

SERA014 Progress Report 2005

GRAPE RESEARCH AND EXTENSION ACTIVITIES UNIVERSITY OF FLORIDA INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES

Mid-Florida Research & Education Center, University of Florida/IFAS

Grapevine Genetics Research Program

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For additional details, please view our website at: <http://www.mrec.ifas.ufl.edu/grapes/>

Muscadine Variety Trial:

Three hurricanes during the harvest season stifled a full harvest in 2004 (the 3rd year of growth). However, the vines are now in their 4th season and a full harvest is now underway (8-17-05). We had a lot of rain this year and it appeared to affect the vines by causing a later than normal ripening date and the vines seem to have less foliage than normal. This may have been due to the rain washing fertilizer away prematurely and/or difficulties in maintenance (it was difficult to apply herbicide). Regardless, the grape yield looks more than adequate for 2005 to assess relative quality between seven selections and approximately eight commercial varieties in replicated trial. The trial is comprised of five replicates of four vines for each of the varieties. The vineyard size is approximately one-acre. In addition to the replicated trial, a number of other selections are being evaluated in a less detailed manner.

Transgenic Grape Research:

We achieved the breakthrough of producing plants that are highly resistant to Pierce's Disease (PD). The level of PD resistance is so high that, in our stringent greenhouse screening test, transgenic plants survive under inoculum conditions that severely affect even resistant Florida hybrids like Blanc du Bois and Tampa rootstock. Details concerning this new research are presented below.

Development of new genes and genetic transformation vectors

Large scale testing of known gene sequences and their modification was made possible through grants from the Florida Department of Agriculture's Viticultural Trust Fund. We identified two useful genes that produce antimicrobial peptides "LIMA" (for PD resistance) and "VVTL-1" (for fungal resistance) and further modified them for efficient function in grapes. In designing the DNA sequences, codons preferably used by *V. vinifera* species were chosen to encode each amino acid residue at the DNA level. Both genes were subsequently cloned into a pUC-19 plasmid vector and nucleotide sequences were confirmed by DNA sequencing.

Both genes were engineered into a patented gene expression system, which also incorporated our Green Fluorescent Protein/antibiotic resistance (EGFP/NPT-II) marker gene expression unit so that transformed plants could be reliably obtained. These gene expression units were introduced into *Agrobacterium tumefaciens* to transform grape.

Production of transgenic plants

Several thousand unique transgenic grape plants lines of Thompson Seedless containing above-mentioned LIMA and VVTL-1 genes have been obtained. Thompson Seedless was used due to its high transformation efficiencies that we obtained routinely in our laboratory. Thompson Seedless also is highly susceptible to PD and most fungal diseases, including powdery mildew, thus representing an ideal model variety for large scale testing of resistance genes. These plants have been established in the greenhouse for in vivo testing. All transgenic plants showed normal transgene expression based on the visualization of GFP-specific fluorescence derived from the expression of the EGFP-NPT-II fusion marker gene.

Analyzing and testing transgenic plants in the greenhouse

Transgenic plants have been analyzed for DNA integration patterns as well as transgenic protein expression levels, and these data were used to correlate with subsequent identification and characterization of transgene-induced disease resistance. Knowledge gained through these analyses will benefit our future attempt to further refine our transformation approach to achieve long lasting high-level transgene-induced disease resistance.

PD screening

Transgenic plants that had been grown in the greenhouse for about one month were inoculated with pathogenic *X. fastidiosa* bacterium. Plants were continuously maintained in the greenhouse under normal growth conditions. Starting about six weeks after inoculation, plants were monitored for the development of PD symptoms, such as marginal burning on affected leaves throughout the plant. PD-susceptible plants began to be severely affected during the next few weeks and many subsequently died as a result of colonization and severe clogging of xylem systems by *X. fastidiosa* bacterium that eventually lead to the loss of all viable leaves.

