VIRUS - INDUCED ER STRESS IN PLANTS

By

OMAR PAUL ARIAS GAGUANCELA

Bachelor of Science in Biotechnology

University of the Armed Forces - ESPE

Quito, Ecuador

2014

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2017
VIRUS - INDUCED ER STRESS IN PLANTS

Thesis Approved:

________________________________________
Dr. Jeanmarie Verchot
Thesis Adviser

________________________________________
Dr. Nathan Walker
Committee Member

________________________________________
Dr. Carla Garzon
Committee Member
ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. Jeanmarie Verchot for her valuable guidance during my master studies at Oklahoma State University – Department of Entomology and Plant Pathology. Dr Verchot constant support and mentoring made possible for me to accomplish several of my dreams as a scientist. Hence, I am and always will be extremely thankful for the opportunity she gave me. I have to thank my committee members Dr. Nathan Walker and Dr. Carla Garzon for their recommendations and advice which were extremely helpful during the development of this thesis. I have to express my gratitude to Dr. Aiming Wang for providing the infectious clone TuMV-GFP. I also thank Dr. Dennis Halterman at the USDA for providing the silencing constructs pHellsgate-bi1si and pHellsgate-bzip60si used in this study.

I want to express my deepest gratefulness to my mother Mrs. Mercy Gaguancela for her love and support throughout my life. My mother is my hero and inspiration, it’s because of her that I always want to improve and be the best version of myself. Last but not least, I have to express my thanks to my friends and ex laboratory members; Evelyn Vazquez, who performed part of the agro-delivery assays in the bZIP mutant Arabidopsis plants, and Lizbeth Peña whose contribution was meaningful for the standardization of the qRT-PCR protocol.
Abstract: The endoplasmic reticulum (ER) is a membrane system that acts as a platform for RNA viral replication and movement. The potexvirus TGB3 and potyvirus 6K2 proteins cause alterations in the ER architecture and protein production, causing ER stress. Embedded in the ER membrane are ER stress sensors that detect malformed proteins and elicit signaling cascades that result in changes in nuclear gene expression, process known as unfolded protein response (UPR). The most conserved ER stress sensors are IRE1 (coded by IRE1a and IRE1b), and type II transmembrane (bZIP) named bZIP60, bZIP17 and bZIP28 transcription factors which enter the nucleus to recognize promoters to activate gene expression of chaperone binding protein (BiP) to restore homeostasis. BI-1 is a cell death suppressor that resides in the ER and is believed to be downstream of IRE1. The contribution of these ER sensors in virus pathogenicity is poorly understood. This study revealed that knockdown of ER stress sensors leads to greater plant virus accumulation. PlAMV-GFP and TuMV-GFP accumulated to higher levels in Arabidopsis plants defective for both IRE1a and IRE1b than in wild-type Col-O. Interestingly, bZIP60 was a host susceptibility factor for both viruses, whereas bZIP28 and bZIP17 knockouts differentially accumulated PlAMV-GFP and TuMV-GFP in Arabidopsis plants. Agro-delivery of potexvirus TGB3 and potyvirus 6K2 resulted in elevated bZIP60, bZIP28 and bZIP17 transcript levels in Arabidopsis and N. benthamiana plants, which led to BiP gene activation. Arabidopsis plants defective for BI-1 were found to have a more significant effect on systemic PlAMV-GFP and TuMV-GFP accumulation than in bZIP60 mutant plants. Similarly, the experiments conducted in BI-1 and bZIP60 silenced N. benthamiana plants infected with PVX-GFP or PVY-GFP showed that knockdown of BI-1 produced higher virus accumulation levels than bZIP60. This data suggest that UPR pathways provide some protection against virus infection.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Objectives</td>
<td>3</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Potexvirus genome</td>
<td>5</td>
</tr>
<tr>
<td>Life cycle of potexvirus</td>
<td>6</td>
</tr>
<tr>
<td>Cell to cell movement of potexvirus</td>
<td>6</td>
</tr>
<tr>
<td>Potyvirus genome</td>
<td>6</td>
</tr>
<tr>
<td>Life cycle of potyvirus</td>
<td>7</td>
</tr>
<tr>
<td>Cell to cell movement of potyvirus</td>
<td>8</td>
</tr>
<tr>
<td>Virus movement into systemic plant tissues</td>
<td>8</td>
</tr>
<tr>
<td>ER stress during viral infection</td>
<td>9</td>
</tr>
<tr>
<td>III. MATERIALS AND METHODS</td>
<td>11</td>
</tr>
<tr>
<td>Plasmids and bacterial strains</td>
<td>11</td>
</tr>
<tr>
<td>Plant material and agroinfiltration</td>
<td>12</td>
</tr>
<tr>
<td>Immunoblot analysis</td>
<td>13</td>
</tr>
<tr>
<td>Semi qRT-PCR and qRT-PCR assays</td>
<td>13</td>
</tr>
<tr>
<td>Image J analysis</td>
<td>15</td>
</tr>
<tr>
<td>IV. RESULTS</td>
<td>21</td>
</tr>
<tr>
<td>Patterns of virus local and systemic infection in <em>IRE1a</em> and <em>IRE1b</em> defective <em>Arabidopsis thaliana</em> plants</td>
<td>21</td>
</tr>
<tr>
<td>qRT-PCR analysis of <em>bZIP60</em>, <em>bZIP17</em> and <em>bZIP28</em> induction following treatment with viral elicitors</td>
<td>28</td>
</tr>
<tr>
<td>Patterns of virus local and systemic infection in <em>bZIP17</em>, <em>bZIP28</em> and <em>bZIP60</em> defective <em>Arabidopsis</em> plants</td>
<td>31</td>
</tr>
</tbody>
</table>
qRT-PCR analysis of ER luminal binding protein (BiP) gene expression following treatment with viral elicitors.................................................................36
Comparison of the patterns of local and systemic PIAMV-GFP and TuMV-GFP infection in mutant BI-1, IRE1a/IRE1b and bZIP60 Arabidopsis plants..........40
Local and systemic PVX-GFP and PVY-GFP infection bZIP60 and BI-1 silenced N. benthamiana plants ..............................................................................46
Chlorosis and necrosis in BI-1 and bZIP60 silenced N. benthamiana plants treated with PVX-GFP or PVY-GFP ..................................................................48

V. DISCUSSION AND CONCLUSION ................................................................53

REFERENCES ..................................................................................................59
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 1</strong></td>
<td>List of primers used for gene expression analysis of \textit{bZIP17}, \textit{bZIP28}, \textit{bZIP60} and \textit{BI-1}</td>
</tr>
<tr>
<td><strong>Table 2</strong></td>
<td>List of primers used for detection of binding protein (BiP) in \textit{A. thaliana}, \textit{N. benthamiana} and \textit{S. tuberosum} leaves treated with potexvirus TGB3 and potyvirus 6K2 elicitors.</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHAPTER IV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figure 1</td>
<td>PIAMV-GFP and TuMV-GFP infecting local leaves of <em>Arabidopsis</em> plants.</td>
<td>25</td>
</tr>
<tr>
<td>Figure 2</td>
<td>PIAMV-GFP infecting systemic tissues of <em>IRE1</em> mutant <em>Arabidopsis</em> plants.</td>
<td>26</td>
</tr>
<tr>
<td>Figure 3</td>
<td>TuMV-GFP systemically infected wild type and <em>IRE1</em> mutant <em>Arabidopsis</em> plants.</td>
<td>27</td>
</tr>
<tr>
<td>Figure 4</td>
<td>Results of qRT-PCR detecting <em>bZIP17</em>, <em>bZIP28</em> and <em>bZIP60</em> in <em>Arabidopsis</em> Col-O and <em>N. benthamiana</em> leaves at 2 and 5 days post inoculation.</td>
<td>30</td>
</tr>
<tr>
<td>Figure 5</td>
<td>PIAMV-GFP and TuMV-GFP infecting local leaves of wild-type Col-O, <em>bZIP17</em>, <em>bZIP28</em>, <em>bZIP60</em>, <em>bZIP60/bZIP17</em> and <em>bZIP60/bZIP28</em> <em>Arabidopsis</em> mutant lines.</td>
<td>33</td>
</tr>
<tr>
<td>Figure 6</td>
<td>PIAMV-GFP systemic infection in wilt-type Col-O, <em>bZIP17</em>, <em>bZIP28</em>, <em>bZIP60</em>, <em>bZIP60/bZIP17</em> and <em>bZIP60/bZIP28</em> mutant lines from 10 to 19 dpi.</td>
<td>34</td>
</tr>
<tr>
<td>Figure 7</td>
<td>TuMV-GFP systemic infection in wilt-type Col-O, <em>bZIP17</em>, <em>bZIP28</em>, <em>bZIP60</em>, <em>bZIP60/bZIP17</em> and <em>bZIP60/bZIP28</em> mutant lines from 10 to 19 dpi.</td>
<td>35</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Results of qRT-PCR detecting <em>BiP</em> genes in detached leaves from <em>Arabidopsis</em> and <em>N. benthamiana</em> and <em>S. tuberosum</em> inoculated with TGB3 potexvirus or potyvirus 6K2.</td>
<td>39</td>
</tr>
<tr>
<td>Figure 9</td>
<td>PIAMV-GFP and TuMV-GFP infecting local leaves of <em>Arabidopsis</em> plants. For comparisons wild-type Col-O, <em>BI-1</em>, <em>IRE1a/IRE1b</em> and <em>bZIP60</em> mutant lines were utilized during the experiments.</td>
<td>43</td>
</tr>
<tr>
<td>Figure 10</td>
<td>PIAMV-GFP infecting <em>Arabidopsis</em> systemic leaves. Wild-type Col-O, <em>BI-1</em>, <em>IRE1a/IRE1b</em> and <em>bZIP60</em> mutant lines were utilized during experiments.</td>
<td>44</td>
</tr>
<tr>
<td>Figure 11</td>
<td>TuMV-GFP infecting <em>Arabidopsis</em> systemic leaves. Wild-type Col-O, <em>BI-1</em>, <em>IRE1a/IRE1b</em> and <em>bZIP60</em> mutant lines were utilized during experiments.</td>
<td>45</td>
</tr>
<tr>
<td>Figure 12</td>
<td>PVY-GFP and PVX-GFP infecting local leaves of <em>N. benthamiana</em> plants.</td>
<td>49</td>
</tr>
<tr>
<td>Figure 13</td>
<td>PVY-GFP and PVX-GFP infecting systemic leaves of <em>N. benthamiana</em> plants.</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure 14  Photos of PVX-GFP infecting *N. benthamiana* plants. Necrosis in inoculated leaves that had co delivery of PVX-GFP with pHellsgate-*bzip60si*, pHellsgate-*bi1si* or *Agrobacterium* lacking the silencing constructs at 6 dpi................................51

Figure 15  Photos of PVY-GFP infecting *N. benthamiana* plants. Necrosis in inoculated leaves that had co delivery of PVY-GFP with pHellsgate-*bzip60si*, pHellsgate-*bi1si* or *Agrobacterium* lacking the silencing constructs at 6 dpi.............................52
INTRODUCTION AND OBJECTIVES

Introduction

The endoplasmic reticulum (ER) is a membrane system that acts as platform for RNA viral replication and movement. Potexviruses are RNA viruses, members of the family Alphaflexiviridae which have a module of three overlapping open reading frames (ORFs) called the “triple gene block” (TGB). These three movement proteins are known as TGB1, TGB2 and TGB3 and enable movement of viral RNA between neighboring cells through plasmodesmata (Bamunusinghe et al., 2009). During potexvirus infection with Potato virus X (PVX), the TGB2 and TGB3 proteins reside in the ER and cause alterations in the ER architecture including the formation of vesicles (Ju et al., 2007). Prior research also indicated that TGB3 specifically activates a signal cascade that alters host gene expression. This cascade is led by ER resident stress sensors. Potyviruses are RNA viruses and members of the Potyviridae and their genomes encode one long open reading frame that generates a polyprotein. The polyprotein is cleaved to produce 11 mature products, one of which is known as the 6K2 protein. The 6K2 protein resides in the ER and causes alterations in the membrane structure that includes formation of vesicles (Jiang et al., 2015).
The 6K2 protein from TuMV anchors the viral replicase to the ER and induces the formation of vesicles that are crucial for the movement of viral RNA and replication proteins (Spetz et al., 2004; Cotton et al., 2009). The potexvirus TGB3 and the potyvirus 6K2 have a single transmembrane domain at the N-terminus (Grangeon et al., 2013; Jiang et al., 2015). It has been reported that the Potato virus X TGB3 and the Turnip mosaic virus 6K2 proteins activate the IRE1/bZIP60 signaling cascade in a manner that regulates virus infection (Ye et al., 2011; Ye et al., 2013; Zhang et al., 2015; Arias Gaguanela et al., 2016).

