TOWARDS IDENTIFYING PIERCE'S DISEASE RESISTANT GENES FROM A NATIVE AMERICAN GRAPE SPECIES (*VITIS SHUTTLEWORTHII)* – A GENOMICS APPROACH

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ABSTRACT

INTRODUCTION
There are over 160,000 grape ESTs in the public data bases and the vast majority of these ESTs were generated from the European grape varieties (*Vitis vinifera*). However, the European grapes are highly susceptible to the Pierce's disease and they are not necessary possessing all the genes required for providing a full protection against the GWSS and *Xf* attack. On the other hand, PD resistant sources exist in some native North American grape species, particularly those species originated in the southeast United States. For example, *Vitis shuttleworthii*, a species originated from the southeast United States, is considered to be one of the most PD resistant grape, which has long been used for developing PD resistant grape varieties for the deep south - a most severe PD infected area. We therefore propose to search for PD resistant genes from the *Vitis shuttleworthii* grape.

The Viticulture Center at Florida A&M University and the USDA-ARS Horticultural Laboratory at Fort Pierce (Florida) jointly initiated a grape EST project from the native American grape -*Vitis shuttleworthii*, aiming to identify and isolate grape disease resistant genes including the Pierce's disease resistant genes. We have sequenced 30,000 ESTs, and have several on-going experiments for expression analysis and marker development for identifying the PD resistant genes.

OBJECTIVES
The objectives of this research are to identify/isolate PD resistant genes from *Vitis shuttleworthii* grapes and develop EST derived molecular markers for PD resistance. Specifically, the project is gearing towards to: 1) discover genes for PD resistance from *Vitis shuttleworthii* grapes; 2) conduct comparative genomics analysis between *V. shuttleworthii, V. vinifera* grapes and other plant species; 3) develop SSR and SNP markers for PD resistance, which will be used for accelerating the development of PD resistant grape varieties.

RESULTS AND CONCLUSIONS
We have sequenced 30,000 ESTs from a clone of *V. shuttleworthii* grape. Blasting analysis revealed that 13% of the *V. shuttleworthii* ESTs are unique when compared to the existing *Vitis vinifera* NCBI databases, and 3% of the ESTs did not find any homologous sequences among all plant ESTs reported in NCBI. Overall, approximately 7% of ESTs were related to disease / pest defense or stress tolerance genes, and it is obvious that these genes are abundant in the *V. shuttleworthii* grape (Table 1, Table 2).

Table 1. Comparison of transcription factor (TF) families in grape (*V. shuttleworthii, V. vinifera*), *Arabidopsis* and *R*ice

V. vinifera	Arabidopis	Rice
124	190	156
69	144	143
114	105	125
67	82	71
131	72	83
34	36	21
31	28	8
121	81	75
10	6	5

Table 2. Comparison of disease resistant gene (R-gene) families in grape (*V. shuttleworthii, V. vinifera*) and *Arabidopsis*

R-gene Class	Number in V. shuttleworthii	Number in V. vinifera	Number in Arabidopis
TIR-NBS-LRR	11	64	85
CC-NBS-LRR	9	51	41
NBS-LRR	9	64	10
TIR-NBS	3	19	17
CC-NBS	5	30	4
TIR	18	82	36

A series of experiments are being conducted to identify and isolate PD resistant genes through gene expression profiling analysis by using DNA microarrays. Specifically, a comparative analysis of transcriptional profiles of 1) unchallenged *V. shuttleworthii* grapes (control), *Xf* challenged *V. shuttlewothii* grapes (samples will be collected on different timeframes after infection).

For marker development, we are developing SNP and SSR markers from our *V. shuttleworhtii* sequence data set and the *V. vinifera* ESTs in the public domain. Aligned sequences will be mined for Single Nucleotide Polymorphism. A preliminary screening of the SNP and SSR marker from the 12,056 *V. shuttleworthii* ESTs indicated that the SNP and SSR markers are abundant in *V. shuttleworthii* grapes, and around 800 candidate SSR and SNP sites have already been identified. Table 3 shows the distribution of the di-, tri-, and tetra- SSRs from *Vitis shuttleworthii* ESTs, and Table 4 shows the abundant SSRs motifs from *Vitis shuttleworthii* ESTs. We have designed and synthesized the PCR primer pairs using computer software such as Primer3 to flank the SSR loci (partially shown in Table 5). Verification of these primers with PCR amplification on selective grape DNA templates is under way.