Since early July of 2004, seven inoculation experiments have been conducted to test hundreds of independent transgenic plant lines and control plants. In these experiments most susceptible control plants began to die within a time period of 8-10 weeks. Significantly, most plants of PD-tolerant control cultivar 'Tampa' also developed severe PD symptoms indicative of progressive disease development, indicating the high degree of stringency of our test conditions.

From numerous tested lines of transgenic plants expressing the LIMA gene, a relatively high number of symptomless plants and plants with lessened PD symptoms were identified. The first of these resistant plants to be identified have remained symptomless for over a year even with repeated inoculations with *X. fastidiosa* bacterium. Bacterial isolation experiments using plant saps and PD bacterium-specific culture medium indicated that most of these resistant plants gave rise to significantly reduced bacterial colony formation as compared to control plants, while some symptomless plants yielded a few or no bacterial colonies. At this time (summer of 2005 and over 12 months after the first inoculations with active PD pathogen), resistant plants continue to produce healthy, robust new shoots. The significant reduction and/or disappearance of introduced *X. fastidiosa* bacteria within the plant system and the normal plant growth after a stringent disease challenge strongly confirmed the correlation between the

expression of the LIMA gene and the significant impediment of bacterial propagation and colonization within the xylem system in these resistant transgenic plants. That 'Tampa' plants were severely affected and transgenic plants survived under these stringent screening conditions strongly suggests that the transgenic plants will be at least as resistant as 'Tampa' under field conditions (note that 'Tampa' normally does not exhibit any PD symptoms and has an indefinite life span in the field). We are continuing to re-test clones of selected lines and screen new independent transformed lines during 2005. We are evaluating the feasibility of conducting our first transgenic field tests during 2006.

Publications:

Book chapters

Gray, D. J., S. Jayasankar and Z. Li, Vitaceae (Grape *Vitis* spp.), Chap. 22, *In*: R. E. Litz (Ed.), Biotechnology of Fruit and Nut Crops, Biotechnology in Agriculture Series, No. 29, CAB International Wallingford, U.K., 2005, pp. 672-706.

Refereed journal articles:

Li, Z., S. Jayasankar and D. J. Gray, Bi-directional duplex promoters with duplicated enhancers significantly increase transgene expression in grape and tobacco. *Trans. Res.* 13, 2004, 143-154.

Gray, D. J., Z. Li and J. Subramanian, Tissue-specific expression of lytic peptides in transgenic grapevines via use of a GFP/NPTII fusion marker, Invited publication, 2nd International Symposium on Biotechnology of Tropical and Subtropical Species, ACTA Hort., 2004, (In Press).

Li, Z. and D. J. Gray, Isolation by improved TAIL-PCR and characterization of a seed specific 2S albumin gene and its promoter from grape (*Vitis vinifera* L.). *Genome* 48, 2005, 312-320.

Jayasankar S., M. Van Aman, J. Cordts, Z. Li and D. J. Gray, Long term storage of suspension culture-derived grapevine somatic embryos and regeneration of plants, *In Vitro Cell. Dev. Biol. Plant* (In Press).

Trade articles:

Gray, D. J., First muscadine harvest at the new Mid-Florida Research and Education Center, *Grape Times*, Fla. Grape Growers Assoc. Newsletter, 2, 2004, 4.

Gray, D. J., A new website to support and promote Florida viticulture, *Grape Times*, Fla. Grape Growers Assoc. Newsletter, 10, 2004, 3.

Abstracts:

Dutt, M., M. Van Aman and D. J. Gray, Liquid culture for rapid in vitro propagation of *Vitis*, 2004 Congress on In Vitro Biology, In Vitro Cell. Dev. Biol. 40, 2004, 56A.

Dutt, M., Z. Li and D. J. Gray, Development of an alternate genetic transformation system for *Vitis*, 68th Ann. Meeting Fla. Acad. Sci., Florida Scientist, 67 ,2004,1.

Gray, D. J., Z. T. Li, D. L. Hopkins, M. Dutt, S. A. Dhekney, M. M. Van Aman, J. Tattersall and K. T. Kelley, Transgenic Grapevines Resistant to Pierce's Disease, 2005 Amer. Soc. Hort. Sci. Ann. Meeting, HortScience, 40, 2005, 1104A.