The ER plays a central role in protein synthesis, as well as carbohydrates and lipids assembling (Duwi Fanata et al., 2013). To enable protein folding and maturation, the ER contains several resident molecular chaperones including ER luminal binding protein (BiP), calnexin (CNX), calmodulin (CAM) and calreticulin (CRT) (Williams et al., 2014a). External factors like drought stress, salt stress, or virus infection can lead to increased translation of proteins and accumulation of malformed proteins in the ER, causing ER stress (Duwi Fanata et al., 2013). Malformed proteins elicit a response by protein chaperones in the ER to refold these proteins or dispose of them. Embedded in the ER membrane are ER stress sensors that detect malformed proteins in the ER and elicit signaling cascades that result in changes in nuclear gene expression, process known as unfolded protein response (UPR). These genetic responses are specifically activated to increase the ER capacity to fold and dispose of proteins (Hollien, 2013). The most conserved ER stress sensor that provide adaptive responses to malformed proteins in the ER include the endoribonuclease inositol-requiring enzyme 1 (IRE1) (Chen et al., 2013). Arabidopsis thaliana has encodes IRE1a and IRE1b which interact with malformed proteins in the ER, and are activated to mediate the splicing of a short intron and frameshift of mRNA basic leucine-zipper (bZIP) 60 transcription factor (Ruberti et al., 2015). The truncated bZIP60 protein enters the nucleus and upregulates expression of genes that contain plant unfolded protein response elements (P-UPRE) in the promoter (Deng et al., 2013a; Korner et al., 2015).
Two additional transcription factors are tethered to the ER, and upon stress are redirected to the nucleus to activate gene expression. These include the type II transmembrane (bZIP) named bZIP17 and bZIP28 (Schäfer et al., 2012; Williams et al., 2014a). The bZIP17 and bZIP28 proteins are tethered to the ER by BiP. During the UPR, BiP moves to the site of the malformed protein and refolds it, releasing bZIP17 and bZIP28. These transcription factors then are translocated to the Golgi apparatus where their transmembrane anchors are removed by the site 1 protease (S1P). The truncated bZIP17 and bZIP28 proteins move to the nucleus where they activate gene expression. Once in the nucleus these bZIP transcription factors will recognize promoters that contain the ER stress responsive cis element (ERSE-1) and P-UPRE (Duwi Fanata et al., 2013).

Bax inhibitor 1 (BI-1), the Bcl2-associated athanogene (BAG) proteins BAG6 and BAG7, and S-phase kinase associated protein 1 (SKP1) are factors associated with protein turnover and cell death regulation (Reape et al., 2008). BI-1 is an ER-resident membrane protein that is highly conserved in eukaryotes and plays a role in plant defense. The atbi-1 mutant A. thaliana plants have showed increase susceptibility to Fusarium graminearum (Ishikawa et al., 2011a).

The goal of this research was to investigate the interactions of the TGB3 and 6K2 proteins with the IRE1/bZIP60 and bZIP17, bZIP28 pathways. The second goal was to broadly examine viral interactions with factors involved in cell death and immune regulation that are also linked in some way to ER stress and protein turnover BI-1.

**Objectives**

1) Evaluate the contribution of UPR regulators IRE1a, IRE1b, bZIP60, bZIP28 and bZIP17 during infection of potexviruses and potyviruses in A. thaliana to determine if potyviruses and potexviruses interact with these machinery in similar or unique ways.
2) Analyze BiP genes induction to TGB3 and 6K2 viral proteins in *A. thaliana*, *N. benthamiana* and *S. tuberosum* in order to understand their role during UPR regulation.

3) Determine if systemic virus accumulation and necrosis are impacted by knocking down host gene expression of *bZIP60* and *Bi-1* in *N. benthamiana*.

4) Investigate the interactions involving potyvirus 6K2 or potexvirus TGB3 viral proteins that enable *IRE1/bZIP60* and *Bi-1* recognition of these elicitors to determine if similar factors are activated by these viruses.
CHAPTER II

REVIEW OF LITERATURE

Potexvirus genome

The potexvirus genome is characterized by the presence of five open reading frames (ORFs) flanked by a 5’ methyl guanosine cap and a 3’ poly (A) tail. The first ORF encodes the replicase (RNA-dependent RNA polymerase) protein that contains three activity domains: methyltransferase, helicase and RNA dependent RNA polymerase (Komatsu et al., 2008; Komatsu et al., 2011). This viral replicase is translated from the (+) ssRNA. The second, third and fourth ORFs encode the “triple gene block” (TGB1, TGB2, and TGB3) movement proteins (Huang et al., 2004). The TGB1 protein is translated from subgenomic RNA (sgRNA) 1 while sgRNA2 is responsible for translation of TGB2 and TGB3. TGB1 has a helicase domain and its functions include suppression of RNA silencing, expansion of plasmodesmata, and membranes remodeling. TGB2 and TGB3 have ER binding domains and they are responsible for cell to cell movement (Tilsner et al., 2012; Heinlein, 2015). The fifth ORF encodes the coat protein (CP) which is located close to the 3’ end of the genome is translated from sgRNA3. CP is involved in encapsidation and movement (Tilsner et al., 2013; Park et al., 2014).
Life cycle of potexvirus

Infection begins when virion particles disassemble in the cellular cytoplasm and release the genomic (+) ssRNA. From this step, (+) RNA recruits host ribosomes and translation of genomic RNA produces the viral replicase. Viral replication complexes (VRCs) form along the ER (Park et al., 2014). The VRCs lead the synthesis of (−) ssRNA which later is used as a template for the production of (+) ssRNA and three subgenomic (+) ssRNA. The first two subgenomic (+) ssRNAs will execute translation of the “triple gene block” proteins while the third subgenomic (+) ssRNA will encode the coat protein (Johnson et al., 2003; Tilsner et al., 2013). The RNA helicase activity of TGB1 will support unwinding between template (−) ssRNA and newly synthesized (+) ssRNA. TGB1 also will associate to CP and (+) ssRNA to form the ribonucleoprotein (RNP) complex or virus like particles which might be transported to neighboring cells (Solovyev et al., 2012; Park et al., 2013; Park et al., 2014).

Cell to cell movement of potexvirus

One of the most accepted models for virus trafficking into other cells states that TGB2 derived vesicles recruit TGB3 and both direct the movement of vesicles from the perinuclear to the cortical side of the ER. TGB2 and TGB3 vesicles align on actin filaments to organize and lead to movement of ribonucleoprotein (RNP) complexes throughout the plasmodesmata (Schoelz et al., 2011; Park et al., 2013; Tilsner et al., 2013). TGB1 which is a member of the RPN, might also be collaborating in expansion of the plasmodesmata pore size which will ultimately support cell to cell movement (Chou et al., 2013).

Potyvirus genome

The potyvirus genome organization encodes a single genome length ORF flanked by a 5’ VPg and a 3’ poly (A) tail and a small ORF. The large ORF translates a polyprotein composed by
the viral proteins P1, HC-Pro, P3, 6K1, CI, 6K2, Nla-VPg, Nla-Pro, Nlb and CP. The small ORF codes for the viral protein P3N-PIPO (Ivanov et al., 2014; Cui et al., 2016). P1 is a protease that cleaves itself from the polyprotein by its C-terminal. P1 functions include enhancement of silencing activity of HC-Pro and systemic spread (Nummert et al., 2017). HC-Pro is a protease that cleaves itself from the polyprotein and functions as a suppressor of host RNA silencing (Mlotshwa et al., 2016; Yang et al., 2016). P3 is involved in RNA replication and has a small ORF embedded in it that encodes PIPO. By ribosomal frameshifting, which is a genome strategy to produce two proteins with overlapping ORFs, P3N-PIPO is produced. P3N-PIPO activity has been correlated with replication and virus movement into systemic tissues (Chung et al., 2008).

The exact function of 6K1 is not well described in the literature, although recent reports suggest its role in virus replication (Cui et al., 2016). CI has ATPase and RNA helicase activities. CI participates in virus replication activity and virus adaptation in the host (Sorel et al., 2014b). 6K2 anchors and associates with the ER to induce the formation of vesicles that will serve as replication complexes, furthermore 6K2 activity might include cell to cell movement (Jiang et al., 2015). The genome-linked viral protein (VPg-Pro) has ATPase activity at the N-terminal domain and a C-terminal protease domain. Nla-Pro is essential for maturation cleavage of sites located on each side of 6K1, CI, 6K2, VPg, Nla-Pro, Nlb and CP. Nlb is the replicase (RNA-dependent RNA polymerase). CP functions for encapsidation and protection of the new progeny of virions (Ivanov et al., 2012; Ivanov et al., 2014).

Life cycle of potyvirus

Potyvirus entry into cells is followed by uncoating, translation of (+) ssRNA to produce the polyprotein, formation of virus replication complexes (VRCs) and delivery of the viral progeny. The formation of the VRCs start with the 6K2 protein interaction with the Nla-Pro, tethering it to the ER. In turn, Nla –Pro recruits Nlb to initiate the synthesis of (-) ssRNA. The 6K2 hijacks the membranes from the ER and the Golgi apparatus forming unique vesicles that
house and protect the VRCs (Jiang et al., 2015). P3 also seems to be involved in the formation of VRCs, since both P3 and 6K2 have been shown to be co-localized in the ER (Cui et al., 2010). The (-) ssRNA that is produced in the VRC, serves as a template for the synthesis of (+) ssRNA. The double strand RNA complexes are unwound by the RNA helicase activity of CI (Sorel et al., 2014a; Sorel et al., 2014b). Then, the newly synthesized (+) ssRNA starts new cycles of translation, replication or can be directed to plasmodesmata to infect other cells (Hong et al., 2007; Ivanov et al., 2014).

Cell to cell movement of potyvirus

The current model suggests that the (+) ssRNA synthesized in the VRC complexes associates to the CP to form ribonucleoprotein (RPN) complexes which will be mobilized to the plasmodesmata along actin filaments. The CP seems to recognize the viral protein CI which allows targeting to the plasmodesmata (Heinlein, 2015). P3N-PIPO also localizes along the plasmodesmata, modulates the formation of cylindrical inclusions at the periphery and facilities movement of the virus. This information supports the notion that potyvirus mobilization is carried out in RNP complexes facilitated by CI and P3N-PIPO rather than in fully encapsidated virions (Wei et al., 2010).

Virus movement into systemic plant tissues

Virus infection into healthy leaves is a process that is mediated by mechanical inoculation or insect vector transmission. Virus spread include short and long distance movements (Hipper et al., 2013). Short distance movement includes cell to cell transportation along nonvascular tissues (epidermis and mesophyll), process that is mediated by plasmodesmata and viral movement proteins such as potyvirus 6K2 and potexvirus “triple gene block” proteins (Verchot-Lubicz et al., 2007; Schoelz et al., 2011; Ivanov et al., 2014). For viruses to reach systemic tissues (long distance movement), they need to reach vascular tissues to multiply in new infection sites. For
such purposes, plant viruses have to move through bundle sheath cells, vascular parenchyma and companion cells, in order to reach sieve elements in the phloem. Transportation through sieve elements is mediated by pore plasmodesmal units which reportedly have wider size pores than regular plasmodesmata. Vascular movement of the virus follows a source (infection foci) to sink (healthy leaves) flux, but it is important to mention that the direction and speed of the viral systemic movement will depend on several factors, such as virus host protein interactions, host resistance and the number of initial infection foci produced (Cheng et al., 2000; Silva et al., 2002; Hipper et al., 2013; Rodrigo et al., 2014).

The study of virus movement has been successful thanks to the used of gene reporter GFP (green fluorescent protein). The use of plant reference models infected with GFP tagged viral infectious clones are highly helpful to monitor virus long distance and short movement in plant tissues, thus proportioning an understanding of virus spread mechanisms and plant-host interactions (Serrano et al., 2008; Harries et al., 2011).

