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Molecular Characterization of Partial Hybrids in Citrus by Microprotoplast Mediated Chromosome Transfer

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Citrus is grown throughout the tropical and subtropical regions of the world. *Citrus* is marked by narrow genetic variability and it has been difficult to develop improved cultivars in *Citrus* spp. by hybridization-selection methods using traditional breeding practices. Conventional breeding programs in citrus have had minimal success because of various biological factors including sterility, self and cross incompatibility, and long juvenile period. Modern breeding techniques have overcome the barriers of conventional breeding, with emphasis on alternative methods such as genetic transformation, somatic hybridization, and microprotoplast mediated chromosome transfer (MMCT). The MMCT technique involves donor microprotoplasts bearing one or two chromosomes being fused with a recipient protoplast to form a partial hybrid. The objectives of the present research are to attempt to make a molecular characterization of two sets of partial hybrids in an effort to study the relationship between the hybrids and also in comparison with the donor and recipient parents. More than 100 primers are being used to characterize the hybrids in comparison with the parents. These investigations will help us arrive at a understanding as to whether the recipient protoplasts are compatible enough to accept the chromosomes from the donor microprotoplasts, and to ascertain the possibility of successfully developing partial hybrids through MMCT in *Citrus* spp.

Gene Expression Analysis of the Cold Responsive Gene from *Poncirus trifoliata* during Acclimation and Deacclimation.

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In Texas, the freezes of 1951 and 1962 together killed 125,000 acres of citrus trees and the freeze of 1983 killed 40,000 acres. Low temperature is one of the most important abiotic stresses to be understood and manipulated molecularly. Cold hardiness is found in the deciduous citrus relative, trifoliate orange (*Poncirus trifoliata*), which can withstand temperatures as low as -26º C when it is cold acclimated. Exposure of the cold hardy trifoliate orange plants to temperatures from 28º C to -5º C enabled us to isolate and characterize one novel citrus low temperature gene (clt) with two transcripts, called *clt-a* and *clt-b* from leaves and twigs. *Clt-a* was produced when plants were subjected to low temperatures (starting at 10º C), while clt-b was constitutively expressed. Both *clt-a* and *clt-b* have the same open reading frame of 165 nucleotides and encode a small (54 deduced amino acid) highly hydrophobic protein. However, *clt-a* has an additional 98 bp nucleotides at the 3'-untranslated region (UTR), which is absent in *clt-b*. Expression analysis using relative quantitative RT-PCR demonstrated that *clt-a* is expressed exclusively at low temperatures, while *clt-b* is expressed constitutively (expression verified from 28º C to -5º C). In the process of deacclimation from -1º C to 28º C, the *clt-a* transcript degraded slowly after 2º C and was completely absent at 28º C, while the *clt-b* transcript remain stable. When the acclimated plant was taken from -1º C to room temperature, the *clt-a* gene degraded within 2 hours. Moreover, when acclimated plant was continuously exposed at -1º C for 20 days, both transcripts *clt-a* and *clt-b* remained stable.

Changes in Spectral Reflectance of Cucumber (*Cucumis sativus)* and Bean (*Phaseolus vulgaris*) Seedlings in Response to Stress Caused by Abiotic and Biotic Factors

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Spectral reflectance measurements were obtained for cucumber (*Cucumis sativus*) seedlings exposed to a series of salt concentrations (0.0, 0.01, 0.1 and 1.0 M). Spectral profiles of cucumbers subjected to all levels of salt stress were similar in all wavebands at the time of treatment, but exhibited substantial differences in reflectance of near-infrared wavebands within a period of 7 days. Spectral reflectance measurements were also obtained for 7-day old bean (*Phaseolus vulgaris*) seedlings that were inoculated with *Rhizoctonia solani* at the crown of the plant. Spectral profiles of both control and inoculated plants were similar in all wavebands at the time of inoculation. Within a period of 14 days, plants successfully infected by the fungus exhibited a substantial decrease in reflectance of both visible and near-infrared wavebands with respect to controls and inoculated but noninfected plants.

Isolation of Grapefruit Furanocoumarins Causing Grapefruit-Drug Interaction Through Preparative HPLC

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Grapefruit juice contains furanocoumarin derivatives which are known to interact with various drugs such as felodipine, leading to the increased bioavailability. Due to very low concentrations in grapefruit juice, isolation of these compounds has been a challenge to researchers. We developed a reliable separation method using preparative HPLC. One liter of white grapefruit juice concentrate was diluted with 2.4 L of distilled water to bring TSS to 26 Brix. Diluted juice was extracted with (1L + 1L) of ethyl acetate and hexane, extraction was repeated twice. Ethyl acetate plus hexane extract was concentrated under vacuum and concentrated extract was reconstituted in HPLC grade methanol. The extract was analyzed in analytical HPLC using dihydrobergamottin (DHB) and bergamottin standards. Three grams of methanol extract was loaded in preparative HPLC and eluted with aqueous methanol (40-45%, over 45 min, 45-60%, over 45 min, 60-90% over 30 min and 90-95% over 10 min) at room temperature, at 25 mL/min flow rate. The elution was monitored at 240 nm. Individual peaks were collected and rotary evaporated to remove organic phase while aqueous phase was removed by freeze drying to obtain solid compounds. Upon analysis by electrospray ionization-mass spectrometry, six furocoumarin isomers including DHB, were confirmed based on structural characterization by collisional activated dissociation. NMR studies are being awaited upon large quantity of isolated compounds.