CITRUS RESEARCH AND EDUCATION CENTER, LAKE ALFRED - *Russ Rouseff & Ozan Gurbuz* (Visiting Scientist from Turkey)

In cooperation with Steve Talcott, work continues to investigate the effects of various enzyme and yeast treatments on wine phenolics in muscadine wine and wine by-products. The influence of fermentation and storage conditions on muscadine phenolics and related antioxidant activity and stability are being evaluated.

FOOD SCIENCE AND HUMAN NUTRITION DEPARTMENT, GAINESVILLE

Enology, Grape Processing & Utilization - *Bob Bates & Charlie Sims*

The continuing goal is to optimize and popularize the beneficial properties of Florida grapes, grape products, and byproducts. There are now over 15 Florida wineries in operation with several more on the drawing board. An additional 100 acre wine grape vineyard and another machine harvester are now operational in state. We work quite closely with established and new commercial wineries and individuals committed to both commercial and hobby winemaking.

In cooperation with Florida Grape Growers Association (FGGA) and FGGA County Chapters, workshops and Hobby Wine Competitions are conducted annually. The FGGA also hosts a commercial wine competition at the Florida State Fair in Tampa every February. This event draws well over 1,000 commercial wines from U.S. and international wineries, as well as several hundred hobby wines from Florida residents.

These activities result in increased membership and participation in the FGGA, greater appreciation of local commercial wines, and a growing demand for Florida grapes cultivars for planting and winemaking.

Muscadine Grape Research - Sue Percival

Our research focuses on the wines made from Muscadine (*Vitis rotundifolia*) and Cabernet Sauvignon (*Vitis vinifera*). We look for health benefits in a variety of models.

Cell culture: We have found that muscadine wine reduces chemically-induced neoplastic transformation (carcinogenesis) better than cabernet (Rowe and Percival, manuscript in preparation). We have also found that a compound unique to muscadine (ellagic acid) in combination with quercetin, are synergistically effective in killing cancer cells. Moreover, combining resveratrol which is a compound normally found at higher levels in muscadine than in cabernet, has even greater anti-cancer activity. This research promotes the influence of whole foods in health.

Mice: In a follow up study to Percival, S.S. and C.A. Sims. *Red Wine Modifies the Effects of Alcohol on Immune Function in the Mouse*. J. Nutr., 130:1091-1094, 2000, we again fed mice muscadine wine, cabernet sauvignon, water or ethanol. We found that the ethanol detrimentally affected several immune parameters, but the same amount of ethanol in the form of muscadine and cabernet sauvignon wine prevented the harmful effects (Rowe, Nantz and Percival, manuscript in preparation).

Human: In a study to examine absorption and short-term benefits of wine, we gave 30 individuals muscadine, cabernet sauvignon, ethanol or water to drink. After 30 and 60 minutes, we drew blood and examined several parameters. Ethanol consumption, but not wine consumption, resulted in greater DNA strand breakage, greater production of reactive oxygen species and greater apoptosis (programmed cell death) in lymphocytes. Plasma antioxidant capacity was greater in wine consumers compared to ethanol or water.

New research collaboration: We are collaborating with a group at Mount Sinai Medical College on the ability of muscadine wine to prevent Alzheimer's disease. To date, several compounds derived from muscadine have been identified and found to prevent β -amyloid plaque formation in cell culture without killing the cell. We have also identified many of the compounds in muscadine by mass spectrometry. Research is

planned to give muscadine wine to a mouse model of Alzheimer's in hope that it will prevent onset or accumulation of β -amyloid plaque formation.

Publications

Mertens-Talcott, S.U., Bomser, J.A., Romero, C., Talcott, S.T., and Percival, S.S. Ellagic acid potentiates the effect of quercetin on p21^{waf1/cip1}, p53 and MAP-kinases without affecting intracellular generation of reactive oxygen species in vitro. *J.Nutr.* 135(3):609-14, 2005.

