

Plant viruses as stressing agents

By, Jozef J. Bujarski
(BIOS 761 - Plant Stress Seminar)

Crop losses due to viral stress

The cost of worldwide crop losses due to plant diseases is estimated at \$60 billion annually.

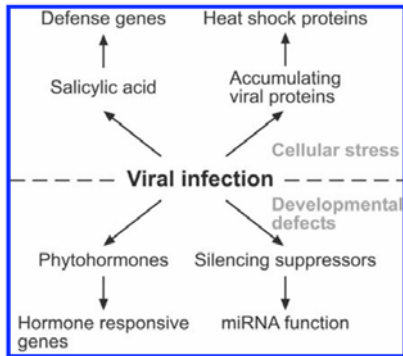
Viruses are generally considered to be the second greatest contributor to those losses (after fungi). The 1000-plus species of plant viruses are responsible for about 20 to 40% of losses due to biotic stress.

Biotic stress: outcome of the disease.

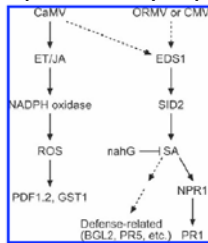
**CATALOGUE OF SYMPTOMS OF
PLANT VIRAL INFECTION**

<http://www.dias.kvl.dk/Plantvirology/esymptoms/symp.html>

Host responses and altered gene expression associated with plant virus infections.



Defense-like responses that are elicited by viruses in susceptible *Arabidopsis* plants.



Cauliflower mosaic virus (CaMV) elicits the expression of jasmonic acid (JA)- and ethylene (ET)-responsive defense-related genes (*PDF1.2* and *GST1*) as well as salicylic acid (SA)-dependent expression of pathogenesis-related genes (*PR-1*). The dashed lines indicate that it is not known if *SID2* or *EDS1* are required to initiate the SA-dependent gene expression in CaMV infections. *Oilseed rape mosaic virus* (ORMV) and *Cucumber mosaic virus* (CMV) elicit the expression of defense-related genes through a pathway dependent on *EDS1*, *SID2*, and SA. The expression of *PR-1* and other defense-related genes (*BGL2* and *PR-5*) occur to branch after SA, because increased *PR-1* expression is strongly dependent on *NPR1*.

REVIEW

**Virus-Induced Disease:
Altering Host Physiology
One Interaction at a Time**

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Annu. Rev. Phytopathol. 2007.45:221-243.

Mechanisms of virus pathogenesis

- Two models
 - resource competition model: usurpation of metabolic resources
 - . e.g. TMV translation accounts for 50% of protein production (shutdown of host genes)
 - . but not for all virus/host systems
 - Disruption of host processes: specific host-virus interactions
 - . significant evidence
 - . Triggering the cascade of events disrupting host cell physiology

TYPES OF VIRUS-HOST INTERACTIONS CAUSING SYMPTOMS

- Consequential interactions
 - Directly contributing to systemic infection and disrupting (TBSV P19 RNAi suppressor-siRNAs; potyviral VPg interacting with eIF4E)
- Inconsequential interactions
 - not contributing to systemic infection but disrupting the physiology indirectly

HORMONE AND DEVELOPMENTAL SIGNALLING

Viral infections can affect the production and accumulation of plant hormones
A typical effect: stunting or leaf curling

AUXIN SIGNALLING

- Auxin controls many developmental and cellular responses
- Interaction of TMV replicase repressors of auxin-responsive factors (see next slide)

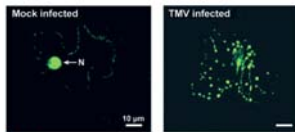


Figure 1
Effect of TMV replicase protein on the localization of an interacting AUX/IAA protein (IAA26) fused to GFP. N denotes the nuclear location of IAA26-GFP in uninfected tissues. In infected tissues, IAA26-GFP is relocalized to cytoplasmic bodies that correspond with the viral replicase protein. Bars = 10 µm.

