



Genomics of Invasiveness: Transcriptomics of Leafy Spurge Reveals Differences in Defense, Growth, and Photosynthesis Between Native and Invasive Populations



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ABSTRACT

Leafy spurge is an invasive perennial rangeland weed. Some evidence suggests introduction of the most invasive genotypes in the Great Plains originated from Eastern Europe (likely with immigrants from Russia and the Ukraine in the late 1880's). We collected leaf material from multiple plants from 4 different populations growing wild in the US near Fargo ND and the Ukraine near Kiev, and subjected the material to microarray analysis using the leafy spurge 23K cDNA arrays. Microarray analysis identified 143 genes that were significantly differentially expressed at a p value of less than 0.005 and 1349 genes were differentially expressed using a p value cutoff of 0.05. Gene set and sub-network enrichment analysis clearly identified processes associated with pathogen responses were up-regulated in Ukrainian samples, and pathways and signals associated with growth and photosynthesis were up-regulated in US samples. These results suggest the increase in aggressive growth in US populations may result from reduction in biotic stress responses due to reduced pathogen levels (consistent with an Enemy Release Hypothesis). However, genetic differences resulting from evolutionary pressure cannot be ruled out.

Experimental Design



Representative Ukrainian spurge

Collect leaf material from leafy spurge growing in the wild from 4 sites near Kiev, Ukraine and 4 sites near Fargo ND, USA (5-6 apparently healthy leaves from 3-4 plants at each site). Prepare RNA from leaf material and make labeled cDNA to probe 23K element spurge/cassava arrays using balanced dye-swap rolling circle hybridization scheme. Analyze results using GeneMaths XT and Pathway Studio.



