complex which belongs to the category of "rapid-acting" primary transcription factors, as it is found in almost all animal cell types in an inactive state and do not require new protein synthesis in order to become activated. This makes it one of the primary responders to harmful cellular stimuli. The finding that the stimulation of TLRs leads to the activation of NF-κB, brings further understanding on how different pathogens activate NF-κB (Hayden et al., 2006).

The processing of the NF-κB is mediated by **the ubiquitin-proteasome pathway** which involves selective degradation of their C-terminal region containing ankyrin repeat motifs of the NF-κB inhibitor (IκB) (Karin and Ben-neriah, 2000). The IκB degradation is triggered in response to stimuli, such as those transduced in TLR activation, which potentiate NF-κB dimer activation in a non-canonical pathway (Basak et al., 2007)(Lo et al., 2006). This process of NF-κB activation occurs solely via the activation of an IκB Kinase (IKK), which phosphorylates serine residues in the IκB regulatory domain. The phosphorylated IκB inhibitor molecules are then modified by a process called ubiquitination, leading to their degradation by a cell structure called proteasome, thus freeing NF-κB to enter the nucleus where it triggers the expression of specific genes leading to immune response (Nelson et al., 2004)(Livolsi et al., 2001).

Proteins linked to the ubiquitin-proteasome pathway: **Ubiquitin**, **Valosin-containing interacting protein 135 (VCIP135)** and **Ubiquitin carboxyl-terminal hydrolase 36**, were significantly increased in *F. occidentalis* after exposure to TSWV. **Ubiquitin** was the most significantly increased protein in terms of abundance after *F. occidentalis* exposure to TSWV (24 fold). It forms the main component of the ubiquitin-proteasome pathway which involves several sequential ATP-dependent enzymatic cascades (Wilkinson et al., 2005)(Komander and Rape, 2012)(Kimura and Tanaka, 2010). Ubiquitination or ubiquitilation refers to the addition of ubiquitin to a substrate protein, which may affect the protein in many ways, like signaling for its degradation via the proteasome, affect its cellular location and activity, as well as prevent or promote protein-protein interactions (Glickman and Ciechanover, 2002)(Mukhopadhyay and Riezman, 2007)(Papers et al., 2003). Only poly-ubiquitination at a defined lysine molecule is linked to the degradation by the proteasome, while the mono-ubiquitinations are involved in the regulation of processes such as translation, inflammation, endocytic trafficking and DNA repair (Mcdowell and Philpott, 2013). Additionally, unanchored-polyubiquitin has also distinct roles such as activation of protein kinases, and signaling. However, the other proteins, **VCIP135** and **Ubiquitin carboxyl-terminal hydrolase 36**, are involved in the de-ubiquitination process in the ubiquitin-proteasome pathway, which leads to the activation of ubiquitin by cleaving of the inactively expressed form of ubiquitin (Turcu et al., 2009).

A-kinase anchor protein 3 which functions as a regulator of protein kinase A signaling cascade, as well as a transmembrane receptor protein of serine/threonine kinase signaling pathway, was also significantly increased in *F. occidentalis* after exposure to TSWV. The pathway involves a series of molecular signals initiated by the binding of an extracellular ligand to a receptor on the surface of the target cell regulating programmed cell death (apoptosis), cell differentiation, among others, by phosphorylating the OH group of serine or

threonine (Vijayaraghavan et al., 1999). Another immune related protein that was significantly increased is **Dermcidin** (10 fold). It has antimicrobial activities and promotes natural cell survival under severe stress (Zeth, 2013). The proposed mechanism for the antimicrobial activities includes an ion gradient decoupling across biological membranes, and proteolytic activity (Song et al., 2013). **Heterochromatin protein 1**, a stress response protein, was also increased in abundance in *F. occidentalis* after exposure to TSWV. Its main function is the repression of transcription by altering the structure of chromatin, also referred to as chromatin silencing e.g. by conversion of large regions of DNA into an inaccessible state often called heterochromatin (Vermaak and Malik, 2009). Additionally, **DNA repair protein REV1** and **Inactive hyaluronidase protein**, both of which are linked to the host defense response, were increased abundantly in response to TSWV infection. **Inactive hyaluronidase proteins** are usually triggered in response to the invasion by foreign bodies or injury, leading to restriction of damage to the tissue attacked or prevention/recovery from the infection, while **DNA repair protein REV1** is required for the induction of mutations as a form of immune reaction in response to physical and chemical agents (UniProtKB/Swiss-Prot, 2015).