- replicase mutant defective in the interaction with Aux/IAA produced attenuated symptoms or silencing of Aux/IAA proteins generated symptom-like phenotype
- Conclusion: TMV repl-Aux/IAA interaction relocalizes the protein leading to abnormalities during infection further affecting 30% of genes displaying transcriptional alterations during TMV infection. This way TMV produces a more favorable environment for replication
- A link to host defense responses also possible
- Other data: downregulation of auxin signalling restricted the growth of *Ps. Syringae* (bacteria).
- Does TMV effect on auxin signalling overcome host resistance?

GIBBERELLIN SYNTHESIS

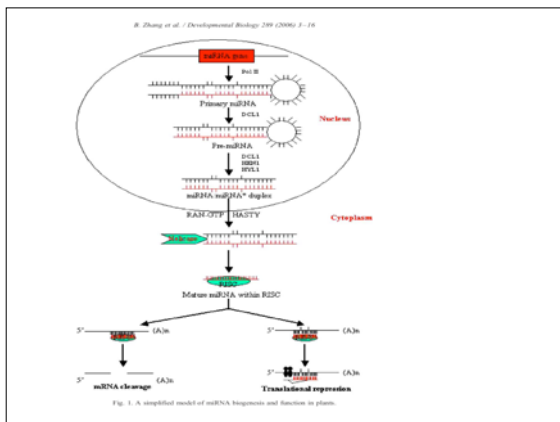
- Rice dwarf virus (RDV) induces stunting and darkening of rice leaves. GA deficient mutants do the same.
- RDV P2 protein (outer capsid protein necessary for leafhopper vector) controls dwarf symptoms but not necessary for RDV replication.
- Interaction between P2 and ent-kaurene oxidase (needed for GA synthesis)
- Significant reduction in GA1 in RDV-infected rice
- Interference in ent-kaurene oxidase may interfere with biosynthesis of phytoalexins making rice more competent for RDV replication.

ETHYLENE

- Ethylene mediates such responses as senescence to defense.
- Viruses increase Et leading to chlorotic or necrotic symptoms.
- CaMV P6 protein induces symptoms of stunting, chlorosis and vein banding
- P6-transgenic Arabidopsis display Et-insensitive phenotype: reduction in apical hook formation, hypocotyl shortening
- Single plant locus modulates the symptoms in P6 transgenic plants, suggesting that P6 mediates changes in Et pathway.
- CaMV in Et-insensitive Arabidopsis mutants in reduced suggesting that Et pathway affects virus biology

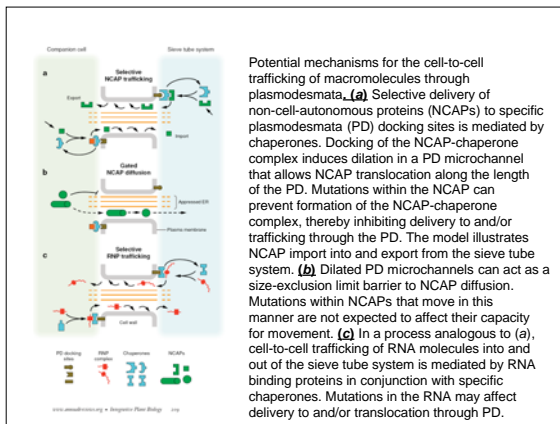
DEVELOPMENTAL SIGNALING

- Viral Suppressors of RNA interference (RNAi) lead to disease
- Those of TuMV, BYV, TBSV and AfrCMV alter the accumulation of miRNA that regulate developmental mRNAs such as auxin-responsive transcription factors ARF8.
- The mechanism is by suppression of miRNA-guided cleavage of host mRNAs that would normally be degraded.
- In contrast TYMV P69 suppressor increased miRNA accumulation and thus increased cleavage of target host mRNAs.
- In general the severity of symptoms due to disruption of RNAi pathways is determined by the strength of suppressor, the RNAi step, the ability to reach meristematic tissues. TYMV is such virus and it affects radial patterning, hormone signalling, meristem identity and flowering.