Suppression of Colon Cancer Development in Sprague Dawley Rats by Natural and Irradiated Grapefruits and their Functional Compounds

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Citrus bioactive components have antiproliferative activity in breast cancer cells and it is hypothesized that citrus also may protect against colon cancer. To test this, rats (n=100) were provided with one of five diets: control diet (AIN-76 containing 60 g/kg pectin), 13.7 g/kg grapefruit pulp powder (GFPP; providing 200 mg/kg naringin), 13.7 g/kg irradiated grapefruit pulp powder (IGFPP; 300 Gy, ^{137}Cs, a proposed treatment against fruit flies), naringin (200 mg/kg) or limonin (200 mg/kg). Following saline or azoxymethane injection (15 mg/kg) during the 3rd and 4th wk after starting the diets, colons were resected (6 wk post 2nd injection) and evaluated for aberrant crypt (AC) formation and cell proliferation. Total number of AC (59.9, 60.3, 62.1, 58.5% of control; P = 0.02), number of high multiplicity AC foci (ACF; 41.0, 48.2, 48.2, 34.5% of control; P = 0.01), and proliferative index (78.4, 85.5, 82.9, 81.7% of control; p=0.02) were lower in GFPP, IGFPP, naringin, and limonin treated rats, respectively. Only GFPP and limonin caused a smaller (P = 0.03) proliferative zone, which may explain their greater numerical reduction in high multiplicity ACF. These results provide in *vivo* evidence for a potential role of grapefruit pulp and its functional components against colon cancer. Funded by USDA # 2001-52102-11257, ATP 003658-0359c-2001, and NIEHS P30-ES09106.

Antioxidant Activity of Citrus Limonoids, Flavonoids and Coumarins

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A variety of *in vitro* models including ß-carotene-linoleic acid, 1,1-diphenyl-2-picryl hydrazyl (DPPH), superoxide, and hamster low density lipoprotrein (LDL) were used to measure the antioxidant activity of 11 citrus phytochemicals. The chemicals included 2 limonoids (limonin and limonin 17-ß-D-gluocpyronoside), 8 flavonoids (apigenin, scutellarein, kaempferol, rutin trihydrate, neohesperidin, neoeriocitrin, naringenin and naringin), and a coumarin (bergapten). In each assay, the concentration of all the compounds was 10μM. Overall, the citrus limonoids and bergapten showed very weak antioxidant activity. Limonin showed relatively stronger antioxidation activity than limonin glucoside, especially in lipid oxidation ($P<0.05$). The flavonoids demonstrated mild, to moderate, to strong antioxidant activity. The results suggested that several of the structural features in the active chemicals were linked to antioxidant activity. Besides those reported features, our data indicated that the hydroxyl group in position 6 of ring A (as in scutellarein) could also increase the antioxidant activity of flavonoids. Compared with the active flavonoids (polyphenols) containing a chromanol ring system, limonoids are highly oxygenated triterpenoids, with fewer hydroxyl groups to stabilize unpaired electrons (or scavenge free radicals). Bergapten lacks a hydroxyl group. This was the first report of limonoids and neoeriocitrin on their antioxidant activity (Based on ISI and Medline database, we did not find any relative report from 1966 to present). The work was in part supported by the USDA-CREES grants #2001-34402-10543 and 2001-52102-11257.

Inhibitory Effect of Citrus Limonoids and Flavonoids on Human CPY450 Isoforms

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Bioactive compounds such as limonoid aglycones, glucosides and flavonoids isolated from molasses, seeds and peels of citrus fruits were studied for their effect on specific substrates of cytochrome P450 isoforms. These compounds are known to exhibit potential anticancer properties and were tested for inhibitory effects of O-deethylase activity, O-demethylase activity and dibenzylflurescein reduction by CYP1A1, CYP1B2, CYP3A4 and CYP19. The total mixtures of both limonoids and flavonoids induced partial to high inhibitory effects at 10μM concentrations. Both limonoids and flavonoids inhibited metabolite formation by human CYP450's, viz. CYP1A2, CYP1B1, CYP3A4 and CYP19, in varied manners. Combination of different limonoids inhibited the enzyme to a greater extent than a single compound. This *in vitro* study provides good evidence to the potential of citrus bioactive compounds for their anticancer properties.