**ER stress during viral infection**

Potexvirus TGB3 and potyvirus 6K2 are involved in the activation of ER transcription factor bZIP60 thus enabling UPR responses via IRE1-bZIP60 cascade. Moreover it has also been seen that the presence of these two viral proteins are linked with the induction of binding protein (BiP) (Williams et al., 2014b; Verchot, 2016). Given the interaction of TGB3 and 6K2 with UPR, it is not clear if these genes enhance or inhibit virus infection. Recent literature, has shown that chaperone CDC48 is induced upon tobacco mosaic virus treatment in A. thaliana, the same chaperone was capable of targeting and moving viral movement proteins out of the ER for degradation purposes, thereby reducing viral infection rate (Niehl et al., 2012). This example highlighted the role of UPR genes in diminishing virus pathogenicity. In contrast in other studies, activation of IRE1 cascade in the ER is linked with virus replication promoting. For example, a
yeast UPR chaperone homolog of HSP70 has been shown to be activated upon viral infection, the upregulation of this chaperone in this case apparently enhances virus replication (Aparicio et al., 2005; Zhang et al., 2015). As observed in the examples given above, there is contradictory information about virus host interactions in the ER. This will hamper the knowledge about this particular topic and will affect further possibilities of designing and developing more virus disease tolerant crops which will ultimately lead to an eventual benefit in crop yields affected by plant viruses.
CHAPTER III

MATERIALS AND METHODS

Plasmids and bacterial strains

All plasmids were maintained in Agrobacterium tumefaciens strain GV3101. Infectious clones of several potyviruses and potexviruses tagged with the green fluorescent protein (GFP), were used to visualize viral movement in plants. pCAMBIA binary plasmids containing Cauliflower mosaic virus (CaMV) 35S promoter and GFP tagging infectious clones of Turnip mosaic virus (TuMV) provided by Dr. Aiming Wang (Southern Crop Protection and Food Research Center – Ontario, Canada) and Plantago asiatica mosaic virus (PlAMV) derived from non-necrotic strain Lily – isolate Li6 (Komatsu et al., 2008) were used for infecting Arabidopsis thaliana. Additionally, a PVX-GFP infectious clone was obtained from Dr. Peter Moffett. The cDNAs of Potato virus Y (PVY) N605 strain genome were contained in pGR106/7 binary plasmids. GFP was inserted into these genomes (Hu et al., 2011; Vassilakos et al., 2016). The pMDC32 binary plasmids expressing the PVY 6K2, TuMV 6K2, PVX TGB3 and PlAMV TGB3 genes were also obtained (Curtis et al., 2003; Arias Gaguancela et al., 2016).
In addition pHellsgate-\textit{bi1si} and pHellsgate-\textit{bzip60si} constructs were prepared by Dr. Dennis Halterman at the USDA and used for silencing experiments in \textit{Nicotiana benthamiana}. Both \textit{BI-1} and \textit{bZIP60} gene sequences used for the assembly of the silencing constructs were derived from PVY-resistant \textit{S. chacoense} clone 39-7 (Arias Gaguancela \textit{et al.}, 2016).

**Plant material and agroinfiltration**

\textit{Arabidopsis thaliana} ecotype Col-O and homozygous (T-DNA) plant lines \textit{ire1a-2} (SALK\_018112), \textit{ire1b-4} (SAIL\_238\_F07), \textit{ire1a-2/1b-4}, \textit{bzip60-1} (SALK\_050203; locus \textit{At1g42990}), and \textit{bi1-2} (CS323793; locus \textit{At5G47120}, \textit{bzip17} (SALK\_104326), \textit{bzip28-2} (SALK\_132285), \textit{bzip17/bzip60} and \textit{bzip28/bzip60} were obtained from the \textit{Arabidopsis} Information Resource Center (Ohio State University, 055 Rightmire Hall and Carmack Rd, Columbus, OH).

\textit{N. benthamiana} plants were used as a hosts for study of virus infection and for carrying out transient assays for silencing of \textit{BI-1} and \textit{bZIP60} genes. \textit{Solanum tuberosum} cultivar Katahdin plants were propagated in Murashige and Skoog (MS) medium and were used to inoculate with PVX-GFP or PVY-GFP. All plant species were grown in growth chambers with 10 h light and 14 h of dark at 23 °C and after three weeks were subjected to \textit{A. tumefaciens} infiltration which was conducted as follows: \textit{A. tumefaciens} harboring the viral constructs grew on YEP media plates and then selected in liquid YEP media at 28 °C, 235 rpm for 16 hours. Cultures were pelleted by centrifugation for 5 min at 5000 rpm. Then, the supernatant was discarded and the pellet re-suspended in infiltration media (10 mM Mg\textsubscript{2}SO\textsubscript{4}, 10 mM MES) brought to pH=5.6 with acetylsyringone (5 mM). The absorbance was adjusted to OD600= 0.5 to 1.0; finally the \textit{A. tumefaciens} solution was incubated at room temperature for one hour and then infiltrated on the abaxial side of the leaf using a needle-free syringe.
**Immunoblot analysis**

Immunoblot analysis for detection of potexvirus and potyvirus CP was carried out using virus infected *Arabidopsis* leaves. Total proteins were extracted using grinding buffer (100 mM Tris HCl, 10mM KCl, 0.4 M Sucrose, 5 mM MgCl₂, 10% glycerol and 10mM β-mercaptoethanol) and then quantified using the Bradford assay (Bradford, 1976). Eighteen micrograms of protein were loaded onto 12% SDS-PAGE gel and then electro blotted to Amersham Hybond P 0.2 PVDF membranes (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) overnight at 200 mA. For visualization of equal protein loading, Ponceau S staining (0.5 % Ponceau S, 1 % acetic acid) was conducted (Arias Gaguancela et al., 2016; Ni et al., 2016). Specific capture antibodies for each virus CP was purchased from AGDIA (Elkhart, IN, USA) and used for immunoblot assay. The secondary antiserum Amersham ECL Rabbit IgG, HRP-linked whole antibody (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). Afterwards, the chemiluminescent reaction was developed using Amersham ECL Western Blotting Reagent Pack (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and the photos of the chemiluminescent blots were taken using a 8.3 megapixel scientific – grade CCD machine (FluorChem E system - Protein Simple, San Jose, CA, USA) applying an exposure of one minute on each blot (Arias Gaguancela et al., 2016).

**Semi qRT-PCR and qRT-PCR assays**

Semi qRT- PCR were conducted for assessment of the level of transient silencing of *bi-1* and *bzip60* genes in *N. benthamiana*. Detached leaves were harvested at 5 dpi and then subjected to RNA extraction with Maxwell 16 LEV simply RNA tissue kit (Promega Corporation, Madison, WI, USA) followed by cDNA preparation using Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc., Carlsbad, CA, USA). Semi qRT- PCR amplification for *bi* and *bzip60* genes were performed using GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 10 µM dNTPs, 25 µM MgCl₂ and 10 µM of each sense and antisense primers (IDT- Integrated DNA Technologies, Coralville, IA, USA) described
in Table 1. Afterwards the semi qRT-PCR reactions were conducted using C1000 Touch Bio Rad thermal cycler (Bio-Rad Laboratories, Inc., Foster City, CA, USA), starting with 1 cycle at 94 °C for 10 min, followed by 35 cycles at 94 °C of denaturation for 30 s, 55 °C for annealing for 30 s and 72 °C of extension for 1.5 min, finalizing with 1 cycle at 72 °C for 10 min (Arias Gaguancela et al., 2016).

qRT-PCR assays were conducted for quantification of gene expression of bzip60 bzip28, bzip17 and bi-1 (Table 1) and BiP gene candidates (Table 2) by using Kleengreen SYBR green PCR Master Mix (IBI Scientific, Peosta, IA) and 5 μM of sense and antisense primers (IDT-Integrated DNA Technologies, Coralville, IA, USA) presented in Table 1. The reactions developed in a Light Cycler 96 Real-Time System (Roche Diagnostics Corporation, Indianapolis, IN) equipment following 1 cycle of preincubation with two steps, the first one at 50 °C for 120 s and second at 95 °C for 600 s, afterwards, the amplification had 40 cycles of 60 °C for 60 s and 95 °C for 15 s, and finally 1 cycle of cooling at 37 °C for 60 s (Arias Gaguancela et al., 2016).

The transcript expression levels were calculated using the comparative quantification \( \Delta \Delta C_t \) method, starting with evaluating the experimental sensitivity and reproducibility with a standard curve and followed by calculation of transcript fold differences, using the following equation: \( \text{fold difference} = 2^{\Delta \Delta C_t} \), where \( \Delta \Delta C_t = \Delta C_{t\text{sample}} - \Delta C_{t\text{reference}} \), and \( \Delta C_{t\text{sample}} = C_t \) of virus infected plant tissue amplified for target gene - \( C_t \) of virus infected plant tissue amplified for housekeeping or endogenous gene (Ubiquitin for A. thaliana and Actin for N. benthamiana and S. tuberosum). In addition, \( \Delta C_{t\text{reference}} = C_t \) of plant tissue inoculated with A. tumefaciens without the viral construct amplified for target gene - \( C_t \) of plant tissue inoculated with A. tumefaciens without the viral construct amplified for housekeeping gene (Ye et al., 2013; Arias Gaguancela et al., 2016). For analyzing statistical differences in the gene expression of the experiments, an analysis of variance between all treatments was calculated in Relative Expression Software Tool - REST 2009, the statistical model that this program utilized was pair wise fixed reallocation which is a parametric test based on comparisons between Ct values of the samples with references with
target genes (ANOVA and Tukey methods). The proportion of how much they differ from the true mean is giving by $P$-values which is an estimate of the standard deviation (Pfaffl et al., 2002).

**Image J analysis**

To determine the percentage of gene knockdowns or silencing for *bZIP60* and *BI-1* in *N. benthamiana* detached leaves at six dpi, the software Image J (Schneider et al., 2012) was used to quantify average integrated density (AvgIntDen) from the scanned agarose gel images (16 bit .tiff format), first the images were uploaded to the program and then using the gel analyzer tool, each band present in the gel was enclosed and integrated density values (IntDen) were produced, these values represent the number of pixels present in a given area (section containing the band to analyze) multiply for the mean gray value of the background. The results were exported to Microsoft Excel - 2010 and then the percentage of gene knockdown was calculated as follows:

AvgIntDen (treated samples; detached leaves inoculated with *A. tumefaciens* containing silencing constructs)/ AvgIntDen (control samples; detached leaves inoculated with *A. tumefaciens* without silencing constructs) *100 for 25, 30, and 35 cycles of amplification. For evaluating statistical differences the ratio of AvgIntDen of control samples and treated samples were paired and the standard deviation of these values resulted in probability values ($P$). If the $P$ value is higher than the significance level (alpha=0.05) the null hypothesis (Ho) is accepted (silencing level is not statistically different) and on the opposite if $P$ value is lower or equal than alpha, the alternative hypothesis (H1) is rejected (silencing level is statistically significant) (Arias Gaguancela et al., 2016).