HOST TRANSPORT SYSTEMS

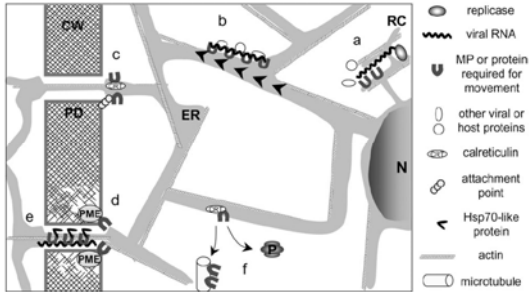
- Viral movement proteins alter permeability and size of exclusion limits in plasmodesmata (PD).
- This affects transport of carbohydrates, small RNAs, and proteins
- Transport of proteins and nucleic acids is significant in affecting cell fate, development, and disease resistance
- In total virus interference with transport disrupts host physiology



CARBOHYDRATE REALLOCATION CELL-TO-CELL TRANSPORT

- Active virus replication sites function as photosynthetic sinks (increase of resources)
- Plasmodesmata (PD)-related changes in MP-transgenic plants: effects depend on the virus system reflecting the different mechanisms of virus transport
- Movement proteins (MP) interact with various cell components (cytoskeleton, transcription factors, enzymes, actin, tubulin chaperones)
- Some MPs function as suppressors of RNAi

Diagram depicting possible explanations for some of the virus–host interactions with host proteins during movement.



WHOLE PLANT COMMUNICATION

- Mobile molecules (non-autonomous proteins [NCAPs], mRNAs, mi-RNAs) control plant development and physiology. These factors are trafficking via PD and phloem, as viruses do.
- NCAPs are similar to viral MPs, and play similar functions in noninfected cells

Are MPs competing for specific targets with NCAPs?

- In infected cells elevated levels of sucrose accumulation were observed. Often a changed mRNA front occurs in advance of the virus infection front.
- MP of WCMV serves as RNAi suppressor and inhibits the systemic spread of RNAi silencing signals.
- Thus plant viruses can affect host physiological balance at a distance (maybe due to interference with host signaling).

The plant vascular system functions as the conduit for long-distance communication between distantly located organs.

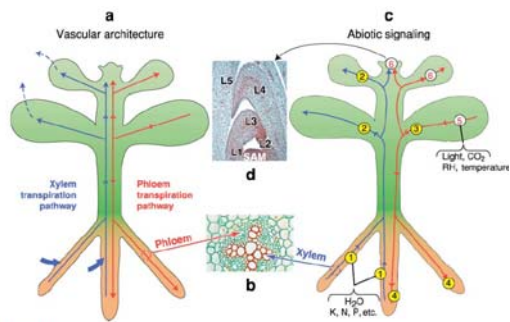


Figure 1

CELL REPROGRAMMING

-Plant viruses depend upon wounds to enter (no specific receptors)
-Plant viruses utilize specific host interactions to reprogram suboptimal host cells to enhance their replication.
-For instance, Geminiviridae, ssDNA viruses, use host-encoded DNA polymerase, which is abundant in meristem cells. To enter other tissues, gemini-viruses alter the cell cycle. Their Rep proteins interact with plant proteins that are responsible for down-regulation of cell cycle. These retinoblastoma-like proteins (pRBs) regulate E2F transcription factors controlling DNA replication/S phase. Rep-pRB interaction causes cells to re-enter S phase and thus to increased DNA replication.
--In TGMV AL1 mutation disrupts pRB binding which greatly alters symptom development.

dsRNA SURVEILLANCE

- In animal cells PKR (PROTEIN KINASE R) binds to dsRNA, phosphorylates ds RNA which then phosphorylates eIF-2alpha, suppressing translation, and inducing cell death.
- In plants P58IPK ortholog interacts with TMV and TEV helicases disrupting this pathway. There are increased levels of eIF-2alpha in infected plant tissue.
- P58IPK knockouts reveal marked necrotic responses to TMV or TEV infections which could be blocked by nonphosphorylable eIF-2alpha.
- The P58IPK-viral helicase interactions likely inhibit PKR-like kinase activity, preventing cell death.
- Chemically-induced ER stress up-regulates P58IPK and reduces eIF-2alpha phosphorylation.
- Interaction between P58IPK and viral replicase may block ER-stress-mediated cell death, allowing the virus to replicate.
- P58IPK functions as cellular suppressor of RNAi implying a link between the PKR-like dsRNA surveillance pathways and RNAi, both affecting viral pathogenicity.