Water Conservation and Fertility Management Strategies on Citrus Cost and Productivity

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South Texas, being a home for citrus for many decades still has limited scientific information on its irrigation and fertilizer requirements when placed under water conservation strategies. This study is being conducted at the South farm of the TAMUK Citrus Center in Weslaco, TX. The crop proposed for the study is Rio Red grapefruit (*Citrus paradisi* Macfad.). Limited studies have been undertaken to determine which type of irrigation system would prove to be the best for grapefruit. The plots are arranged in a randomized complete block design for different treatments. Three different irrigation systems are being utilized for the citrus crops namely flood, drip and microjet spray. Similarly different fertilizer combinations including a new fertilizer called X tend and compost are under investigation. Apart from this, the study involves the continuous monitoring of water usage and soil moisture status within the root zone of citrus. The research will involve an economic evaluation of water conservation and fertility management strategies on citrus productivity.

Increasing Rate of Spread of Citrus Psorosis Disease of Grapefruit in Texas

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Citrus psorosis disease has been known for more than 100 years. It was once considered a complex of several diseases that show similarity of symptoms. However, the current knowledge describes three types of psorosis (Psorosis A, B, and ringspot) are caused by strains of the same virus *Citrus psorosis virus* (CPsV), the type member of the *Ophiovirus* genus. Visual symptoms of Psorosis A are bark scaling and flaking on trunk and limbs and internally it produces gum impregnation of wood. Psorosis B causes widespread, rapidly expanding bark lesions, and twigs with gum impregnation. Ringspot symptoms develop on young leaves and fruit. Psorosis is a disease that is distributed through grafting of contaminated buds. However, convincing evidence of natural spread of this disease has been reported from Argentina and Uruguay. The result we discuss here originates in the blocks where L.W. Timmer suspected a natural spread of psorosis in Texas several years ago. Our studies have generated new information associated with the constant presence of an *Olpidium*-like fungus in the feeder roots of infected trees in at least four grapefruit blocks. The rate of psorosis spread in these blocks may be explained as a result of virus spread through: 1) contaminated buds, 2) natural transmission through a vector, and 3) transmission through contaminated tools used for hedging and pruning operations. However, our observations and data support that the first and the third explanations have less merit and all circumstantial evidence indicate a possible link with the second explanation. Comparison of the rate of spread of symptoms, ELISA tests for virus detection, biological indexing on indicator plants, microscopy for fungus *Olpidium*, and PCR test for virus are shedding new information that fills the gap of knowledge in the search for the factor(s) behind this alarming spread of psorosis in some blocks. In future, we will be testing the hypothesis of *Olpidium* as a vector of CPsV.

Lethal and Sublethal Effects of Two Insect Growth Regulators on Adult *Delphastus catalinae* (Coleoptera: Coccinellidae)

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Pyriproxyfen, a juvenile hormone analog, and buprofezin, a chitin synthesis inhibitor, are considered as selective and effective insecticides for controlling whiteflies. *Delphastus catalinae* (Horn) is a commercially produced predator considered to be a valuable biological control agent of whiteflies in greenhouses. The compatibility of these two control strategies was tested by evaluating lethal and sublethal effects on adult ladybeetles of ingesting insect growth regulator (IGR) residues on treated *Bemisia tabaci* eggs in the laboratory. Feeding on pyriproxyfen-treated whitefly eggs caused no significant decrease in longevity of either male or female, whereas feeding on buprofezin-treated eggs reduced longevity significantly. Likewise, preoviposition period was not affected by pyriproxyfen, but was lengthened 3-6 d by buprofezin. Buprofezin reduced *D. catalinae* egg production and oviposition period while a 28-d treatment with the low rate of pyriproxyfen actually increased these parameters. Both IGRs reduced *D. catalinae* egg fertility, especially the higher rate of pyriproxyfen and both rates of buprofezin. However, the process was largely reversed in the case of pyriproxyfen by a switch of *D. catalinae* to water-treated whitefly eggs. Since *D. catalinae* fecundity was actually increased by 28 d exposure to the low rate of pyriproxyfen, there was no net effect on viable egg production. Thus, while both IGRs negatively impacted *D. catalinae*, fewest side effects were observed with pyriproxyfen, and the worst of these, egg sterility, was reversible following removal of treated eggs.

