Additional Abstracts of Some Papers Presented at the 2008 Annual Meeting of the Kentucky Academy of Science

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AGRICULTURAL SCIENCES

The Influence of Light on Annonaceous Acetogenin Activity in Pawpaw (Asimina triloba) Stem and Leaf Tissue. EMERALD W. GATES*, JEREMIAH D. LOWE, KIRK W. POMPER, and SHERI B. CRABB-TREE, Land Grant Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

The pawpaw [Asimina triloba (L.) Dunal] is a native Kentucky tree-fruit that contains Annonaceous acetogenins in the twigs and fruit which display antitumor and pesticidal effects. This tree is usually found in the forest understory and prefers growing in low-light conditions. Our working hypothesis was that high light levels stress the pawpaw plant and induce high acetogenin activity in the stem and leaf tissue. Higher extractable acetogenin levels would be desirable for future product development. The objective of this study was to determine if there was a positive correlation between increased light level and acetogenin activity in the stems and leaves of pawpaw seedlings. Three month old greenhouse grown seedlings were subjected to three light treatments using no shade cloth (100% ambient light), 35% shade cloth (65% ambient light), and 80% shade cloth (20% ambient light). A randomized block design was used in the experiment with three replicate seedlings in each treatment in three replicate blocks (3 plants X 3 treatments X 3 blocks) for a total of 27 plants. The plants were destructively harvested after 6 weeks; stems and leaves were dried at 50°C, ground, and extracted with 95% ethanol. The Brine Shrimp Test (BST) bioassay was employed to assess acetogenin activity of the pawpaw extracts. Brine shrimp mortality at 0, 5, 10, 50, and 100 ppm of extract after 24 hours was used to determine the LC50 for each treatment. A negative correlation between extract LC50 and shade was found and we rejected our working hypothesis.

Soluble Solids Content Varies by Pawpaw (Asimina triloba) Variety. SHERI B. CRABB-TREE*, ANTHONY MCCORMICK, CHARLENE DANIELS, and KIRK W. POMPER, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

The pawpaw [Asimina triloba (L.) Dunal] is the largest tree fruit native to the United States and is in the initial stages of commercialization as a unique, high-value fruit crop for fresh-market sales or processing. As the satellite site for the USDA National Clonal Germplasm Repository for Asimina species, priorities of the Kentucky State University (KSU) pawpaw research program include description and classification of unique germplasm. Soluble solids content (SSC), or Brix, is a measure of the approximate sugar content of fruits, vegetables, juices, and wines. SSC has not been previously examined for major pawpaw selections. The objective of this study was to determine SSC (°Brix) in 31 pawpaw selections. Five ripe fruit were harvested from 31 different pawpaw selections at the KSU Research Farm in September 2006, skin and seeds removed, and flesh pureed and frozen. Three ~2 ml samples of each selection were thawed, and °Brix was determined using a refractometer. Differences in SSC among pawpaw selections were observed. The selections Potomac, KS-2, 9-47, Susquehanna, 5-5, Overleese, and Taytwo had the highest SSC (Brix >23). The selections 3-21, Mitchell, and PA-Golden had the lowest SSC (Brix <17). Brix can be correlated with perceived sweetness in fruits, which can affect consumer taste preference. Classifying pawpaw varieties by SSC could improve cultivar recommendations for pawpaw growers and aid the Repository in description of fruit characteristics of germplasm material.

Assessment of Variation in Annonaceous Acetogenin Activity in Pawpaw (Asimina triloba) Cultivars. JEREMIAH D. LOWE*, KIRK W. POMPER, SHERI B. CRABB-TREE, and JESSICA DURHAM, Land Grant Program, Kentucky State University, Atwood Research Facility, Frankfort, KY 40601.