HOST mRNA TARGETS

- RNAi degrades dsRNA or hairpins, being targeted by siRNAs.
- Viral-derived siRNAs could disrupt selected host mRNAs (via sequence similarity)
- PSTVd virulence region is similar to host sequences suggesting disease induction through mRNA disruption.
- Indeed expression of virulence region as hairpin induced viroid-like symptoms in tobacco.
- Similarly, TCV-mRNA similarities corresponded to the reduced accumulation of the corresponding mRNAs
- In total: targeted mRNA degradation by similar viral sequences may alter gene expression and host physiology.

PROTEIN MODIFICATION AND PROCESSING

- Posttranscriptional modifications on proteins include:
 - Phosphorylation
 - Acetylation
 - Myristoylation
 - Ubiquitination
 - Glycosylation

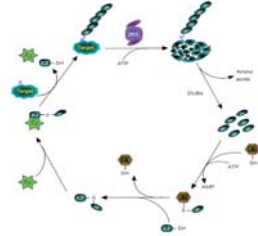
ACETYLATION

- In geminiviruses interactions between viral nuclear shuttle protein (NSP) and MP are required for DNA transport
- This process is regulated by viral CP
- NSP interact with acetyltransferase protein (NSI) in Arabidopsis.
- Acetyltransferases regulate DNA-associated processes
- Viral NSP acetylates viral CP
- Viral NSP recruits NSI away from cellular targets to acetylate viral CP
- Acetylated CP shows reduced affinity to viral DNA allowing NSP to export viral DNA from the nucleus.
- NSP reduces the activity of NSI which directly affects plant physiology (NSP mutants reduced interaction with NSI and produce attenuated symptoms).

PROTEASOME/DEGRADATION

- Ubiquitin-mediated proteasome pathways contribute to cell development and pathogen defense.
- Ploverovirus F-box protein P0 (suppressor of RNAi) interacts with Skp-1, a ubiquitin ligase. Likely P0 targets some RNAi components into proteolysis pathway.
- FBNYV nanovirus 20kD F-box protein interacts with Skp-1 homolog in Medicago and with pRBR proteins. This suggests that FBNYV targets the pRBR to the proteasome.
- Geminivirus Rep protein interacts with SCE1, a SUMO conjugating enzyme (a ubiquitin-like polypeptide). Does this also affect pRBR functions?
- Virus-encoded proteins are also targeted to the proteasome, e.g. TMAV 30K MP or TYMV 69K MP.
- HC-Pro from LMV inhibits the activity of the proteasome.

The Ubiquitin/26S proteasome pathway.



The protein ubiquitination begins with the activation of the ubiquitin molecule (Ub) in an ATP-dependent manner. The activated ubiquitin is then transferred to the active site of the ubiquitin-conjugating enzyme (E2). Finally, an ubiquitin-ligase (E3) binds E2 and catalyzes the formation of an isopeptide linkage between the activated ubiquitin and the Lysine residue of the substrate protein. After a chain of multiple ubiquitins is attached to the target protein, it is usually destined to the 26S proteasome, where the target protein is degraded and the ubiquitin monomers are reclaimed by the action of de-ubiquitination enzymes (DUBs).

HOST PROTEIN RELOCALIZATION

- . Relocalization of host proteins by viral components is likely a main mechanism affecting plant physiology.
- . One system is TMV replicase blocking the nuclear localization of Aux/IAA proteins.
- . Another system: CP of TCV interacts with TIP transcription factor which induces hypersensitive reaction of HRT gene in Arabidopsis. TIP works as a guard protein to HRT and interaction with TCV CP blocks transport to nucleus.
- . Third system: TBSV P19 protein interacts with ALY proteins in tobacco. P19 is RNAi suppressor, and MP. ALY transport RNAs from nucleus but also activate transcription. ALY2 and ALY4 re-localize from nucleus after interaction with P19. But also reverse: ALY re-localize P19 to the nucleus which however impairs the RNAi suppressor activity of P19. Thus re-localization could be directed by either viral or host components, and both the virus and the host modulate infection.