The Potential Impacts of Mandatory Country-of-Origin Labeling on Texas

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We examined crucial issues in fruits and vegetables as a result of the mandatory country-of-origin labeling (MCOOL) in the Food Security and Rural Investment Act of 2002 (FSRIA). While voluntary beginning October 2002, these provisions were originally scheduled to become mandatory in October 2004. The U.S. Congress may delay the implementation of MCOOL until October 2006. Retailers will be required to label selected products by their country of origin. For fruits and vegetables, this includes fresh and frozen products. The US imported 4.5 million metric tons (mmt) of vegetables and 3.4 mmt of fruits in 2002. Mexico is the leading supplier. All covered products must have their country-of-origin listed on the label. The labels may be in the form of standard labels, stickers, stamps, or placards on packages, containers, or bins. Major exceptions to the MCOOL provisions include exemptions for commodities that enter the Hotel/Restaurant/ Institutional (HRI) trade, commodities that are processed or used as ingredients in further processing, and retailers selling less than $230,000 per year of any perishable commodity. Exported fruits and vegetables are also exempt from labeling. Products that are of exclusive U.S. origin may be labeled as "Product of U.S.A.", or simply U.S.A. Products grown outside the United States will be required to have their country of origin listed. Examples of these are onions produced in Mexico and melons from Mexico and Central America. Each of these products sold at retail grocery outlets will have to have "Product of Country X" on the label. The country's name alone will be sufficient. Mixed-origin and blended products, are slightly more complicated. For blended products, a package which contains the same product from different countries packaged in the same container must be labeled by country listed alphabetically. For instance, frozen bags of broccoli may contain product from the United States only, or may have broccoli from Mexico and/or Chile. The degree of foreign content will depend on harvest times in other countries and relative costs of procurement. At one point during the year, the bag might contain only product of the "U.S.A." At other times of the year, the bag might have a label that says "Chile, Mexico, U.S.A.," with other combinations possible during different times of the year. Similar situations can be imagined for other products such as bagged salad lettuce from the United States and Mexico. It is unclear if U.S. consumers prefer domestic over foreign products as well as the costs associated with new labeling. Much of the foreign produce marketed in U.S. groceries is now labeled as to country of origin and sales are increasing. It is unlikely that MCOOL will have major positive or negative consequences for Texas produce since most products are currently labeled and firms have adjusted to the additional costs. One possible negative impact could be that MCOOL forces foreign suppliers to label and differentiate their product, thereby establishing a premium price niche in the market. Finally, the potential for retaliation by Mexico, Canada, and other foreign suppliers should be considered if they implement more restrictive import procedures or challenges in the World Trade Organization.

The Opportunity Cost of Inefficient Irrigation Water-Use in Chihuahua, Mexico

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Since NAFTA began in 1994, U.S.-Mexico trade barriers have declined or been eliminated on most high value agricultural products. Trade growth has spurred significant population increases in the U.S.-Mexico border region, placing greater stress on food production and infrastructure. Agricultural production in Mexico has increased to serve both the U.S. market for high value products and the growing border population in both countries. The state of Chihuahua is one area where increased agricultural production has occurred. Growth in crops bound for the U.S. market and alfalfa for Mexican dairy cattle, which produce milk for the border population, is profit driven. Many of these new crops are more water-intensive than crops previously produced in Chihuahua. Increased production, coupled with a prolonged drought, has placed greater pressure on the Rio Conchos basin, which supplies eighty percent of the water for irrigation in Chihuahua and is the main Mexican tributary of the Rio Grande. Complicating matters is the 1944 water treaty between the United States and Mexico under which Mexico's accumulated water debt with the United States, 1.4 million acre feet in 2003, a debt built up during a recent prolonged drought. The purpose of this poster is to present an assessment of the economic and environmental impacts of increased irrigation in Chihuahua on water availability and to discuss policy options to mitigate these impacts. Using secondary data and the Penman-Montieth method of determining water use, it was estimated that irrigated acreage in Chihuahua has increased from 554,613 acres in 1980 to 782,019 in 2002. Irrigation water use increased to 3.51 million acre feet over this same time. The result was production of 3.61 million metric tons of commodities valued at $618 million. Chihuahua irrigation systems are estimated to be forty percent efficient. If the irrigation systems were improved to achieve sixty percent efficiency, irrigation water use would be cut to 2.34 million acre feet per year for the same level of production. Further, it was calculated that, on average, an acre foot of water in Chihuahua produced output valued at $440 in 2002. Therefore, using an irrigation water system which is only forty percent efficient costs the Chihuahua economy $264 per acre foot of water use, or $927 million, when compared to a 100 percent efficient irrigation water system. The water debt owed by Mexico also adversely affects agricultural producers on both the Texas and Mexico side of the lower Rio Grande. U.S. policy options to correct negative impacts caused by the Mexican water debt include: direct producer compensation; higher tariffs on Mexican produce; withholding U.S. deliveries of Colorado River water to western Mexico under the 1944 treaty; linkage to U.S. drug certification and immigration reform; and provision of NADBank funds to improve Mexican irrigation efficiencies.