Pawpaw [Asimina triloba (L.) Dunal] is a tree fruit that has potential as a new niche crop for small farmers in the eastern United States. Pawpaw contains Annonaceous acetogenins, which are promising new anti-tumor and pesticidal agents, present in extracts of twigs, fruit, seeds, roots, and bark of pawpaw. Ripe fruit potentially represent a large source of biomass for the extraction of acetogenin compounds. Identification of pawpaw cultivars displaying a high acetogenin activity would be beneficial for farmers wishing to grow pawpaw as a source of these compounds. The objective of this study was to assess the variation in acetogenin activity of 16 different pawpaw genotypes. Five ripe fruit were harvested from each of the pawpaw cultivars Middletown, Mitchell, NC-1, Potomac, Sunflower, Susquehanna, Taylor, Taytwo, Wahash, Wells, and Zimmerman as well as the advanced selections 2-10, 3-11, 10-35, 11-13, and K2-7. Fruit pulp was homogenized, placed in ziplock bags, and stored at −15°C until extraction. Pulp was extracted with 95% ethanol and the Brine Shrimp Test (BST) bioassay was employed to assess acetogenin activity. The BST identified acetogenin activity in the pulp of all cultivars examined. The ripe fruit pulp of the cultivar NC-1 had the highest activity while the cultivars Sunflower and Wells displayed the lowest activity.
activity. Other cultivars showed activity levels that were intermediate. BST can serve as a rapid screening method in identifying high acetogenin pawpaw genotypes.

Clonality of Pawpaw (Asimina triloba) Patches in Kentucky. KIRK W. POMPER, JEREMIAH D. LOWE, LI LU, SHERI B. CRABTREE, and LAUREN A. COLLINS, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Pawpaw [Asimina triloba (L.) Dunal] is a fruit tree native to the southeastern region of the United States. As part of Kentucky State University USDA Pawpaw Repository efforts, assessing genetic diversity across the pawpaw’s native range is a high priority. Pawpaw is usually found in large patches in the understory of hardwood forests. Because root suckering is often observed, these patches are believed to be clonal in nature. In this study we wished to test the hypothesis that native pawpaw patches are clonal. The objective of this study was to utilize inter-simple sequence repeat (ISSR) DNA-PCR fingerprinting techniques to determine if DNA fingerprint patterns indicated pawpaw patches contained genetically different trees (seedlings) in a patch. DNA was extracted from leaf samples collected from 20 trees each from six native patches in central Kentucky. Two ISSR primers yielded three polymorphic markers, 841T-1470, 841C-2800, and 841C-750, and six monomorphic markers, 841T-1380, 841T-670, 841C-1945, 841C-1830, 841C-1550, and 841C-1480 in the six patches (A–F). Patches B, C, and D did not display any polymorphic markers in each patch, suggesting these patches were clonal. However, Patches A, E, and F did show polymorphic markers within each patch, indicating these patches were not clonal and contained trees of at least two genotypes within each patch. With 50% of the pawpaw patches that we examined not being clonal, we reject of our hypothesis that native patches are clonal. This study suggests that to assess the genetic diversity of populations, more intensive sampling strategies will be required.

SCIENCE EDUCATION

Molecular Biology and Biotechnology Courses and Opportunities at Kentucky State University. LI LU*, KIRK W. POMPER*, KARAN KAUL, NARAYANAN RAJENDRAN, and JAMES TIDWELL, Community Research Service, Land Grant Program, Kentucky State University, Frankfort, KY 40601, Carver Hall, Kentucky State University, Frankfort, KY 40601, Division of Aquaculture, Land Grant Program, Kentucky State University, Frankfort, KY 40601.

Modern molecular biology and biotechnology impact multiple areas of biology and chemistry, such as genetics, biochemistry, cell biology, medicine, and agriculture. Training in biotechnology and molecular biology techniques is critical for students who wish to pursue careers in the life sciences and agriculture. In 2005, a USDA 1890 Institution Capacity Building Grant titled “Development of Biotechnology Courses to Enhance Aquaculture and Life Science Programs and Recruit Students to Kentucky State University” was funded with the objectives to 1) support the instruction and development of two courses, “Understanding Biotechnology” and “Advanced Techniques in Biotechnology”, 2) enhance laboratory experiences of the course “Cell Biology” with molecular techniques, 3) support undergraduate student research projects in biotechnology, and 4) support recruitment of undergraduate Biology and Aquaculture Master’s students at KSU through high school recruiting visits, a biotechnology website, and increased KSU biotechnology library holdings. About 50 students have already participated in classes supported by this grant. In the course “Understanding Biotechnology”, students extract DNA from plant and aquaculture species, and conduct techniques such as Southern blotting, Western blotting, PCR, and bacterial transformations. In the course “Advanced Techniques in Biotechnology” (renamed as “Advanced Molecular Biotechnology”), the students received additional training in modern techniques including purification of DNA from agarose gel; ligation of DNA fragments to create new constructs; tissue culture and transformation of model plant Arabidopsis; and usage of bioinformatics databases and software, such as Genbank, EMBL, and BLAST.

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