Table 3. Distribution of EST derived SSRs from *Vitis shuttleworthii*

Number of ESTs	Number of SSR-ESTs	Motifs		
		di-	tri-	tetra-
10,995	401(3.651[1])	82(20.32[2])	306(76.5)	13(3.2)

[1.] SSR-EST percentage in total EST
[2.] di-nucleotide motif percentage in SSR-EST.

Table 4. Distribution of the abundant (>5) SSR-ESTs among the *V. shuttleworthii* EST data set.

SSR Motif	Number of ESTs
GA/CT	36
AT/TA	13
CAA/GTT	90
ACC/TGG	34
TCT/AGA	19
CAG/GTC	15
AAG/TTC	14
CAC/GTC	14
CTT/GAA	13
CCA/GGT	12
CCT/GGA	12
TGA/ACT	9
TCC/AGG	8
CAT/GTA	7
GAT/CTA	6
TGC/ACG	6
CTC/GAG	5
Total	313

Table 5. A selective set of SSR primer pairs from the *Vitis shuttleworthii* ESTs

Repeat	*Left Sequence*	*Right Sequence*	*Product Size*
GTCGTCGTCGTCGTCGTCGTC	TACAAGAGCCAAGAGGGATT	GGATAACGAAGGAGACAGAGT	245
AGCAGCAGCAGC	AGGGAGATGACAAAGATGAAG	CCAAACACCGTAGGAGAGA	367
AACAACAACAACAAC	AATAATAAGAAGGAGATGCGG	GTTGTGGTGGTCGTGAAG	367
AGCAGCAGCAGC	CAGAGTGTCAGCACAGCA	GCGTTTCTCAAGGTTCTACTT	368
AACAACAACAACAAC	TGACTGGCATACTGATTTACC	CCCAATGAACTACCTTTACCT	368
CGGCGGCGGCGGCGGCGG	ACCCAATGAACTACCTTTACC	AGGAACAAGACAAACAATACACT	113
CCTCCTCCTCCTCCTCCTCCT	TTTATCCCAACAATCAGG	CTTTCACAGCAGAAGAGTT	226
CCTCCTCCTCCTCCTCCTCCT	GCCTTGGACCGAACTATC	CTAAGAAACACCATTCATCAG	226
GAGAGAGAGAGAGAGAGAGAGA	CGACCTAAGAAACACCATTC	CCTTGGACCGAACTATCTG	292
ACCACCACCACCACCACC	CGCATCAGAAGTCATCAAC	ACCCTCACTCTCACACTCAC	238
TCCTCCTCCTCCTCCTCC	ACGGAAGAAGAGAAGAAAGAG	ATCCACCGAAACAAACTTAC	133
AGAAGAAGAAGAAGAAGA	ACAAAGCAGGTAAGTAGCAAA	AAGACGGAAGAAGAGAAGAAA	233
TCTCTCTCTCTC	GTGATTGTTACCGACCTTGA	ATTCCCTTCTTCTCCTTTACC	195
TCTCTCTCTCTC	CCTCGGAAACAAACTTACA	CGAAGAAGAGAAGAAAGAGAAA	195
TGATGATGATGATGATGAGGATGATGA	AAGACCGAAGAAGAGAAGAAA	TAATACCGTGGAAATCACAAA	281
ACCTACCTACCTACCTACCT	TTACCCGACACTGGACAC	ACTTACCACCGAGATGAGG	266

After the potential SNP-EST and SSR-EST are verified, PD segregating populations will be used for marker development. Several populations derived from the hybridization of Native American species/hybrids and *V. vinifera* grapes will be candidates for this purpose. For example, a 183-seedling population of N18-6 x 'Cabernet Sauvignon' has been evaluated for PD resistance for several years in our vineyard. 'N18-6' is a breeding line highly resistant to PD while 'Cabernet Sauvignon' is the best known wine grape variety highly susceptible to PD. Segregating analysis revealed that three dominant genes provide full resistance to the Pierce's disease**.**

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