SUMMARY

Table 1 Virus-host interactions affecting host physiology

Virus component*	Host component	Effect on host physiology	References
TMV replicase	Aux/IAA proteins	Alterations in auxin response pathways, developmental symptoms	(90, 91)
TMV replicase	P35 ^{ORF} (inhibitor of dRNA activated PKR)	Regulation of cell death	(13)
TiMV P1-Hc-Pro, BYV p21, TBSV p19, ACMV AC4, TYMV p69	RNAi pathway components	Misregulation of miRNA targeted mRNAs, developmental symptoms	(23-25, 61)
RDV P2	ent-Kaurene oxidase	Gibberellin synthesis, dwarfing	(140)
Geminivirus Rep proteins	Retinoblastoma protein (pRBR)	Cell cycle reprogramming	(65)
PSTVd derived siRNA	Host mRNA	Misregulation of host mRNA, induction of disease	(128)
TBSV p19	ALY proteins (nuclear shuttle proteins)	Unknown	(17, 119)
Geminivirus NSP (nuclear shuttle protein)	AcNSI (Acetyltransferase)	Disruption of AcNSI acetylation activity	(18, 19, 82)
Geminivirus NSP (nuclear shuttle protein)	NIK kinases	Reduce NIK kinase activity, disrupt defense response?	(35)
FRNYV 20-kDa protein (F-box protein)	Skp-1 and pRBR	Degradation of pRBR? Cell cycle reprogramming?	(2)

*TMV, Tobacco mosaic virus; TiMV, Tomato mosaic virus; TBSV, Tobacco etch virus; BYV, Beet yellow virus; ACMV, Apple chlorotic mosaic virus; RDV, Rice dwarf virus; PSTVd, Potato spindle tuber virus; FRNYV, Faba bean necrotic yellow virus; LAMV, Lettuce mosaic virus.

SUMMARY

- Specific virus-host interactions cause physiological defects toward disease.
- However, not all interactions affect host physiology.
- A number of such interactions have been mapped.
- Virus-host interactions target a broad array of host processes.
- Emerging picture: pathogenic interactions are complex and some of them are direct to promote virus replication whilst others function indirectly.

SUMMARY, Cont.

- Development of symptoms is a complex interplay between replication, movement, resistance suppression, and cellular reprogramming.
- These and other interactions – all contribute to the disease.
- Specific interactions of host-virus components play major role in disrupting host physiology rather than general metabolic perturbations (virus has limited genetic information).
- Minimal perturbations would be evolutionarily favored leading to loosing disease symptoms. However, symptoms develop suggesting a necessary outcome.

Cont.

. In fact some disease symptoms result from virus attempts to avoid resistance.
e.g. localization of RNAi suppression, disease as a means to reprogram the cell to create a favorable host physiology (like for Geminivirus Rep).
-thus display of disease symptoms may depend on whether disruptive physiology is advantageous for the virus.
-One strategy to virus resistance would be to ameliorate disruptive disease symptoms and decouple them from virus accumulation.
-Another approach: enhance symptoms so much as to inhibit virus replication at a localized level (used already by some plants as e.g. hypersensitivity reaction).
-Future study: identify all complex effects leading to disease (symptoms), especially the hosts's downstream changes in the proteome and in the transcriptome. Explore the effect of multicomponent virus//host complexes on infection and disease. Attenuate or enhance the interactions and use them to develop new disease resistance strategies.

Table 1. Selected studies employing different methods to profile host gene expression in response to viral infection.