To analyze virus GFP on infected plant tissue, photos of the plant material were taken using Nikon D3100 DSLR camera with 18-55 mm lens and then the images were exported to Image J which selected the sections of the plant tissue presenting GFP and returned fluorescence values (FVs). FVs are equal to the Area (number of pixels per millimeter square) multiply by the
Mean gray value (sum of gray values of pixels in the section measured). FVs obtained from six plants were averaged and compared against each other. All area measurements were optimized by normalization with an internal length reference scale of 83.38 and 56.36 pixels/mm² for local and systemic leaves, respectively. ANOVA and Tukey methods were used to evaluate statistical differences between average FVs between wild type and mutant plants.
Table 1: List of primers used for gene expression analysis of *bZIP17*, *bZIP28*, *bZIP60* and *BI-1*.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
<th>Target gene</th>
<th>Host</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin_F</td>
<td>5’- AAA GAC CAG CTC ATC CGT GGA GAA -3’</td>
<td>Actin</td>
<td><em>N. benthamiana</em></td>
<td>(Zhang et al., 2015)</td>
</tr>
<tr>
<td>Actin_R</td>
<td>5’- TGTGGTTCATGAATGCCAGCAGC -3’</td>
<td></td>
<td><em>S. tuberosum</em></td>
<td></td>
</tr>
<tr>
<td>UBQ10_F</td>
<td>5’- GTACTTTGCGGATTACAAACATC-3’</td>
<td>Ubiquitin</td>
<td><em>A. thaliana</em></td>
<td>(Arias Gaguancela et al., 2016)</td>
</tr>
<tr>
<td>UBQ10_R</td>
<td>5’- GAATACCTCCTTGATTTGATCT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nb-bZIP28 FWD Set1</td>
<td>5’-CATGGAGCCGCTGATAAGAA-3’</td>
<td><em>bZIP28</em></td>
<td><em>N. benthamiana</em></td>
<td>Developed in this study</td>
</tr>
<tr>
<td>Nb-bZIP28 REV Set1</td>
<td>5’-CACTGCCGGACTTGATCT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benth-bZIP17 qPCR FWD</td>
<td>5’-CCTATGGCTCTCAATGCCCTAT-3’</td>
<td><em>bZIP17</em></td>
<td><em>N. benthamiana</em></td>
<td>Developed in this study</td>
</tr>
<tr>
<td>Benth-bZIP17 qPCR REV</td>
<td>5’-GGATGTCTCAAGGGTTTCT-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qPCR_AtbZIP28 28 FWD Set1</td>
<td>5’-GAGAGATAGTGTGAGAGAGGATA-3’</td>
<td><em>bZIP28</em></td>
<td><em>A. thaliana</em></td>
<td>Developed in this study</td>
</tr>
<tr>
<td>qPCR_AtbZIP28 28 REV Set1</td>
<td>5’-GGGACATAGAGAGAGCAAGAGAG-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primer name</td>
<td>Sequence</td>
<td>Target gene</td>
<td>Host</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------------------------</td>
<td>-------------</td>
<td>--------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>AtbZIP17 FWD</td>
<td>5'-CCTATGGCTCAATGCCCTAT-3'</td>
<td>bZIP17</td>
<td>A.thaliana</td>
<td>Developed in this study</td>
</tr>
<tr>
<td>AtbZIP17 REV</td>
<td>5'-GGATGTCTCAAGGGTGGTCC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SolbZIP60_FWD</td>
<td>5'-CCTGCTTTGGCTATGGGCTCAT-3'</td>
<td>bZIP60</td>
<td>N. benthamiana</td>
<td>(Zhang et al., 2015)</td>
</tr>
<tr>
<td>SolbZIP60_REV</td>
<td>5'-AGAAGACCGTGGTCTCTGCTTCG-3'</td>
<td></td>
<td>S. tuberosum</td>
<td></td>
</tr>
<tr>
<td>SolBI-1for SolBI-1rev</td>
<td>5'-TCCGCCAGATCTCCTCCTT-3' 5'-AGCCACACTATGCTCCCAC-3'</td>
<td>BI-1</td>
<td>N. benthamiana</td>
<td>(Arias Gaguancela et al., 2016)</td>
</tr>
<tr>
<td>bZIP60_FWD</td>
<td>5'-GCCTATCCCCCTATATGTCAC-3'</td>
<td>bZIP60</td>
<td>A.thaliana</td>
<td>(Moreno et al., 2012)</td>
</tr>
<tr>
<td>bZIP60_REV</td>
<td>5'-GAACCTTCATCTCCGACTAAC-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AtBI-1_FWD</td>
<td>5'-GTAGCAAGCAGCGAGGAGT-3'</td>
<td>BI-1</td>
<td>A.thaliana</td>
<td>(Arias Gaguancela et al., 2016)</td>
</tr>
<tr>
<td>AtBI-1_REV</td>
<td>5'-CTTCAAGTGCCACACACG-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVY-F</td>
<td>5'-TGGAGTGTGGCTCGCTATG-3'</td>
<td>PVY gRNA</td>
<td>N. benthamiana</td>
<td>(Arias Gaguancela et al., 2016)</td>
</tr>
</tbody>
</table>
Table 2: list of primers used for detection of binding protein (BiP) in *A. thaliana*, *N. benthamiana* and *S. tuberosum* leaves treated with potexvirus TGB3 and potyvirus 6K2 elicitors.

<table>
<thead>
<tr>
<th>Host</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. thaliana</em></td>
<td>qPCR_AtBiP1/2 F</td>
<td>5’-TCACTTGGGAGGTGAGGACTTT-3’</td>
<td>AtBiP1</td>
</tr>
<tr>
<td></td>
<td>qPCR_AtBiP1/2 R</td>
<td>5’-CTCACATTCCCTTCGGAGCTTA-3’</td>
<td>AtBiP2</td>
</tr>
<tr>
<td><em>A. thaliana</em></td>
<td>AtBIP3- F2</td>
<td>5’-GTGGTGAAAGTGGAAGAAGAAA-3’</td>
<td>NbBiP3</td>
</tr>
<tr>
<td></td>
<td>AtBIP3- R2</td>
<td>5’-CACCCTGTTCCTCGGATATTT-3’</td>
<td>AtBiP3</td>
</tr>
<tr>
<td><em>N. benthamiana</em></td>
<td>NbBLP1g022 FWD Set4</td>
<td>5’-GCTGTCTCAATCTTCTTCTTCTCTC-3’</td>
<td>NtBiP1</td>
</tr>
<tr>
<td></td>
<td>NbBLP1g022 REV Set4</td>
<td>5’-TGATGCCCCTACAGTCTTC-3’</td>
<td>NtBiP2</td>
</tr>
<tr>
<td><em>N. benthamiana</em></td>
<td>NbBLP2g022 FWD Set3</td>
<td>5’-AGACTGCTCCACCTCTTCTTCTTCTTACT-3’</td>
<td>NtBiP1</td>
</tr>
<tr>
<td></td>
<td>NbBLP2g022 REV Set3</td>
<td>5’-GTCGATGAGAAGGAGAAGATTG-3’</td>
<td>NtBiP3</td>
</tr>
<tr>
<td><em>N. benthamiana</em></td>
<td>NtBiP3 FWD Set1</td>
<td>5’-GCGGTGAAAGGAGACTATG-3’</td>
<td>NtBiP3</td>
</tr>
<tr>
<td></td>
<td>NtBiP3 REV Set1</td>
<td>5’-GCTCCGCGCTTCTTTGATATG-3’</td>
<td>NtBiP1</td>
</tr>
<tr>
<td><em>N. benthamiana</em></td>
<td>NtBLP4/5/8 F</td>
<td>5’-AAGGAGGCTGGAGGAGTTTGC-3’</td>
<td>NtBiP4</td>
</tr>
<tr>
<td></td>
<td>NtBLP4/5/8 R</td>
<td>5’-TCAGCAGCTCTGGTTCGTC-3’</td>
<td>NtBiP5</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Host</th>
<th>Primer name</th>
<th>Sequence</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NbBiP4</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NbBiP5</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NbBiP8</strong></td>
</tr>
<tr>
<td><em>N. benthamiana</em></td>
<td>benthBIPS_00005</td>
<td>5'-GCTCCACTGACTCTTTGGTATTG-3'</td>
<td><strong>NbBiP5</strong></td>
</tr>
<tr>
<td></td>
<td>FWD Set</td>
<td>5'-GTTGTCTGCTGATCCTGGTAAG-3'</td>
<td><strong>NbBiP4</strong></td>
</tr>
<tr>
<td></td>
<td>benthBIPS_00005</td>
<td></td>
<td><strong>NbBiP5</strong></td>
</tr>
<tr>
<td></td>
<td>REV Set</td>
<td></td>
<td><strong>StBiP-D5</strong></td>
</tr>
<tr>
<td><em>S. tuberosum</em></td>
<td>Potato BIP7710</td>
<td>5'-CTCTCTTCGATGGTGTGGATTT-3'</td>
<td><strong>NtBiP5</strong></td>
</tr>
<tr>
<td></td>
<td>qPCR FWD</td>
<td>5'-GCCTTCTTGACAGAGTCATT-3'</td>
<td><strong>NtBiP2</strong></td>
</tr>
<tr>
<td></td>
<td>Potato BIP7710</td>
<td></td>
<td><strong>NbBiP4</strong></td>
</tr>
<tr>
<td></td>
<td>qPCR REV</td>
<td></td>
<td><strong>StBiP-D5</strong></td>
</tr>
<tr>
<td><em>S. tuberosum</em></td>
<td>Potato BIP1937</td>
<td>5'-GAGGGTGGAGACGAAACTAAAG-3'</td>
<td><strong>NtBiP1</strong></td>
</tr>
<tr>
<td></td>
<td>qPCR FWD</td>
<td>5'-GGGACTTCTTGGGTGGGATAA-3'</td>
<td><strong>NtBiP2</strong></td>
</tr>
<tr>
<td></td>
<td>Potato BIP1937</td>
<td></td>
<td><strong>NtBiP3</strong></td>
</tr>
<tr>
<td></td>
<td>qPCR REV</td>
<td></td>
<td><strong>NbBiP1</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>NbBiP2</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>StBiP-D3</strong></td>
</tr>
<tr>
<td><em>S. tuberosum</em></td>
<td>PGSC42780 FWD</td>
<td>5'-CTAGTGACAAGTCGCTCTAAG-3'</td>
<td><strong>SiBiP-A1</strong></td>
</tr>
<tr>
<td></td>
<td>Set1</td>
<td>5'-GAGCATCCACCTTCTCCTTAC-3'</td>
<td><strong>StBiP-A2</strong></td>
</tr>
<tr>
<td></td>
<td>PGSC42780 REV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Set1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER IV

RESULTS

Patterns of virus local and systemic infection in \textit{IRE1a} and \textit{IRE1b} defective \textit{Arabidopsis thaliana} plants

IRE1 is a transmembrane protein that is a sensor of ER stress and malformed proteins. IRE1 leads a signaling cascade to limit accumulation of malformed proteins and maintain appropriate ER functions. \textit{Arabidopsis} has two IRE1 proteins, IRE1a and IRE1b which are responsible for regulating \textit{bZIP60} mRNA splicing (Chen \textit{et al.}, 2012, 2013; Korner \textit{et al.}, 2015). The \textit{Potato virus X} (PVX) TGB3 or \textit{Turnip mosaic virus} (TuMV) 6K2 proteins specifically activate \textit{IRE1} to splice the \textit{bZIP60} mRNA but the mechanism for IRE1 to recognize these viral proteins is not known (Ye \textit{et al.}, 2011; Ye \textit{et al.}, 2013; Zhang \textit{et al.}, 2015; Arias Gaguancela \textit{et al.}, 2016). It was undertaken investigations to characterize the roles of \textit{IRE1a} and \textit{IRE1b} in potexvirus (\textit{Plantago asiatica mosaic virus}; PlAMV) and potyvirus (TuMV) infection in \textit{Arabidopsis} plants.

It was examined whether \textit{IRE1a} and \textit{IRE1b} restrict PlAMV-GFP infection in inoculated leaves. Six leaves per wild-type and T-DNA tagged mutant lines \textit{ire1a-2}, \textit{ire1b-4} and
ire1a-2/ire1b-4 were inoculated with PLAMV-GFP. At 6 days post inoculation (dpi) leaves were detached, then photographed and subjected to Image J software analysis to quantify the average fluorescence values (FVs) across the leaf area.

The average FVs for PLAMV-GFP in mutant lines were compared with each other and the average FVs in wild-type Col-O using ANOVA. The FVs was 3- to 4-fold higher in ire1a2 (P<0.01) or ire1a-2/ire1b-4 (P<0.001) mutants than in wild-type Col-O leaves (Figure 1A). The FVs in ire1b-4 mutant and Col-O leaves were not significantly different (P≥0.1). These data indicated that PLAMV-GFP accumulation is higher in ire1a2 mutant plants suggesting that IRE1a but not IRE1b restricts virus movement in the inoculated leaves.

To further examine whether PLAMV-GFP infection is restricted in inoculated leaves it was used immunoblot detection to examine the levels of viral CP at 6 dpi. Immunoblot analysis revealed that the PLAMV CP accumulated to higher levels in ire1a2 or ire1a-2/ire1b-4 mutants than in ire1b-4 or Col-O (Figure 1B). These combined data indicated that knockout of IRE1a but not IRE1b leads to greater levels of PLAMV-GFP accumulation in inoculated leaves.

Next, it was examined whether virus accumulation was altered in systemically infected leaves. It was monitored the number of plants that became systemically infected over a time course of 24 dpi (Figure 2A). It was recorded the percentage of infected 20 plants at 10 and 12 dpi (Figure 2B) and it was measured the average FVs in systemic leaves to monitor the levels of virus accumulation over the 24 day time course (Figure 2C). At 10 dpi, 40-50% of Col-O, ire1a2 or ire1b-4 plants were systemically infected, whereas 100% of the mutant ire1a-2/ire1b-4 plants were infected (Figure 2B). At 12 dpi, 55% of Col-O or ire1b-4 plants while 85% of ire1a2 plants were systemically infected. These data suggested that knock out of the IRE1a gene, has a greater effect than knock out of IRE1b to limiting the systemic movement of PLAMV-GFP.
PlAMV-GFP was inoculated to six wild-type Col-O and mutant plants, photographed the whole plant canopy at 10, 12, 17, 19 and 24 dpi (Figure 2A), and used Image J software to obtain the average FVs as a measure of systemic virus accumulation. The average FVs were plotted over time and compared using ANOVA. The average FVs were significantly higher in ire1a2 (P<0.05) or ire1a-2/ire1b-4 (P<0.01) than Col-O. PlAMV-GFP accumulation in wild-type Col-O and ire1b-4 mutant plants were similar (P≥0.1; Figure 2C). These combined data indicated that IRE1a plays a greater role in PlAMV-GFP infection than IRE1b.