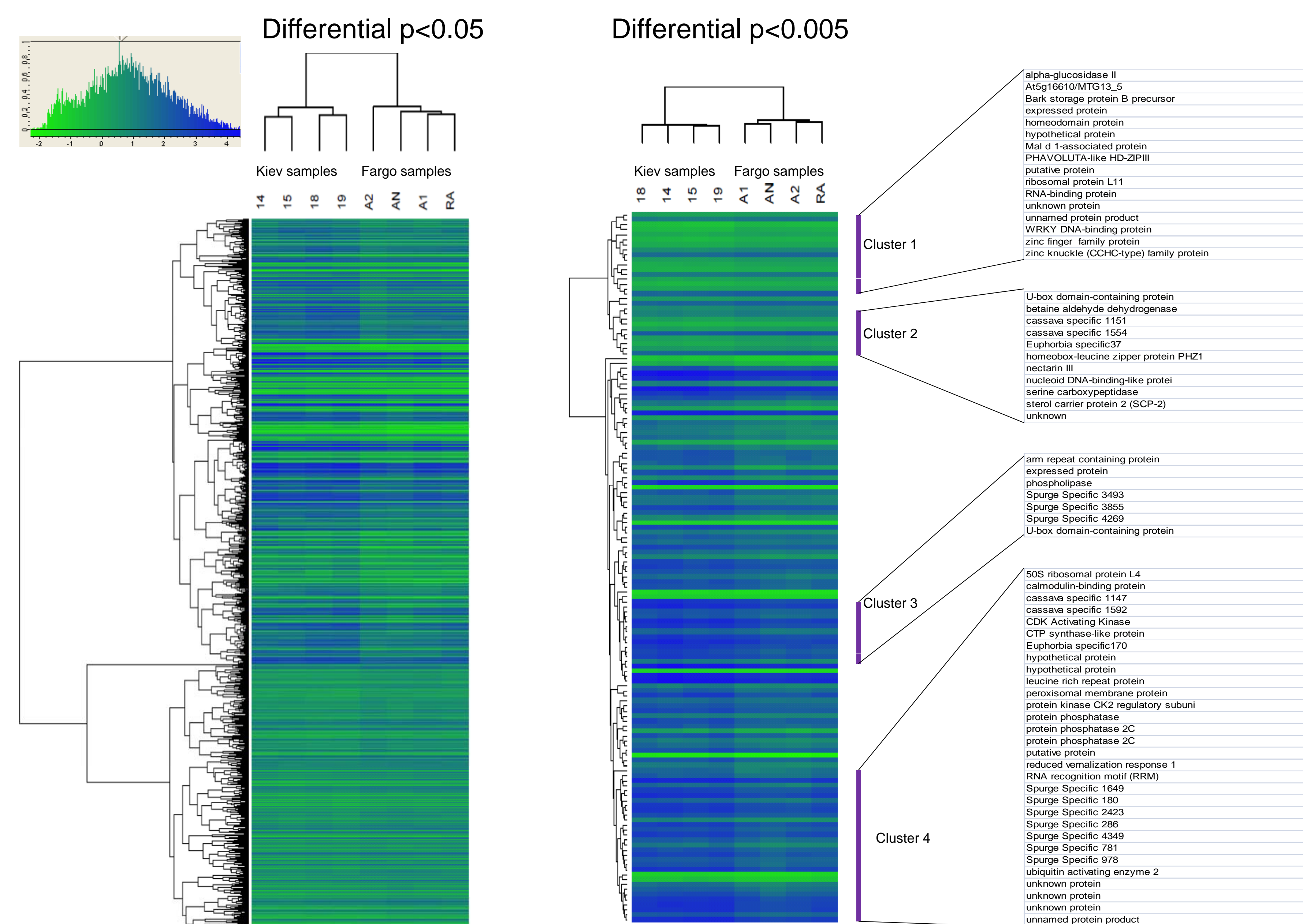
Representative US spurge

Up in US Name	p-value	Gene Set Category	Up in Ukraine Name	p-value	Gene Set Category
Superpathway of acetyl-CoA biosynthesis	0.008913	ArAcyc	Triacylglycerol degradation	0.025722	ArAcyc
Pyrimidine salvage pathway	0.015976	ArAcyc	Superpathway of sucrose degradation to pyruvate	0.031651	ArAcyc
Homogalacturonan biosynthesis	0.005904	ArAcyc	Glycolysis I (plant cytosol)	0.0318	ArAcyc
Starch biosynthesis	0.017691	ArAcyc	Aerobic respiration	0.016972	ArAcyc
cell-cell signaling	0.023566	biological_process	β-LUX and β-HPL pathway	0.035007	ArAcyc
microtubule-based process	0.033791	biological_process	Superpathway of polyamine biosynthesis	0.044484	ArAcyc
carotenoid biosynthetic process	0.004612	biological_process	Systemin Signaling	0.024554	Ariadne Pathways
starch biosynthetic process	0.027706	biological_process	cell death	0.010801	biological_process
ubiquitin cycle	0.034892	biological_process	microtubule-based movement	0.01811	biological_process
transcription initiation	0.048104	biological_process	toxin catabolic process	0.035637	biological_process
RNA splicing	0.046969	biological_process	auxin polar transport	0.022179	biological_process
dephosphorylation	0.026537	biological_process	metal ion transport	0.03737	biological_process
sycytium formation	0.029999	biological_process	response to fungus	0.048575	biological_process
carbohydrate metabolic process	0.039375	biological_process	defense response	0.043402	biological_process
carbohydrate biosynthetic process	0.024575	biological_process	response to heat	0.020513	biological_process
lipid metabolic process	0.006314	biological_process	response to abscisic acid stimulus	0.033554	biological_process
response to other organism	0.025955	biological_process	response to salicylic acid stimulus	0.040375	biological_process
double fertilization forming a zygote and endosperm	0.035655	biological_process	response to wounding	0.035554	biological_process
response to UV-B	0.011869	biological_process	mitochondrial membrane	0.045438	cellular_component
response to water deprivation	0.042685	biological_process	vacuolar membrane	0.046712	cellular_component
response to abscisic acid stimulus	0.048017	biological_process	nucleolus	0.036887	cellular_component
cytoplasmic membrane-bounded vesicle	0.011869	biological_process	respiratory chain complex I	0.045438	cellular_component