Increase in abundance **of the structural proteins** and those involved in anatomical development, in response to viral infection, could be associated with the rapid growth and development reported in *F. occidentalis* after exposure to TSWV (Belliure et al., 2005)(Belliure et al., 2008)(Ogada et al., 2013)(Shalileh et al., 2016). Additionally, the proteins associated with translation that were increased abundantly in response to TSWV could be linked to the replicating virus within the (Stumpf and Kennedy, 2007)(Nault, 1994). For instance, **Eukaryotic translation initiation factor 5A-1**, one of the abundantly increased translation protein in this study, is an mRNA-binding protein, involved in the regulation of mRNA turnover by controlling the actin dynamics and cell cycle progression (RNA decay). It is also involved in stress response and maintenance of cell integrity. Additionally, it regulates

TNF-alpha-mediated apoptosis (UniProtKB/Swiss-Prot, 2015). Moreover, the proteins involved in cellular lipids and nucleotides metabolic processes that were abundantly increased in *F. occidentalis* after exposure to TSWV, could also be associated with the replicating virus in the vector (Stumpf and Kennedy, 2007)(Nault, 1994). For example, **Guanine nucleotide-binding subunit beta-like protein**, which is involved in nucleotide metabolism, functions by recruiting, assembling and/or regulating of a variety of signaling molecules. It is also involved in the positive regulation of viral genome replication by the host, which is initiated in response to viral infection. This selective mRNA translation opens up a target for the development of broad antiviral intervention in the host (Majzoub et al., 2014).

The use of L1s of *F. occidentalis* which were not exposed to TSWV, enabled us to clearly identify the differentially expressed proteins in response to viral infection. The proteins that were decreased in abundance in TSWV exposed *F. occidentalis*, were clearly those proteins which are basically involved in the normal anatomical growth and development of *F. occidentalis*. Thus, we can conclude that thee differentially expressed proteins are naturally present at basal levels in expression and are involved in multifaceted roles in the invertebrate physiology, but they are only activated significantly in response to stress or pathogen attack (Badillo-Vargas et al., 2012).

Despite being able to see clearly the involvement of immune related proteins in response to viral infection, the use of other Metazoan databases in the functional description of the differentially expressed protein in *F. occidentalis*, only offers basic information. Availability and use of *F. occidentalis* transcriptome database related to the innate immune system would enable a more specific and detailed analysis. We can therefore conclude that, the hereby presented results indicate that the exposure of *F. occidentalis* to TSWV triggers production of certain proteins which are associated with the innate immune response of the vector to viral infection. This substantiates the research findings that have reported improved fitness in *F.*

occidentalis exposed to TSWV, and thus, improving our understanding to the complex virus-vector-host plant interaction.