Similar experiments were carried out using the TuMV-GFP infectious clone provided by Dr. Aiming Wang (Southern Crop Protection and Food Research Center) to examine if IRE1a and/or IRE1b plays a role in virus accumulation. In the inoculated leaves, the average FVs were 4- to 5-fold higher in ire1a2 (P<0.05) and ire1b-4 (P<0.01) mutant leaves than Col-O leaves. The average FVs in ire1a-2/ire1b-4 double mutant lines (P<0.001) was 6-fold greater than in Col-O leaves (Figure 1C). The immunoblot analysis revealed that TuMV CP was higher in ire1a2, ire1b-4 or ire1a-2/ire1b-4 than in Col-O leaves (Figure 1D). These data indicated that IRE1a and IRE1b comparably influence TuMV-GFP accumulation in inoculated leaves. There did not appear to be a preferred dependence upon IRE1a as for PlAMV-GFP.

Next, it was examined TuMV-GFP accumulation in systemically infected leaves. It was monitored the number of plants that became systemically infected over a time course of 24 dpi (Figure 3A). It was recorded the percentage of infected 18 plants at 10 and 17dpi (Figure 3B) and it was measured the average FVs in systemic leaves to monitor the levels of virus accumulation over the 24 day time course (Figure 3C). At 10 dpi, 0% of wild-type Col-O were systemically infected whereas 44% of ire1a, and 100% of ire1b-4 and ire1a-2/ire1b-4 plants were systemically infected (Figure 3B). At 17 dpi, approximately 50% of Col-O plants were systemically and 100% of ire1a2, ire1b-4 and ire1a-2/ire1b-4 plants were systemically infected (Figure 3B). The average FVs in six TuMV-GFP systemically infected plants were obtain at 10,
12, 17, 19 and 24 dpi. TuMV-GFP accumulated to higher levels in *ire1b-4* (*P*<0.05) and *ire1a-2/ire1b-4* (*P*<0.01) than Col-O. TuMV-GFP accumulation was not significantly different among *ire1a-2* and wild-type Col-O plants (*P*≥0.1; Figure 3C). These data indicated that *IRE1b* has a greater role in restricting TuMV-GFP systemic accumulation than *IRE1a*.

These data indicated that *IRE1a* restricts PlAMV-GFP movement in both local and systemic plants tissues. *IRE1a* might participated in recognition of PlAMV TGB3. In the case of TuMV-GFP infection, virus restriction was equally affected by mutations in either *IRE1a* or *IRE1b* in inoculated leaves but *IRE1b* played a greater role in restricting systemic accumulation. These results suggested that both *IRE1* genes might participate in recognition of TuMV 6K2.
Figure 1: PlAMV-GFP and TuMV-GFP infecting local leaves of Arabidopsis plants. (A) and (C) show representative images of PlAMV-GFP and TuMV-GFP inoculated leaves at 6 days post inoculation (dpi). Fold of increase of average fluorescence values (FVs) for each mutant line with respect to Col-O are written below each photo. Each photo was taken under UV light. Healthy leaves auto fluorescence red under UV light, while green fluorescence shows the pattern of PlAMV-GFP or TuMV-GFP fluorescence. (B) and (D), Immunoblots detected viral CP in PlAMV-GFP and TuMV-GFP inoculated leaves. Lane 1b is ire1b-4, lane 1a is ire1a-2, lane 1a/b is ire1a-2/ire1b-4 mutant, lane C is Col-O and lane C (-) corresponds to healthy control. Ponceau S staining below immunoblots show equal loading of ribosomal protein in each well.
Figure 2: PiAMV-GFP infecting systemic tissues of IRE1 mutant Arabidopsis plants. (A) Representative images of PiAMV-GFP systemic infected Arabidopsis wild-type, ire1a-2, ire1b-4 and ire1a-2/ire1b-4 mutant lines taken between 10 and 24 days post inoculation (dpi). Photos were taken with a UV lamp. Leaves auto fluorescence red under UV light, while green fluorescence shows the pattern of PiAMV-GFP fluorescence. Red squares around the photo outlines the first day that systemic infection was first observed. (B) Percent of systemically infected plants at 10 or 12 dpi calculated from 20 plants per wild-type and mutant line. (C) Graphical representation of the average fluorescence values (FVs) in PiAMV-GFP infected systemic leaves (six plants per wild-type and mutant line) recorded at 10, 12, 17, 19 and 24 dpi.
Figure 3: TuMV-GFP systemically infected wild type and IRE1 mutant Arabidopsis plants. (A) Representative images of TuMV-GFP systemically infected Arabidopsis between 10 and 24 days post inoculation (dpi); wild type, ire1a-2, ire1b-4 and ire1a-2/ire1b-4 mutant lines. Photos were taken with a UV lamp. (B) Percent of systemically infected plants at 10 or 17 dpi calculated from 18 plants per wild-type and mutant line (C) Graph of the average fluorescence values (FVs) (six plants per wild-type and mutant line) represent TuMV-GFP accumulation recorded at 10, 12, 17, 19 and 24 dpi.
qRT-PCR analysis of bZIP60, bZIP17 and bZIP28 induction following treatment with viral elicitors

bZIP17, bZIP28 and bZIP60 are transcription factors tethered to the ER. Activation of bZIP60 is dependent upon IRE1 splicing the bZIP60 mRNA to remove the transmembrane encoding domain. Project collaborator Dr Aiming Wang previously showed that bZIP60 expression is upregulated following delivery of the PVX TGB3 or TuMV 6K2 genes (Ye et al., 2011; Ye et al., 2013; Zhang et al., 2015). It was used qRT-PCR to learn if bZIP17 or bZIP28 transcripts were elevated following agro-delivery of PLAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 to Arabidopsis (Col-O ecotype) and N. benthamiana leaves. Control leaves were treated with Agrobacterium only.

qRT-PCR assays were performed using RNA extracted from inoculated leaves at 2 and 5 days post inoculation to observe changes in the levels of bZIP17, bZIP28, and bZIP60 transcripts (Figure 4A). At 2 dpi, the level of bZIP60 transcripts in PVX TGB3 or PVY 6K2 infiltrated Arabidopsis Col-O leaves was 2.6-fold above the control (P<0.05) and there was no change in bZIP60 transcripts following delivery of PLAMV TGB3 or TuMV 6K2 (P<0.05). There was no change in bZIP17 and bZIP28 transcript accumulation in Arabidopsis leaves following delivery of PLAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 (P<0.05; Figure 4A).

qRT-PCR assays were performed at 5 dpi and the level of bZIP17 transcripts was ~2-fold above the control following agro-delivery of the PLAMV TGB3, TuMV 6K2 or PVY 6K2 (P<0.05) but not PVX TGB3 genes (P<0.05). For bZIP28, an average transcript level was ~2-fold above the control following agro-delivery of TuMV 6K2 or PVY 6K2 (P<0.05) but not PLAMV TGB3 or PVX TGB3 (P≥0.01; Figure 4A). bZIP60 transcript level was between 2.4 and 3.6-fold above the control following agro-delivery of TuMV 6K2 or PVY 6K2 (P<0.05) but not following agro-delivery of PLAMV TGB3 or PVX TGB3 (P≥0.01; Figure 4A). The two potyvirus 6K2
genes appeared to induce expression of \textit{bZIP60}, \textit{bZIP17} and \textit{bZIP28} at either 2 or 5 dpi. The two potexvirus TGB3 genes appeared to induce either \textit{bZIP60} or \textit{bZIP17} at 2 or 5 dpi.

Next, qRT-PCR assays were performed using RNA extracted from \textit{N. benthamiana} leaves following agro-delivery of these four virus genes (Figure 4B). At 2 dpi following agro-delivery of PiAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 genes, there was no change in the levels of \textit{bZIP17} transcripts ($P<0.05$), whereas \textit{bZIP28} was elevated 2.3- to 3- fold and \textit{bZIP60} was elevated 6.6- to 11.3- fold ($P<0.05$; Figure 4B). At 5 dpi, \textit{bZIP17} transcripts were 3.2 to 4- fold following agro-delivery of PVX TGB3 or PVY 6K2. \textit{bZIP28} transcripts were elevated by 2.8 to 5-fold following agro-delivery of PiAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 genes above the control ($P<0.05$). The \textit{bZIP60} average transcript was 2.3-fold following agro-delivery of PVX TGB3 ($P<0.05$) but not following agro-delivery of PiAMV TGB3, TuMV 6K2 or PVY 6K2 genes ($P \geq 0.05$; Figure 4B). These data obtained in \textit{N. benthamiana} plants indicated that the four viral proteins induce \textit{bZIP60} and \textit{bZIP28} and two viral proteins appeared to activate \textit{bZIP17}. In both \textit{Arabidopsis} and \textit{N. benthamiana}, the transcript levels for each gene changed, however the timing and levels of transcript accumulation in \textit{N. benthamiana} was somewhat different than in \textit{Arabidopsis}. 


Figure 4: Results of qRT-PCR detecting bZIP17, bZIP28 and bZIP60 in Arabidopsis Col-O (A) and N. benthamiana (B) leaves at 2 and 5 days post inoculation (dpi). Key below graphs identify bars corresponding to each viral elicitor treatment. Control leaves were infiltrated with Agrobacterium (Agro) that does not deliver a viral gene. Each colored bar is an average of three independently infiltrated leaves. Error bars represent the level of standard deviation in each treatment. ANOVA and Tukey methods were used for evaluating statistical differences. Bars that were statically different from the control were identified with the letter “a” for a significance level of ($P<0.05$).
Patterns of virus local and systemic infection in \textit{bZIP17}, \textit{bZIP28} and \textit{bZIP60} defective \textit{Arabidopsis} plants

It was investigated PlAMV-GFP accumulation in inoculated and systemically infected leaves of wild-type Col-O and \textit{bzip17}, \textit{bzip28} and \textit{bzip60}, \textit{bzip17/bzip60}, and \textit{bzip28/bzip60} plants. Evelyn Vasquez, a visiting scholar at OSU (Department of Entomology of Plant Pathology) was in charge of agro-inoculating and monitoring the patterns of infection in these plants. I performed the (photography) and Image J analysis to obtain average FVs. The average FVs compared using ANOVA to determine if knock down of these genes altered the accumulation of PlAMV-GFP.

The average FVs in PlAMV-GFP inoculated leaves were calculated for six leaves of wild-type or mutant \textit{Arabidopsis} plants at 5 dpi (Figure 5A). In \textit{bzip17}, \textit{bzip60}, or \textit{bzip17/bzip60} inoculated leaves, the average FVs were 7- to 9- fold higher than Col-O infected leaves \((P<0.001)\). For \textit{bzip28}, \textit{bzip28/bzip60} mutant plants, the average FVs were ~2- to 3- fold greater than Col-O infected leaves (Figure 5A). These data showed that in \textit{Arabidopsis} \textit{bZIP17} or \textit{bZIP60} have a greater role in restricting PlAMV-GFP accumulation in local leaves.

\textit{Arabidopsis} plants that were systemically infected with PlAMV-GFP were photographed at 10, 12, 17 and 19 dpi, using a six plants per wild-type and mutant line. The average FVs were determined using Image J software analysis as in prior experiments involving \textit{IRE1}-defective plants (Figure 6A). Statistical analysis of the trend lines indicated that the average FVs were higher in \textit{bzip17}, \textit{bzip60} or \textit{bzip17/bzip60} mutant plants than in wild-type Col-O plants \((P<0.001\); Figure 6B). At 19 dpi the average FVs for PlAMV-GFP in the \textit{bzip17/bzip60} mutant plants was \(~60 \times 10^3\) FVs, in \textit{bzip17} mutant plants was \(31 \times 10^3\) FVs and in \textit{bzip60} was \(57 \times 10^3\) FVs (Figure 6B). The trend lines for PlAMV-GFP systemic accumulation in \textit{bzip28} and \textit{bzip28/bzip60} was different from all other plants \((P<0.005)\) (Figure 6B). At 19 dpi the average FVs in \textit{bzip28} mutants was \(17.4 \times 10^3\) FV and in \textit{bzip28/bzip60} mutant plants was \(13.2 \times 10^3\) FVs (Figure 6B).
The average FVs in wild-type Col-O at 19 dpi was $8.4 \times 10^3$ FVs. These data demonstrated that knocking out $bZIP17$ and/or $bZIP60$ expression led to higher levels of systemic PlAMV-GFP accumulation than in wild-type or $bzip28$ knock out plants. Importantly knocking out $bZIP28$ also led to greater accumulation of PlAMV-GFP, but not to the same extent as $bZIP17$ or $bZIP60$ mutations.