Mertens-Talcott, S.U. and Percival, S.S. Ellagic Acid and Quercetin Interact Synergistically with Resveratrol in the Induction of Apoptosis, and Cause Transient Cell Cycle Arrest in Human Leukemia Cells. *Cancer Letters* 218:141-151, 2005.

Mertens-Talcott, S.U. Talcott, S.T., Percival, S.S. Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells, *J Nutr.* 133:2669-2674, 2003.

Grape Pigments and Phytochemical Research - Steve Talcott

Currently, three graduate students are conducting research full- or part-time on muscadine grapes, organized into the following projects:

Processing and modeling enzyme inhibition (PPO) for high hydrostatic pressure processing of muscadine grape juice.

Maturity and cultivar evaluation of ellagitannins and antioxidant polyphenolics in muscadine grapes and grape juices.

Anthocyanin copigmentation reactions for nutrient retention in processed muscadine grape juice.

Isolation and identification of antioxidant polyphenolics responsible for color degradation (enzymatic and non-enzymatic) in muscadine grape juice.

Accomplishments: Modifying functional properties of natural pigments (anthocyanins) are valuable to the food industries in the U.S. due to its potential impact in multiple areas. Specifically, preventing degradation of antioxidant phytochemicals, alleviation of visual color loss, reducing visible sediments, reduction of processing loss, and overall retention of nutritional and quality characteristics are paramount. The concept of anthocyanin copigmentation for incorporating antimicrobial, antioxidant, and enzyme

inhibiting compounds into anthocyanins for novel uses within the food industry has thus far proved effective as a means of extending shelf life characteristics.

Student Work: Danielle Pires graduated from a MS project with grapes entitled: "Polyphenolic and Quality Retention in Fortified Muscadine Grape Juice." Ms. Janelle McGuinness is now employed at Schriber Foods (Gainesville, GA) after conducting her research project entitled: "Isoflavonoid Copigmentation Reactions and the Functional Properties of Muscadine Wine".

Research Papers Published or Submitted for Publication:

Red Clover Isoflavonoids Act As Copigments to Muscadine Grape Anthocyanins. Stephen T. Talcott, Janelle E. McGuinness, and Carmen H. Brenes.

Phytochemical and Color Retention of Copigmented and Processed Muscadine. Grape Juice. S. T. Talcott, Carmen H. Brenes, D. M. Pires, and David Del Pozo-Insfran.

Ellagic Acid and Flavonoid Antioxidant Content of Muscadine Wine and Juice. S.T. Talcott and J.H Lee

Ellagic Acid and Ellagitannins Affect on Sedimentation in Muscadine Juice and Wine. Joon-Hee Lee and Stephen T. Talcott

Fruit Maturity and Juice Extraction Influences Ellagic Acid Derivatives and Other Antioxidant Polyphenolics in Muscadine Grapes. Joon-Hee Lee and S. T. Talcott

Polyphenolic and Antioxidant Content of Muscadine Grapes Influenced by Cultivars and Ripening. Joon-Hee Lee and Stephen T. Talcott

EXTENSION ACTIVITIES

Mid-Florida Research and Education Center

Dennis Gray - Receives several calls per week from growers, hobbyists and potential new growers. Advises on varieties and directs clients to other sources of information. Serves on the Board of Directors of the Florida Grape Growers Association and as a committee member on the Florida Department of Agriculture and Consumer Services Viticultural Advisory Council.

Don Hopkins - Provides growers with disease diagnoses and recommends control practices.

Food Science and Human Nutrition Department

Charlie Sims – As Department Chair allocates resources and encourages grape programs

Bob Bates - Retired with emeritus status 6/30/03. Still responds to grower and winemaker inquiries and judges state and regional wine competitions.

Statewide Extension Service

Extension agents assist growers and hobbyists on an ad hoc basis. The following agents present yearly programs to assist growers: John Jackson & Charles Fedunak - Lake County Grape Field Day each year in August for the past six years.

Plant Disease Clinic Monday & Friday 9-4. Max Griggs - Escambia County.