chloroplast thylakoid	0.048189	cellular_component	chlorophyll binding	0.025122	molecular_function
extracellular region	0.020075	cellular_component	cysteine-type peptidase activity	0.036887	molecular_function
copper ion binding	0.038171	molecular_function	Expression Targets of CCA1	0.042032	expression targets of
manganese ion binding	0.034986	molecular_function	Binding Partners of AG	0.0179	binding partners of
phosphoinositide binding	0.012132	molecular_function	Neighbors of MEK1	0.009808	neighbors of
hydrolase activity	0.041149	molecular_function	Neighbors of ubiquitin-protein ligase	0.017605	neighbors of
hydrolase activity, acting on ester bonds	0.033103	molecular_function	Neighbors of okadaic acid	0.033369	neighbors of
carboxylesterase activity	0.011056	molecular_function			
protein tyrosine/serine/threonine phosphatase activity	0.013237	molecular_function			
hydrolase activity, hydrolyzing O-glycosyl compounds	0.035584	molecular_function			
beta-galactosidase activity	0.049925	molecular_function			
ATP-dependent peptidase activity	0.017899	molecular_function			
aldo-keto reductase activity	0.029459	molecular_function			
transaminase activity	0.027892	molecular_function			
signal transducer activity	0.049844	molecular_function			
receptor activity	0.012825	molecular_function			
channel receptor activity	0.012411	molecular_function			
water channel activity	0.000115	molecular_function			
Expression Targets of DREB1A	0.023653	expression targets of			
Expression Targets of G11	0.032719	expression targets of			
Expression Targets of HY5	0.039914	expression targets of			
Binding Partners of histone deacetylase	0.019167	binding partners of			
Binding Partners of FLC	0.029306	binding partners of			
Neighbors of ABIS	0.002742	neighbors of			
Neighbors of HYDRAZINE	0.007785	neighbors of			
Neighbors of GL1	0.009843	neighbors of			
Neighbors of ribulose 1,5-bisphosphate carboxylase	0.013134	neighbors of			
Neighbors of OPR3	0.017112	neighbors of			
Neighbors of cyclin	0.019421	neighbors of			
Neighbors of Gibberellin	0.028617	neighbors of			
Neighbors of Proanthocyanidin	0.036707	neighbors of			
Neighbors of GL3	0.039535	neighbors of			
Neighbors of Methyl viologen	0.043662	neighbors of			
Neighbors of RGS1	0.045506	neighbors of			
Neighbors of H2O	0.045965	neighbors of			
Neighbors of LSD1	0.049772	neighbors of			