TuMV-GFP was inoculated in the same wild-type and mutant plants to examine whether $bZIP17$, $bZIP28$ or $bZIP60$ play a role in virus restriction in inoculated leaves and systemic plants. FVs were measured in a six inoculated leaves per wild-type and mutant line at 8 dpi (Figure 5B). The FVs in the inoculated leaves of $bzip28$ ($P<0.01$) or $bzip60$ ($P<0.05$) mutant plants were 3- fold higher than in wild-type Col-O plants. The average FVs for TuMV-GFP accumulation in the inoculated leaves of $bzip17$ or $bzip17/bzip60$ mutants plants and wild-type Col-O plants were not significantly different ($P\geq0.1$; Figure 5B). These data suggested that $bZIP28$ and $bZIP60$ restrict TuMV-GFP infection.

TuMV-GFP systemically infected Arabidopsis plants were photographed at 10, 12, 17 and 19 dpi. The average FVs in six plants per wild-type and mutant line were calculated and plotted as in prior experiments involving IRE1 mutant plants (Figure 7A). Statistical analysis of the trend lines indicated that the average FVs were higher in $bzip28$, $bzip60$, $bzip17/bzip60$ and $bzip28/60$ mutant plants than in Col-O wild-type plants ($P<0.001$; Figure 7B). The trend lines for $bzip17$ and Col-O wild-type plants were not significantly different ($P\geq0.05$; Figure 7B). In a lower scale, $bzip28$, $bzip60$ and $bzip17$ accumulated TuMV-GFP in a greater level than Col-O wild-type plants ($P<0.001$). These data indicated that knocking out $bZIP28$, $bZIP60$ or the combined genes leads to greater systemic accumulation of TuMV-GFP.
Figure 5: PIAMV-GFP and TuMV-GFP infecting local leaves of wild-type Col-O, *bzip17*, *bzip28*, *bzip60*, *bzip60/bzip17* and *bzip60/bzip28* Arabidopsis mutant lines. (A) and (B) show representative images of PIAMV-GFP and TuMV-GFP inoculated leaves at 5 and 8 days post infiltration (dpi), respectively. Fold of average fluorescence values (FVs) of each plant line respect to Col-O are written below each photo. Each photo was taken under UV light.
Figure 6: PlAMV-GFP infecting systemic tissues of Arabidopsis plants. (A) shows a sequence of representative images representing the progress of PlAMV-GFP systemic infection in wilt-type Col-O, bzip17, bzip28, bzip60, bzip60/bzip17 and bzip60/bzip28 mutant lines from 10 to 19 days post infiltration (dpi). Photos were taken with a UV lamp. (B), trend line graph of average fluorescence values (FVs) represent the level of PlAMV-GFP accumulation in the systemic tissues of six Arabidopsis plants recorded at 10, 12, 17 and 19 dpi.
**Figure 7:** TuMV-GFP infecting systemic tissues of *Arabidopsis* plants. Section (A) shows a sequence of representative images representing the progress of TuMV-GFP systemic infection in wilt-type Col-O, bzip17, bzip28, bzip60, bzip60/bzip17 and bzip60/bzip28 mutant lines from 10 to 19 days post infiltration (dpi). Photos were taken with a UV lamp. (B), trend line graph of average fluorescence values (FVs) represent the level of PIAMV-GFP accumulation in the systemic tissues of six *Arabidopsis* plants recorded at 10, 12, 17 and 19 dpi.
qRT-PCR analysis of ER luminal binding protein (BiP) gene expression following treatment with viral elicitors

The ER luminal binding protein (BiP) belongs to a family of hsp70 chaperones and its expression can be induced by the transcription factors bZIP60, bZIP28, and bZIP17. BiP is a master regulator in the ER lumen involved in protein translocation and protein folding and is linked with protection against several forms of abiotic and biotic stress (Carolino et al., 2003; Carvalho et al., 2014). Dr Verchot’s laboratory reported that BiP gene expression increases following agro-deliver of the PVX TGB3 or following PVX-GFP inoculation to N. benthamiana plants (Ye et al., 2011; Ye et al., 2013). Each plant species has several copies of BiP genes. In Arabidopsis plants there are AtBiP1, AtBiP2 and AtBiP3. In N. benthamiana, there are NbBiP1, NbBiP2, NbBiP3, NbBiP4/5/8 and NbBiP5. Dr Dennis Halterman (USDA-ARS) used the SPUD database (Potato Genomics Resource) to identify candidate BiPs in S. tuberosum and Dr Verchot used these sequences to conduct phylogenetic analysis to relate these genes to those in Arabidopsis and N. benthamiana, N. tabacum and potato. Dr Halterman identified three BiP candidate genes recognized by the accessions numbers: PGSC003DMP400042780, PGSC0003DMT400031937, and PGSC003DMT400047710. For the purposes of this study, they were named as StBiP-A-2, StBiP-D3, and StBiP-D5, respectively.

It was performed qRT-PCR assays to determine if BiP gene expression in Arabidopsis, N. benthamiana increases following agro-delivery of the PIAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 genes. In S. tuberosum, BiP gene expression was measured following agro-delivery of PVX TGB3 or PVY6K2. Control leaves were treated with Agrobacterium only. RNA was extracted from the inoculated leaves at 2 and 5 days from Arabidopsis and at 2 days from N. benthamiana and S. tuberosum leaves. RNA was used for the qRT-PCR assays.

It was used two sets of qRT-PCR primers for detecting Arabidopsis genes (Figure 8A, Table 2). AtBiP1 and AtBiP2 have 99% similarity therefore one set of PCR primers (AtBiP1/2)
detect both genes. A second set of primers detect AtBiP3 only. With regard to the combined AtBiP1 and AtBiP2 transcripts, there was no variation in transcript accumulation following delivery of any viral elicitor at 2 dpi. At 5 dpi, the average level of the combined AtBiP1 and AtBiP2 was elevated ~2-fold above the average level in control leaves following agro-delivery of TuMV 6K2 or PVY 6K2 but not PLAMV TGB3 or PVX TGB3. Increased AtBiP1/2 accumulation was a specific response to the potyvirus 6K2 gene ($P<0.05$; Figure 8A).

With regard to AtBiP3 transcripts, the average level at 2 dpi was ~2-fold higher in leaves following agro-delivery of PVX TGB3 compared to control leaves, but there was no variation in the average levels following delivery of PLAMV TGB3, TuMV 6K2 or PVY 6K2. At 5 dpi following agro-delivery of PVX TGB3, PLAMV TGB3, TuMV 6K2 or PVY 6K2, the average levels of AtBiP3 transcripts increased to levels between 2.5- and 3.9-fold above the control ($P<0.05$; Figure 8A).

Five sets of qRT-PCR primers were used for detecting N. benthamiana BiP transcripts (Figure 8B, Table 2) and all data was collected at 2 dpi. Four sets of PCR primers were generated to distinguish NbBiP1, NbBiP2, NbBiP3, and NbBiP5 transcripts. Another primer set named NbBIP4/5/8 detects the combined NbBiP4, NbBiP5, and NbBiP8 transcripts. The average levels of NbBiP1 and NbBiP2 transcripts following agro-delivery of PVX TGB3, PLAMV TGB3, TuMV 6K2, or PVY 6K2, were ~3 to 6-fold above the control ($P<0.05$; Figure 8B). Interestingly, the average levels of NbBiP3 transcripts were 7.7 to 10-fold following agro-delivery of PVX TGB3, PLAMV TGB3, TuMV 6K2, or PVY 6K2 ($P<0.05$). The average levels of the combined NbBiP4/5/8 transcript fell into two categories. For PVX TGB3 and PVY 6K2 treated leaves, the combined transcripts were highly elevated, 17.1 and 13.5-fold over the control, respectively ($P<0.001$). For PLAMV TGB3 and TuMV 6K2 the combined transcripts were elevated to 6.1 and 3.1-fold above the controls, respectively ($P<0.05$). Using NbBiP5 specific primer set, the average levels were 20.7 and 16.6-fold over the control following agro-
delivery of PVY 6K2 and PVX TGB3 ($P<0.001$). The average levels of NbBIP5 transcripts were 9.6 and 6.1-fold above the control following agro-delivery of PIAMV TGB3 and TuMV 6K2, respectively ($P<0.05$; Figure 8B). In *N. benthamiana* the transcript levels for each BiP candidate was elevated. Notably the combined detection of NbBiP4, NbBiP5, and NbBiP8 transcripts, which are not related phylogenetically to AtBiP1, AtBiP2, or AtBiP3 responded most dramatically to the viral elicitors.

In *S. tuberosum*, three primer sets were used to detect StBiP-A, StBiP-D3, and StBiP-D5 candidate genes identified by Dr. Halterman. All qRT-PCR assays were conducted at 2 dpi. The average levels of StBiP-D3 and StBiP-D5 transcripts following agro-delivery of PVX TGB3 and PVY 6K2, were ~2.3 and 3.1 fold above the control ($P<0.05$; Figure 8C). With regard to StBiP-A transcripts, there was no variation in transcript accumulation following delivery of any viral elicitor. These genes are phylogenetically distinct from the *Arabidopsis* AtBiP1, AtBiP2, or AtBiP3 genes.
**Figure 8:** Results of qRT-PCR detecting BiP genes in detached leaves from *Arabidopsis* (A) and *N. benthamiana* (B) and (C) *S. tuberosum* inoculated with TGB3 potexvirus or potyvirus 6K2. Key below graphs identify bars corresponding to each viral elicitor treatment. Control leaves were infiltrated with *Agrobacterium* (Agro) that does not deliver a viral gene. Error bars represent the level of standard deviation in each treatment. ANOVA and Tukey methods were used for evaluating statistical differences. Bars that were statically different from the other colored bars were identified with the letter “a” or an asterisk “∗” for significance levels of (*P*<0.05) and (*P*<0.001), respectively.
Comparison of the patterns of local and systemic PlAMV-GFP and TuMV-GFP infection in BI-1, IRE1a/IRE1b and bZIP60 defective Arabidopsis plants

BI-1 is a cell death suppressor that resides in the ER. It was hypothesized that BI-1 expression was controlled by IRE1 and bZIP60 based on reports from (Lisbona et al., 2009; Ishikawa et al., 2011b). To test this hypothesis, my colleague Liz Peña conducted qRT-PCR assays to determine if BI-1 expression was altered in ire1a-2/ire1b-4 and wild-type Arabidopsis plants. Based on her results it was not possible to conclude that there was any pattern of gene expression linking BI-1 to IRE1a, IRE1b or bZIP60. In this study, plants that were defective for bi-1, ire1a-2/ire1b-4, and bzip60 were inoculated with PlAMV-GFP or TuMV-GFP to determine if these genes similarly impact the ability of each virus to spread locally and systemically. If BI-1 were downstream of IRE1 or bZIP60, the timing of infection and the accumulation of GFP and CP would be comparable in mutant lines. If BI-1 was independent of IRE1 or bZIP60, GFP and CP accumulation would be unique.

The FVs for PIAMV-GFP in bi-1, irea1a-2/ire1b-4, bzip60 mutant and wild-type Col-O plants were examined and compared. Six leaves per wild-type and T-DNA tagged mutant lines were inoculated with PIAMV-GFP. At 6 dpi, the average FVs for PIAMV-GFP accumulation in mutant lines were compared with each other and the average FVs in wild-type Col-O using ANOVA. The average FVs showed that PIAMV-GFP accumulation was 3 to 4-fold higher in bi-1, irea1a-2/ire1b-4, and bzip60 mutant plants than in Col-O wild-type leaves (P<0.001; Figure 9A). BI-1, IRE1a, IRE1b, and bZIP60 similarly restricted PIAMV-GFP accumulation.