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Figure

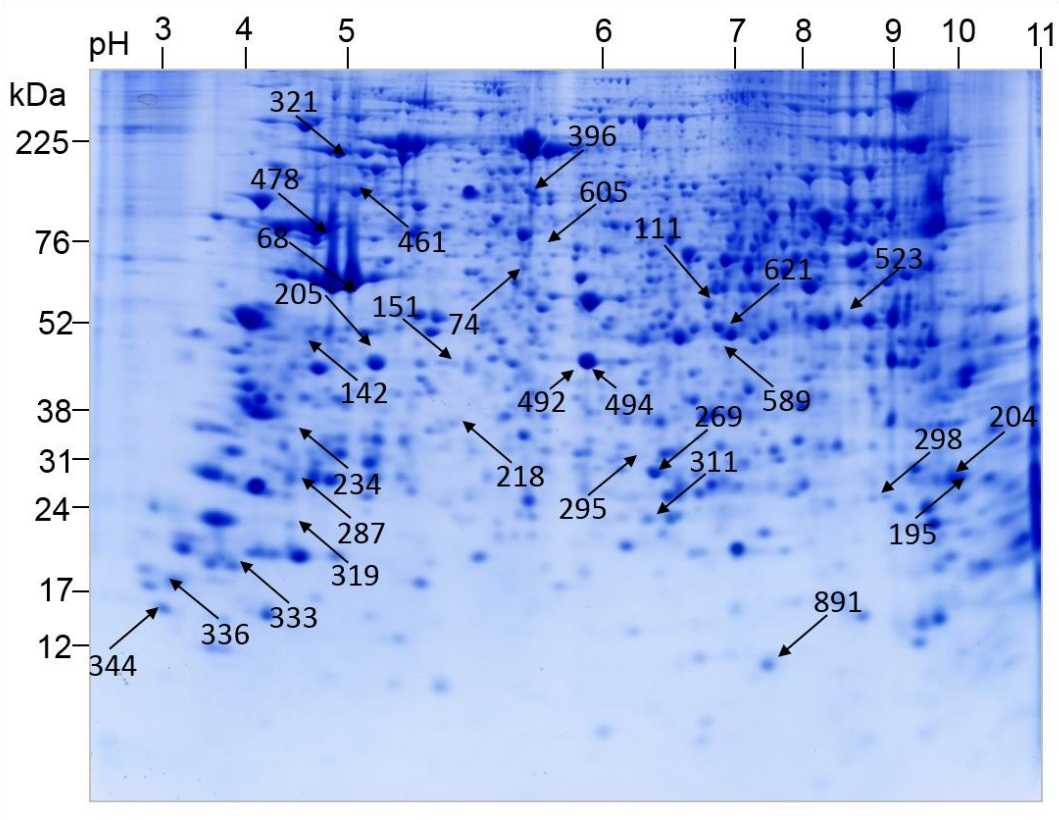

Figure 1: Two-dimensional reference gel map for *F. occidentalis* proteome. A 3-11 non-linear immobilized pH gradient (IPG) strip was used to separate total soluble proteins by isoelectric focusing (pIs are given above the 2D gel), followed by their separation according to molecular mass by SDS-PAGE (the masses of standard proteins are given to the left of the 2D gel). Coomassie brilliant blue G250 was used to stain the gels. The arrows indicate spots which are significantly altered in volume, and the numbers represent spot IDs (www.gelmap.de/1290 password: FoProt).

Table 1: Protein identifications from spots with changed volumes between the TSWV exposed and the non-exposed (24 hours) *F. occidentalis* L1. Student's t-test (p-value ≤ 0.05) was used to determine significant changes in spot volume between the treatments based on normalized relative spot volumes. Changes in spot volume ≥ 1.5 were considered to represent alterations. MASCOT search algorithm was used for protein identification (www.matrixscience.com) against the (i) Ensembl Metazoa (www.metazoa.ensembl.org), (ii) Flybase (www.flybase.org), (iii) SwissProt (www.uniprot.org), and (iv) NCBInr (www.ncbi.nig.gov) databases. A) Proteins of increased abundance in *F. occidentalis* after the 24-hour exposure to TSWV, and B) Proteins of decreased abundance in *F. occidentalis* after the 24-hour exposure to TSWV (www.gelmap.de/1290 password: FoProt).