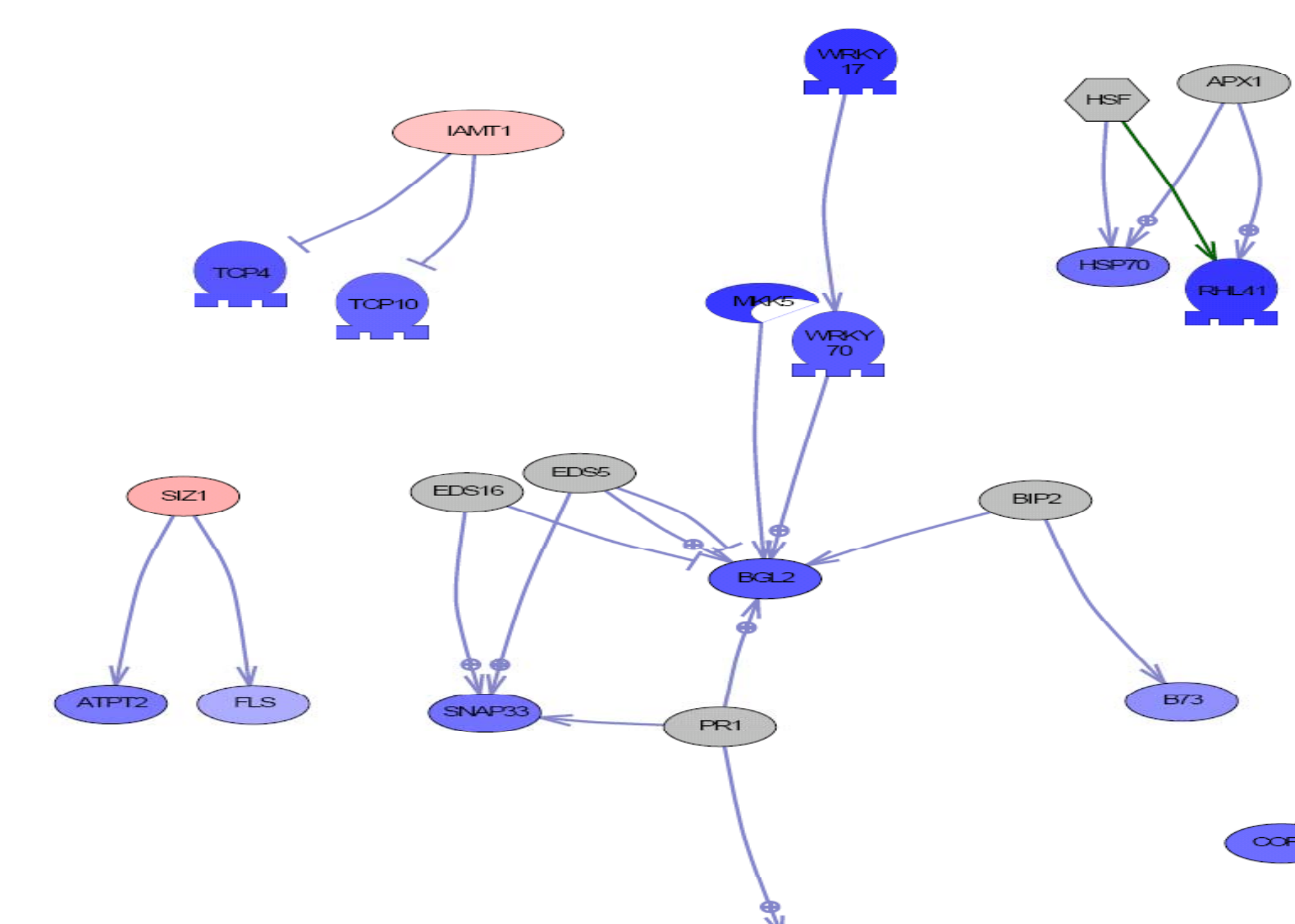
Gene set enrichment identified many more ontologies associated with growth/flowering (yellow) and photosynthesis (green) among genes that were up-regulated in plants growing in the invaded range, and many more ontologies associated with disease resistance (orange) among genes up-regulated in plants growing in their native range. Sub-network expression analysis identified WKRY-type transcription factors as being involved in regulating differential responses in both native and invaded ranges-suggesting a possible role in connecting defense responses with growth and photosynthesis regulation.

Results

Of the 23937 probes on the array, 23641 hybridized at levels > two standard deviations above background in >75% of the hybridizations from either the US or Ukrainian samples. Only two genes had q-values <20%, likely because these were collected from the field and thus had greater variability between samples. However, there were 1349 with p-values <0.05 and 143 with p-values <0.005. A cluster analysis (Pearson correlation with UPGMA) is shown for all genes with p-values <0.05. note that many of the strongly genes up-regulated in the invaded range are of a regulatory nature, while many of the genes in the native range are species-specific- possibly suggesting roles in response to specific pathogens.



Expression networks associated with significantly differentially expressed genes that were up-regulated in the invaded range.



Expression networks associated with significantly differentially expressed genes that were up-regulated in the native range.