To further examine whether virus infection is restricted in inoculated leaves, immunoblot detection was used to examine the levels of viral CP in BI-1 or bZIP60 leaves at 6 dpi. Immunoblot analysis revealed that PIAMV CP accumulated to higher levels in BI-1 or bZIP60 than in wild-type Col-O leaves (Figure 9B). These data indicated that BI-1, IRE1, and bZIP60 comparably influence PIAMV-GFP accumulation in inoculated leaves.
Next, it was examined whether virus accumulation was altered in systemically infected leaves. The number of plants that became systemically infected were monitored over a time course of 24 dpi (Figure 10A). The percentage of 20 systemically infected plants were observed at 10 and 12 dpi (Figure 10B) and the average FVs were measured in systemic leaves to monitor the levels of virus accumulation over the 24 day time course (Figure 10C). At 10 dpi, between 45% and 50% of wild type Col-O and bzip60 plants were systemically infected whereas 100% of bi-1 mutant lines were systemically infected. At 12 dpi, systemic infection reached 50 to 80% for Col-O and bzip60 plants (Figure 10B). PlAMV-GFP was inoculated to six wild-type Col-O and mutant plants, photographed the whole plant canopy at 10, 12, 17, 19 and 24 dpi (Figure 10A), and used Image J software to obtain the average FVs. The average FVs were plotted over time and compared using ANOVA. PlAMV-GFP accumulate to higher levels in bi-1 mutant lines than in ire1a-2/ire1b-4, bzip60 and in Col-O wild-type plants (P<0.05; Figure 10C). These data indicated that bi-1 has a greater role in restricting systemic PlAMV-GFP accumulation than ire1a-2/ire1b-4 or bzip60. Thus suggesting that BI-1 might not be involved in regulating events upstream of IRE1 or bZIP60 expression.

Similar experiments were carried out using the TuMV-GFP infectious clone provided by Dr. Aiming Wang (Southern Crop Protection and Food Research Center). In the inoculated leaves, the average FVs values in bi-1 mutant plants were 11-fold greater than Col-O. TuMV-GFP accumulation in ire1a-2/ire1b-4 and bzip60 mutant plants (P<0.01) was 3- and 5-fold greater than wild-type Col-O leaves. The immunoblot analysis revealed that the levels TuMV CP were similar in wild-type and mutant lines. Bases on the FVs data, it seemed that BI-1 might played a greater role in TuMV-GFP infection than IRE1 or bZIP60.

Next, TuMV-GFP accumulation was examined in systemically infected leaves. The number of plants that became systemically infected were monitored over a time course of 24 dpi (Figure 11A). The percentage of 18 infected plants was recorded at 10 and 17dpi (Figure 11B).
and the average FVs were measured in systemic leaves to monitor the levels of virus accumulation over the 24 day time course (Figure 11C). At 10 dpi 0% of wild-type Col-O plants were systemically infected whereas 22% of bi-1 and 67% of bzip60 mutant plants were systemically infected. At 17 dpi, 45% of wilt-type Col-O plants were systemically infected whereas 100% of either bi-1 or bzip60 plants were systemically infected at the same time point (Figure 11B). The average FVs in TuMV-GFP systemically infected plants were obtain at 10, 12, 17, 19 and 24 dpi. TuMV-GFP accumulated to higher levels in bi-1, bzip60 and in ire1a-2/ire1b-4 mutant plants than in wild-type Col-O plants (P<0.05; Figure 11C). These data suggested that TuMV-GFP was equally restricted by BI-1 or bZIP60.
Figure 9: PLAMV-GFP and TuMV-GFP infecting local leaves of Arabidopsis plants. For comparisons wild-type Col-O, bi-1, ire1a-2/ire1b-4 and bzip60 mutant lines were utilized during the experiments (A) and (C) show representative images of PLAMV-GFP and TuMV-GFP infecting local leaves at 6 days post infiltration (dpi). Average fluorescence values (FVs) from each mutant line with respect to Col-O were written below each photo. All photos were taken with a hand UV lamp. (B) and (D), immunoblots showed the amount of viral coat protein (CP) detected in inoculated leaves with PLAMV-GFP and TuMV-GFP. Lane C represents Col-O and lane C (-) corresponds to healthy controls. Ponceau S staining below immunoblots show equal loading of plant ribosomal protein in each well.
Figure 10: PlAMV-GFP infecting Arabidopsis systemic leaves. Wild-type Col-O, bi-1, ire1a-2/ire1b-4 and bzip60 mutant lines were utilized during experiments. (A) shows a sequence of representative images of PlAMV-GFP systemic infection in Arabidopsis plants from 10 to 24 days post infiltration (dpi). Photos were taken with a UV lamp. Healthy tissues present red color while green tissues represent the virus infected plant tissues. (B), percentage of systemically infected plants at 10 or 12 dpi calculated from 20 plants per wild-type and mutant line. (C), the trend line graph of average fluorescence values (FVs) from six plants per wild-type and mutant line represent the level of PlAMV-GFP accumulation in the systemic tissues of Arabidopsis plants at 10, 12, 17, 19 and 24 dpi.
Figure 11: TuMV-GFP infecting Arabidopsis systemic leaves. Wild-type Col-O, bi-1, ire1a-2/ire1b-4 and bzip60 mutant lines were utilized during experiments. (A) shows a sequence of representative images of TuMV-GFP systemic infection in Arabidopsis plants from 10 to 24 days post infiltration (dpi). Photos were taken with a UV lamp. Healthy tissues present red color while green tissues represent the virus infected plant tissues. (B), percentage of systemically infected plants at 10 or 17 dpi calculated from 18 plants per wild type and mutant line. (C), the trend line graph of average fluorescence values (FVs) from six plants per wild-type and mutant line represent the level of TuMV-GFP accumulation in the systemic tissues of Arabidopsis plants at 10, 12, 17, 19 and 24 dpi.
Local and systemic PVX-GFP and PVY-GFP infection *bzip60* and *bi-1* silenced *N. benthamiana* plants

Experiments were conducted to determine if silencing *bZIP60* or *BI-1* in *N. benthamiana* leaves leads to greater levels of PVX-GFP or PVY-GFP in inoculated leaves. GFP fluorescence and CP immunoblot analysis were used to monitor virus accumulation in silenced leaves. Dr Dennis Halterman (USDA) prepared pHellsgate-*bzip60si* or pHellsgate-*bi1si* to transiently silence these two genes in *N. benthamiana* plants. Leaves were infiltrated with *Agrobacterium* carrying the pHellsgate-*bzip60si*, pHellsgate-*bi1si* constructs. Semi quantitative RT-PCR assays were conducted to verify that the *bZIP60* and *BI-1* genes were knockdown at 6 days. Samples were amplified for 25, 30, and 35 cycles and then loaded onto 1.5% agarose gels (Figure 12A). The results viewed between 30 and 35 cycles indicated that *bZIP60* or *BI-1* transcripts were reduced 80 to 90% verifying that gene silencing was effective (*P*<0.05; Figure 12A).

PVX-GFP or PVY-GFP with pHellsgate-*bzip60si*, pHellsgate-*bi1si* or *Agrobacterium* (lacking the silencing constructs) were co-delivered to *N. benthamiana* leaves. It was used three samples per treatment and the experiments were repeated three times, and GFP was visualized at 6 dpi. Semi-quantitative RT-PCR was again used to verify knockdown of gene expression in virus inoculated plants. The fluorescence in *bZIP60* or *BI-1* silenced leaves infected with PVX-GFP was greater than in control leaves (Figure 12B). PVY-GFP accumulates slower than PVX-GFP in inoculated leaves and fluorescence was not observed in control or silenced leaves. GFP fluorescence for PVY experiments was not as useful as for PVX-GFP.

Following co-delivery of PVX-GFP or PVY-GFP with the silencing constructs, immunoblot detection was used to determine if the viral CPs were increased in silenced leaves at 6 dpi. Immunoblot analysis revealed higher levels of PVX CP and PVY CP in *bZIP60* or *BI-1* silenced leaves than in control leaves (Figure 12C and D). These data indicated that PVX-GFP and PVY-GFP accumulation increases in the absence of *bZIP60* or *BI-1* in *N. benthamiana*, as for
Arabidopsis plants. These overall data suggested that both bZIP60 and BI-1 can restrict PVX-GFP or PVY-GFP infection in inoculated leaves in N. benthamiana and Arabidopsis.

Systemic PVX-GFP or PVY-GFP infection was monitored to determine if silencing BI-1 or bZIP60 leads to increased systemic virus accumulation. GFP from PVX-GFP or PVY-GFP in nine systemic leaves was examined at 14 dpi. GFP fluorescence due to PVX-GFP or PVY-GFP in BI-1 silenced leaves was higher than in bZIP60 silenced leaves or control leaves (Figure 13A, B). These data indicated that BI-1 has a greater role for restricting systemic PVX-GFP or PVY-GFP infection.

Systemic fluorescence was not observed in PVY-GFP infected plants, although the plants were symptomatic. One possibility is that the virus titer was too low to view GFP using a handheld UV lamp. Therefore qRT-PCR was used to quantify virus genomic RNA accumulation in systemic leaves. At 16 dpi total RNA was extracted and primer sets (Table 1) overlapping the PVY CP ORF were used for qRT-PCR analysis. The levels of PVY genomic RNA in bZIP60 and BI-1 silenced leaves were ~3- and 8-fold higher than in control leaves (P<0.05; Figure 13C). These data indicated that BI-1 restricts PVY-GFP to a greater level than bZIP60.
**Chlorosis and necrosis in bi-1 and bzip60 silenced N. benthamiana plants treated with PVX-GFP or PVY-GFP**

It was observed that silenced plants showed more virus related chlorosis and necrosis than healthy plants. At 6 dpi, PVX-GFP infected and *BI-1* silenced leaves showed severe chlorosis compared to the *bZIP60* silenced or control leaves (Figure 14A). There were no obvious symptoms on PVY-GFP inoculated leaves (Figure 15A).