Host	Virus ^a	Focus of study	Tissue	Approach ^b	Reference	
In situ hybridization	PSMV	Down-regulated genes	Embryonic	5	Wang and Maule 1995	
	PSMV	HSP70 Ubiquitin	Embryonic	5	Aranda et al. 1996	
	PSBMV, PEHV, WCMV, BCTV	HSP70 Ipolyogenase	Embryonic	5	Escaler et al. 2006b	
<i>Caraculba pepo</i>	CMV	HSP70 NADP-ME	Cotyledons	5	Havelka and Maule 2000	
Differential display	Arabidopsis	CMV, CaMV gene VI	Global profiling	Systemic leaves	1, 4	Giri et al. 1999
	Chenopodium	TMV	Global profiling	Inoculated leaves	2	Cooper 2001
Serial analysis of gene expression (SAGE)	Cucurbit	ACMV	Global profiling	Mixed leaves	1	Freyre et al. 2004
	Macroum	PSTVd, TMV	Global profiling	Systemic leaves	1, 2	Bayra et al. 2002
Predicted by differential display	Arabidopsis	CMV	Global profiling	Inoculated leaves	1	Ishihara et al. 2004
	Macroum	CMV, CaMV, ORMV	Transcription factors	Inoculated leaves	1, 2	Chen et al. 2002
Arabidopsis	PVX, TCV, TaMV	Global profiling	Inoculated leaves	1, 2	Whitman et al. 2003	
	CMV, ORMV, PVX, TCV, TaMV	Global profiling	Inoculated leaves	1, 2	Whitman et al. 2003	
Arabidopsis	TMV	Global profiling	Systemic leaves	1	Gelfand and Calver 2003	
Arabidopsis	CMV	Global profiling	Inoculated leaves	1	Morales et al. 2004	
Arabidopsis	CMV, ORMV	388 Pseudoclass genes	Inoculated leaves	2, 3	Huang et al. 2005	
<i>N. benthamiana</i>	INSV, SYNV	Global profiling	Systemic leaves	1, 2	Scallan et al. 2005	
Arabidopsis	AC7 protein of MYMV	Global profiling	Protoplasts	4	Trinks et al. 2005	
Proteomics	RYMV	Global profiling	Cell suspension	1, 3	Venturello-DeBout et al. 2004	
Rice	RYMV	Global profiling	Cell suspension	1, 3	Venturello-DeBout et al. 2004	

^a PSMV = *Pea seedborne mosaic virus*; PEHV = *Pea early flowering virus*; WCMV = *White clover mosaic virus*; BCTV = *Bartlett cherry top virus*; CMV = *Cucumber mosaic virus*; CaMV = *Cauliflower mosaic virus*; TMV = *Tobacco mosaic virus*; ACMV = *African cassava mosaic virus*; PSTVd = *Plum pox virus*; INSV = *Influenza virus*; SYNV = *Symplectic virus*; ORMV = *Onion ring necrosis virus*; PVX = *Pea vein clearing virus*; TCV = *Tomato virus C*; TAVV = *Tomato virus A*; TaMV = *Tomato mosaic virus*; INSV = *Influenza virus*; AC7 = *Arabidopsis chloroplast virus*; MYMV = *Mung bean yellow mosaic virus*; RYMV = *Rice yellow mosaic virus*.
^b Experimental approach that the study is consistent with: 1 = Profiling host responses to wild type or engineered viruses; 2 = comparative analysis of host responses to different viruses, pathogen, and abiotic stresses; 3 = host mutants or genotypes; 4 = expression of individual virus proteins and nucleic acids; 5 = Spatial and temporal relationships between viral infection and host responses.

Table 2. Genes with common functions that are induced by positive- and negative-strand RNA viruses in *Arabidopsis* and *Nicotiana benthamiana*.