A) Proteins of increased abundance in *F. occidentalis* after the 24-hour exposure to TSWV

ID	Accession	Name	Organism	MM calc [kDa]	pI calc	MASCOT Score	Sequence Coverage (%)
			Structural protein				
74	ARP2_DROME	Actin-related protein 2	*Drosophila melanogaster*	44.7	5.8	293	16
74	ACT2_MOLOC	Actin, muscle-type	*Molgula oculata*	42.2	5	275	17
74	ACT1_BACDO	Actin, indirect flight muscle	*Bactrocera dorsalis*	41.7	5.2	267	21
			Anatomical development				
589	HORN_HUMAN	Hornerin	*Homo sapiens*	282.2	10.4	39	1
333	HAND2_DANRE	Heart- and neural crest derivatives	*Danio rerio*	23.2	9.7	41	3
205	CATL_SARPE	Cathepsin L	*Sarcophaga peregrina*	37.8	6	47	4
333	IDGF3_DROME	Chitinase-like protein	*Drosophila melanogaster*	49.2	7.8	36	1
74	HDX_HUMAN	Highly divergent homeobox (DNA Binding)	*Homo sapiens*	77.2	5.5	43	2
			Translation				
111	RLA0_CERCA	60S acidic ribosomal protein P0	*Ceratitis capitata*	33.9	7.6	224	10
111	RLA0L_HUMAN	60S acidic ribosomal protein P0-like	*Homo sapiens*	34.3	5.3	112	6
111	RLA0_CAEEL	60S acidic ribosomal protein P0	*Caenorhabditis elegans*	33.8	6.3	69	6
111	SYEP_DROME	Bifunctional glutamate/proline--tRNA ligase	*Drosophila melanogaster*	189.3	9.4	42	1
195	RL12_BOVIN	60S ribosomal protein L12	*Bos taurus*	17.8	10.3	56	5
204	RL12_BOVIN	60S ribosomal protein L12	*Bos taurus*	17.8	10.3	53	5
204	EIF3J_DROVI	Eukaryotic translation initiation factor 3, subunit J	*Drosophila virilis*	27	4.6	41	4
287	IF5A1_BOVIN	Eukaryotic translation initiation factor 5A-1,	*Bos taurus*	16.8	4.9	49	5
523	RL5_LYSTE	60S ribosomal protein	*Lysiphlebus testaceipes*	34.4	10.1	48	5
621	RLA0_CERCA	60S acidic ribosomal protein	*Ceratitis capitata*	33.9	7.6	150	8
			Stress response				
74	HSP7D_DROME	Heat shock 70 kDa protein	*Drosophila melanogaster*	71.1	5.2	317	11
74	HSP7C_DROME	Heat shock 70 kDa protein	*Drosophila melanogaster*	72.2	5.1	273	6
74	HSP7E_DROME	Heat shock 70 kDa protein	*Drosophila melanogaster*	74	6	209	8
			Signaling				

151	FIBA_CHICK	Fibrinogen alpha chain	*Gallus gallus*	82.4	5.6	39	1
195	AKAP3_MOUSE	A-kinase anchor protein 3	*Mus musculus*	95.5	5.9	40	1
195	UBIQ_CAMDR	Ubiquitin	*Camelus dromedarius*	8.6	7.6	83	24
621	VCIP1_MOUSE	Valosin-containing interacting protein 135 (VCIP135)	*Mus musculus*	134.4	6.8	42	1
621	GBLP_DROME	Guanine nucleotide-binding subunit beta-like	*Drosophila melanogaster*	35.6	7.9	53	3
		Defense response					
298	REV1_HUMAN	DNA repair protein REV1	*Homo sapiens*	138.2	9.5	39	0
74	HUGAB_VESVU	Inactive hyaluronidase B	*Vespula vulgaris*	40	9.8	38	2
195	DCD_HUMAN	Dermcidin	*Homo sapiens*	11.3	6.1	44	10
195	DCD_HUMAN	Dermcidin	*Homo sapiens*	11.3	6.1	37	10
311	UBP36_HUMAN	Ubiquitin carboxyl-terminal hydrolase 36	*Homo sapiens*	122.6	10.4	52	1
311	HP1_DROME	Heterochromatin protein 1	*Drosophila melanogaster*	23.2	4.9	36	4
		Nucleotide metabolism					
523	GMPR_ASCSU	GMP reductase	*Ascaris suum*	39.2	6.8	76	3
621	GBLP_DROME	Guanine nucleotide-binding subunit beta-like	*Drosophila melanogaster*	35.6	7.9	53	3
		Cellular lipid metabolic process (NADP)					
621	ALD1_MOUSE	Aldose reductase-related protein 1	*Mus musculus*	36	6.9	56	3
		Actin binding protein					
621	CORO7_RAT	Coronin-7 (Fragment)	*Rattus norvegicus*	48.9	5.3	41	2