At 14 dpi, PVX-GFP and PVY-GFP systemically infected *BI-1* silenced plants showed severe stunting, chlorosis and necrosis in the upper crown leaves. PVX-GFP and PVY-GFP systemically infected *bZIP60* silenced plants displayed mosaic symptoms (Figure 14B, C and 15B, C). This information indicated that *BI-1*, a known cell death suppressing protein, protection against virus induced necrosis in *N. benthamiana* plants.
Figure 12: PVY-GFP and PVX-GFP infecting local leaves of *N. benthamiana* plants. (A), semi-quantitative qRT-PCR gels for evaluating *bZIP60* and *BI-1* gene silencing efficiency. *Actin II* was utilized as loading control. Amplifications were carried in 35, 30 and 25 cycles. Expected sizes of 100-200 and 400-500 base pairs (bp) are were found for *bZIP60* and *BI-1* genes, respectively. Lanes titled as “Agro” represent samples treated with *Agrobacterium* and no silencing constructs. Lanes named as “bzip60si” and “bi1si” were *N. benthamiana* leaves agro-infiltrated with pHellsgate-*bzip60si* and pHellsgate-*bi1si* constructs. (B), PVX-GFP infected leaves of *N. benthamiana* at 6 days post infiltration (dpi), photos were taken using UV light. (C) and (D), Immunoblots for detection of PVY-GFP and PVX-GFP viral coat protein (CP) accumulation in detached leaves at 6 dpi (upper image). Ponceau S staining was utilized for assessing equal protein loading in all lanes (lower image). Lanes with the tag H represent healthy control leaves.
Figure 13: PVY-GFP and PVX-GFP infecting systemic leaves of *N. benthamiana* plants. (A), Images of PVX-GFP infecting *bzip60* and *bi-1* silenced *N. benthamiana* plants, agro delivery without the silencing constructs was also used as control. (B), Images of PVY-GFP infecting *bzip60* and *bi-1* silenced *N. benthamiana* plants, agro-delivery without the silencing constructs was used as control. All images were taken using a UV lamp at 14 days post infiltration (dpi). (C), relative RNA levels of PVY-GFP infecting systemic leaves were measured with qRT-PCR; “N” and “Agro” represent *N. benthamiana* that were not silenced, “*bzip60si*” and “*bi1si*” columns represent systemic leaves treated with pHellsgate-*bzip60si* and pHellsgate-*bi1si* constructs. RNA from systemic leaves was extracted at 16 dpi. Error bars represent the level of standard deviation in each treatment. ANOVA and Tukey methods were used for evaluating statistical differences. Bars that were statically different were identified with the letter “a” for a significance level of ($P<0.05$).
Figure 14: photos of PVX-GFP infecting *N. benthamiana* plants. (A) necrosis in inoculated leaves that had co delivery of PVX-GFP with pHellsgate-*bzip60si*, pHellsgate-*bi1si* or *Agrobacterium* lacking the silencing constructs at 6 days post infiltration (dpi). (B) and (C) necrotic symptoms in systemic leaves at 14 dpi.
Figure 15: photos of PVY-GFP infecting *N. benthamiana* plants. (A) necrosis in inoculated leaves that had co delivery of PVY-GFP with pHellsgate-*bzip60si*, pHellsgate-*bi1si* or *Agrobacterium* lacking the silencing constructs at 6 days post infiltration (dpi). (B) and (C) necrotic symptoms in systemic leaves at 14 dpi.
PIAMV-GFP or TuMV-GFP infection levels were different depending upon the $IRE1$ gene. PIAMV-GFP accumulation in inoculated and systemic leaves is higher in plants defective in $IRE1a$ than in wild type or $IRE1b$ defective plants. TuMV-GFP accumulation is equally dependent upon the active presence of $IRE1a$ and $IRE1b$ in inoculated leaves, and systemic accumulation seems to be greatly affected by mutations in $IRE1b$ than in $IRE1a$. Mutations in both $IRE1a$ and $IRE1b$ lead to higher local PIAMV-GFP and TuMV-GFP accumulation levels which suggest that one gene can provide support when the other gene is deleted. The IRE1 protein has two enzymatic domains, an RNAse domain for splicing bZIP60 mRNA or for RIDD mRNA targets, and a kinase domain for auto-phosphorylation (Deng et al., 2013b; Korner et al., 2015). $IRE1a$ and $IRE1b$ proteins have different kinase loops which may mean they do not have the same phosphorylation targets (Moreno et al., 2012). Researchers reported that $IRE1b$ plays a greater role in $bZIP60$ mRNA splicing than $IRE1a$ during ER stress (Deng et al., 2011; Humbert et al., 2012). This suggested that the two $IRE1$ genes may act preferentially on certain functions. There are factors identified in eukaryotes that bind IRE1 proteins, but this list is incomplete.
It is known that CPR5 in plants interacts with IRE1 and is activated by salicylic acid during defense (Meng et al., 2017). In mammals, BiP binds IRE1, but it is not known if that interaction occurs in plants (Chen et al., 2013). BiP is also associated with co-chaperone AtERDj3A and it is reasonable to consider that the cochaperone may be involved in IRE1 interactions along with BiP (Chen et al., 2012).

The PLAMV TGB3 preferentially interacts with IRE1a recognition strategies whereas TuMV 6K2 seems to engage both IRE1 proteins in the inoculated leaves. These data suggested that these viral proteins may engage different domains of IRE1 or intermediary factors leading to activation of UPR and bZIP60 mRNA splicing. IRE1a and IRE1b have been observed to provide some unique and some overlapping activities in response to ER stress in Arabidopsis after exposure with ER stress chemical inducer tunicamycin (Chen et al., 2012; Wan et al., 2016). Although it was not discovered whether either IRE1 gene restrict viral replication, further experiments should be conducted to establish potential roles of IRE1 in virus multiplication.

ER localized transcription factors bZIP17, bZIP28 and bZIP60 are key components of UPR responses during ER stress signaling (Deng et al., 2013a). Their transcript accumulation levels were evaluated following agro-delivery of the potexvirus TGB3 or potyvirus 6K2 in Arabidopsis and N. benthamiana. Prior studies demonstrated that bZIP60 mRNA is induced by the PVX TGB3 and TuMV 6K2 (Ye et al., 2011; Ye et al., 2013; Zhang et al., 2015). The data presented in this thesis confirms bZIP60 mRNA is induced by more than one potexvirus TGB3 or potyvirus 6K2 in two different host species. These data also indicated that gene activation is a conserved function of these viral proteins.

Prior reports indicated that bZIP17 and bZIP28 are primarily induced by abiotic stressors (Liu et al., 2007b, 2007a; Liu et al., 2010). This is the first evidence that bZIP17 and bZIP28 transcripts are elevated following agro-delivery of potexvirus TGB3 or potyvirus 6K2. Although
bZIP17 is not induced by PVX TGB3 in either host, it is elevated in Arabidopsis (but not N. benthamiana) following delivery of TuMV 6K2. bZIP17 and bZIP28 typically are activated by abiotic factors such as ABA, heat, or salt (Liu et al., 2007b, 2007a; Zhou et al., 2015). bZIP60 is more often associated with SA and plant innate immunity as well certain other abiotic stressors. One study showed that treatment of Arabidopsis leaves with the polyamine, spermine led to transcriptional upregulation of bZIP60, bZIP28 and bZIP17 (Sagor et al., 2015). This study demonstrated that spermine is a general activator of these three UPR related pathways. Spermine synthase is activated in RCY1 resistance gene to Cucumber mosaic virus (CMV) strain Y (Mitsuya et al., 2009; Sagor et al., 2015). Increase in spermine in the cell can cause calcium release from the ER lumen which leads to UPR (Pottosin et al., 2014). In this study it is proposed that the potexvirus TGB3 and potyvirus 6K2 proteins, which insert into the ER membrane, may cause some changes in ER calcium stores and this could indirectly lead to general activation of bZIP28 and bZIP17 related pathways. However, there is not direct evidence that bZIP60, bZIP28, or bZIP17 move from the ER to the nucleus.

Virus accumulation in Arabidopsis plants that were defective for bZIP17, bZIP28 or bZIP60 were analyzed and compared. It was found that PlAMV-GFP accumulation was higher in plants defective for bZIP60 and/or bZIP17 than wild-type or bzip28-mutant plants. TuMV-GFP accumulation was higher in plants defective for bZIP60 and/or bZIP28 than in wild-type or bzip17 mutant plants. These data suggested that bZIP60 is a factor in host susceptibility to both viruses whereas bZIP28 and bZIP17 differentially restrict potyvirus and potexvirus infection. The ability of bZIP17 and bZIP28 to respond differently to certain stressors has been documented (Ruberti et al., 2015; Wan et al., 2016). It is known that bZIP28 reacts mainly to heat and cold stress while bZIP17 is mostly involved in salt stress (Liu et al., 2007b, 2007a; Deng et al., 2011; Wan et al., 2016). Further experiments are needed to determine if these factors differently engage the TGB3 and 6K2 proteins.
Prior research conducted in yeast two hybrid systems have demonstrated that \( bZIP17 \), \( bZIP28 \) and \( bZIP60 \) form heterodimers (Liu et al., 2010). These heterodimers exist within a transcription activation complex sitting down on a promoter to activate certain ER stress responsive genes (Llorca et al., 2014). Based on this model, it was suggested a scenario in which bZIP60 and bZIP17 associate to activate genes that restrict PIAMV movement. Alternatively bZIP60 and bZIP28 could associate to activate genes that restrict TuMV movement.

*Arabidopsis* and *S. tuberosum* have three *BiP* candidate genes and *N. benthamiana* has six *BiP*, these are differentially activated by \( bZIP17 \), \( bZIP28 \) and \( bZIP60 \). It was conducted qRT-PCR experiments to measure the transcript level of *BiP* candidates in *Arabidopsis*, *N. benthamiana* and *S. tuberosum* in response to agro-delivery of PIAMV TGB3, PVX TGB3, TuMV 6K2 or PVY 6K2 genes. In *Arabidopsis*, agro-delivery of each TGB3 and 6K2 led to higher levels of \( AtBiP3 \), but \( AtBiP1 \) and \( AtBiP2 \) transcript accumulation was elevated following agro-delivery of TuMV 6K2 or PVY 6K2. IRE1 splices \( bZIP60 \) mRNA and the transcription factor bZIP60s moves to the nucleus and upregulates \( BiP3 \) transcription (Liu et al., 2010).

bZIP17 and bZIP28 also regulate transcription of \( BiP1 \), 2, and 3 (Iwata et al., 2008; Henriquez-Valencia et al., 2015). BiP1 and BiP3 proteins bind bZIP28 and bZIP17 in the ER and suppress their release from the ER membrane (Srivastava et al., 2013). This becomes an autoregulatory process whereby the bZIP transcription factors activate BiP expression to increase protein folding in the ER lumen, but also to shut down their own activation in the ER and restore the cell to homeostasis. BIP2 binds another factor known as AtBAG7, which is a cell death regulating protein located in the ER (Williams, et al., 2010). AtBAG7 can form a heterodimer with bZIP28 (Li et al., 2016c). This is interesting because it appears that the potyvirus 6K2 proteins may play a role in activating *BiP2* which potentially could lead to downregulation of the cell death regulator *AtBAG7*.  

56
In *N. benthamiana*, the transcript levels of *NbBiP1, NbBiP2, NbBiP3, NbBiP5* and *NbBiP8* orthologues and in *S. tuberosum*, *StBiP-D3* and *StBiP-D5* but not *StBiP-A* were induced upon treatment with PVX TGB3 or PVY 6K2. Activation of *BiP* mediated by viral genes resembles prior studies in which treatments with treatments with PVX TGB3 demonstrated a significant *BiP* induction (Ye *et al.*, 2011; Ye *et al.*, 2013). Therefore, this study sheds light on the identification of new *BiP* candidates that can activate in response to virus assault.

This study wanted to determine if cell death suppressor *BI-1*, and UPR genes *IRE1* and *bZIP60* similarly impact the ability of PlAMV-GFP or TuMV-GFP to spread locally and systemically in *Arabidopsis* plants. Prior reports proposed a model in which *BI-1* binds to *IRE1* and downregulates *bZIP60* mRNA splicing (Lisbona *et al.*, 2009; Ishikawa *et al.*, 2011b). Based on this model it would expect that knockout of *BI-1, IRE1* or *bZIP60* should similarly alter the patterns of virus accumulation in local and systemic leaves. Moreover, in this study it was hypothesized that *BI-1* expression was controlled by *IRE1* and *bZIP60*. The data indicated that PlAMV accumulation in local leaves is comparably higher in *bi-1, ire1a-2/ire1b-4*, and *bzip60* mutant plants however TuMV infection was 11 fold higher than in wild-type leaves and 2-3 fold higher than in *ire1a-2/ire1b-4* and *bzip60* mutant plants. One hundred percent of *bi-1* inoculated plants were systemically infected at 10 and 17 dpi with PlAMV-GFP and TuMV-GFP. These data suggested that *bi-1* has a more significant effect on systemic accumulation than *bZIP60*. Similarly, the experiments conducted in *bi1si- and bzip60si- N. benthamiana* plants infected with PVX-GFP or PVY-GFP revealed *BI-1* greater role for restricting virus movement than *bZIP60*.

Prior work has shown that *BI-1* is linked to plant defense responses and functions in the ER (Eichmann *et al.*, 2012). In two studies *BI-1* knockdowns led to enhanced susceptibility to *P. syringae* and *B. graminis* (Kawai-Yamada *et al.*, 2009; Weis *et al.*, 2013). *bZIP60* is also engaged in regulating susceptibility to *P. syringae* (Moreno *et al.*, 2012). The *E. coli* bacterial effector NleH can target *BI-1* to inhibit cell death responses (Robinson *et al.*, 2016). Evidence that
bacterial effectors control activation of BI-1 and other ER stress responses, suggests that the ability of the TGB3 and 6K2 to act on these pathways, may suggest that these viral proteins are also effectors. Pathogen effectors are proteins that interact with a host protein involved in defense recognition (Gouveia et al., 2016). The data provided in this thesis showed that knockdown of the ER stress sensors led to greater virus accumulation. While there was lack of evidence of direct interactions between the viral proteins and these ER stress sensors, it is reasonable to suggest that the UPR pathways provide some protection against virus infection and that the TGB3 and 6K2 proteins may act as effectors of these viral suppressive pathways.
REFERENCES


60


VITA

OMAR PAUL ARIAS GAGUANCELA

Candidate for the Degree of
Master of Science

Thesis: VIRUS-INDUCED ER STRESS IN PLANTS

Major Field: Entomology and Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in July, 2017

Completed the requirements for the Bachelor of Science in Biotechnology at University of the Armed Forces - ESPE, Quito, Ecuador in 2014

Experience:

Graduate Research Assistant, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, August 2015 to July 2017

Undergraduate Research Assistant, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, February 2014 to June 2014

Undergraduate Research Assistant, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, January 2013 to March 2013