Description	<i>Arabidopsis</i> AGI number	<i>Solanum tuberosum</i> GenBank number ^a	Function ^b
Aldolase reductase family protein	AT2G57770	BQ119151	Carbohydrate metabolism
Potative protein methyltransferase	AT1G11580	BQ106460	Cell wall
Potative aminotransferase	AT2G24550	BQ118210	Metal handling
Potative cyclohexane P450	AT2G45730	BQ110466	Miscellaneous
Potative phytylase	AT2G39420	BQ116784	Not assigned
Glucanase 6, phloem 1, 4-β-D-glucanase	AT1G24280	BQ108097	Oxidative phosphorylation
Similar to rice methyltransferase	AT1G24140	BQ111463	Protein degradation
Ubiquitin activating enzyme-like protein	AT2G24940	BQ112099	Protein degradation
Polysphingolipin protein	MS20628	BQ114984	Protein degradation
Ubiquitin family protein	AT2G24940	BQ118413	Protein degradation
HeV protein	AT2G24940	BQ108074	Protein folding
Potative Ser/ Thr phosphatase 2C	AT4G08260	BQ112763	Protein modification
Potative copper/zinc superoxide dismutase	AT1G12520	BQ114488	Redox
Thioredoxin h	AT1G4145	BQ108086	Redox
Potative glutathione S- transferase	AT2G02930	BQ115272	Redox
Potative glutathione S- transferase	AT4G03230	BQ115272	Redox
Protein disulfide-isomerase-like protein	AT2G4960	BQ116026	Redox
WRKY6	AT1G62300	BQ117451	RNA transcription
SRE1	AT1G17020	BQ117001	Secondary metabolism
Potative ligand-gated ion channel protein	AT2G29120	BQ110910	Signaling
Potative receptor-like protein kinase	AT2G11800	BQ105516	Signaling
Potative calcium-binding protein	AT2G43290	BQ118008	Signaling calcium
Calreticulin	AT1G08400	BQ111161	Signaling calcium
Calmodulin	AT2G41110	BQ118170	Signaling calcium
Heat shock protein 70	AT2G12580	BQ112815	Stress abiotic
Like embryogenesis abundant protein homolog	AT4G01300	BQ111428	Stress abiotic
Heat shock protein 83	AT2G52640	BQ118115	Stress abiotic
Thaumatin-like protein	AT1G70400	BQ115360	Stress biotic
Pathogenesis-related PR-1-like protein	AT2G14610	BQ119120	Stress biotic
Potative oxidohematinase	AT2G14570	BQ118720	Stress biotic
Class IV chitinase	AT2G4420	BQ114435	Stress biotic
Beta-1,3-glucanase	AT2G57260	BQ111760	Stress biotic

^a *S. tuberosum* designation is given, because *N. benthamiana* data were collected using the potato microarray from TIGR (Reinold and Baul 2005). The genes highlighted in bold share significant sequence similarity in BLAST searches ($E < e^{-9}$) but are not necessarily orthologous. The other genes share a common description between *Arabidopsis* and potato, but sequence similarity is low ($E < e^{-9}$).
^b Function based on *Arabidopsis* annotation using the MAPMAN program (Thimm et al. 2004).

PHOSPHORYLATION-KINASE ACTIVITY

- MP and replicase phosphorylation regulates their activity.
- Plasmodesmal PK phosphorylates TMA MP but not clear if this affects host physiology
- Geminivirus NSP interacts with host receptor-like kinases (NIK) reduces NIK activity and Arabidopsis NIK mutants show enhanced infection.
- This suggests that NIK mediates antiviral responses while NSP disrupt this defense response
- NSP interacts with extensin-like PK that functions to enhance geminivirus infectivity by up-regulating NSP

Plant Stress Seminar

Spring 2009

Tuesdays, Noon, room 109 in Montgomery Hall
 Coordinator: Jozef Bujarski tel: 753-0601 or jbujarski@niu.edu

Tentative Schedule

January 13	Organizational			
January 20	Jozef Bujarski	"Plant Stress" a general overview		
January 27	Joel Stalstrom	Abiotic stress	Journal club	Article by Weber C. Nover L, Fauth M. (2008) Plant stress granules and mRNA processing bodies are distinct from heat stress granules. Plant J. 56:517-530.
February 3	Melvin Duval	Abiotic Stress	"C4 adaptations evolved in parallel among diverse lineages of grasses in response to falling CO2 during the Oligocene."	
February 10	Gabriel Holbrook	Abiotic stress		tba
February 17	Thomas Sims	Abiotic Stress	"Effects of Heat Stress, Carbon Dioxide, and Light Regime on the Breakdown of Gametophytic Self-Incompatibility"	
February 24	Jozef Bujarski	Biotic stress	"Viruses as plant stressing agents"	
March 3	Ana Calvo	Biotic stress: fungi	"Role of the VeA system in Aspergillus and Fusarium pathogenesis"	
March 10	Spring break			
March 17	S. Hill	Biotic stress	bacteria	tba
March 24	Jon Warnock	Reed Scherer	grad student	"Diatom culturing"
March 31	Coup, Cheryl Marie		grad student	tba
April 7	Karczynski, Elisabeth		grad student	tba
April 14	Ladethouse, Jeramy		grad student	tba
April 21	Moore, Anni		grad student	tba
April 28	Olson, Jessica Mary Grace		grad student	tba
May 5	Shrestha, Pratima		grad student	tba