B) Proteins of decreased abundance in *F. occidentalis* after the 24-hour exposure to TSWV

ID	Accession	Name	Organism	MM calc [kDa]	pI calc	MASCOT Score	Sequence Coverage (%)
		Structural protein					
68	ACT5_BACDO	Actin-5, muscle-specific	*Bactrocera dorsalis*	41.7	5.2	1332	50
68	ACT2_MOLOC	Actin, muscle-type	*Molgula oculata*	42.2	5	1308	51
68	ACTB_BOVIN	Actin, cytoplasmic 1	*Bos taurus*	41.7	5.2	1251	49
68	ACTB_ORYLA	Actin, cytoplasmic 1	*Oryzias latipes*	41.7	5.2	1199	49
68	ACTG_ANSAN	Actin, cytoplasmic 2	*Anser anser*	41.9	5.3	1192	47
68	ACTA_STRPU	Actin, cytoskeletal 1A	*Strongylocentrotus purpuratus*	41.8	5.2	1153	42
68	ACTC_BRALA	Actin, cytoplasmic	*Branchiostoma lanceolatum*	41.7	5.1	1144	39
68	ACTBL_HUMAN	Beta-actin-like protein 2	*Homo sapiens*	42	5.3	499	22
396	HORN_HUMAN	Hornerin	*Homo sapiens*	282.2	10.4	85	2
478a	TBAT_ONCMY	Tubulin alpha chain, testis-specific	*Oncorhynchus mykiss*	50	4.8	1155	42
478a	TBA1_DROME	Tubulin alpha-1 chain	*Drosophila melanogaster*	49.9	4.9	1151	42

478a	TBA3_HOMAM	Tubulin alpha-3 chain	*Homarus americanus*	50	4.8	806	28
478a	TBA1_HOMAM	Tubulin alpha-1 chain	*Homarus americanus*	50	4.9	776	25
478a	TBB_PIG	Tubulin beta chain	*Sus scrofa*	49.8	4.6	275	14
142	ACTA_BOVIN	Actin, aortic smooth muscle	*Bos taurus*	42	5.1	47	3
			Protein processing				
321	GRP78_BOVIN	78 kDa glucose-regulated protein	*Bos taurus*	72.4	4.9	243	9
			Sugar metabolism				
605	ENO_DROME	Enolase	*Drosophila melanogaster*	54.3	9.4	186	11
			Energy metabolism				
68	ACTBL_HUMAN	Beta-actin-like protein 2	*Homo sapiens*	42	5.3	499	22
478a	ATPB_DROME	ATP synthase subunit beta, mitochondrial	*Drosophila melanogaster*	54.1	5	705	33
478a	VATB_DROME	V-type proton ATPase subunit B	*Drosophila melanogaster*	54.5	5.1	52	2
461	ATPB_DROME	ATP synthase subunit beta, mitochondrial	*Drosophila melanogaster*	54.1	5	844	33
			Amino acid metabolism				
605	SAHH_ANOGA	Adenosylhomocysteinase	*Anopheles gambiae*	47.6	5.5	82	6
605	SAHHA_XENLA	Adenosylhomocysteinase A	*Xenopus laevis*	47.7	6	53	6
			Cell cycle				
605	CDC45_MOUSE	Cell division control protein 45 homolog	*Mus musculus*	65.3	5.1	48	1