

**Research Progress Report
Foods for Health, Texas
USDA Special Grant 2000-2001**

Vegetable & Fruit Improvement Center
Texas Agricultural Experiment Station

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Biotechnology

Enhancing Calcium Levels of Tomato, Carrot and Potato

Investigators:

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Objectives:

To develop tomato fruits, carrot and potatoes with elevated levels of calcium

- a. Transform plants with CAX1
- b. Evaluate calcium levels in the plants

Tomato Progress:

Published transformation protocols were tested using Micro-Tom and Red Cherry including those of McCormick et al. (1986) and Meissner et al. (1997). We found the tobacco feeder layer did not enhance transformation. Pre-culture and co-cultivation on a medium with 1 mg/l BA and 0.2 mg/l IAA followed by a medium with 2 mg/l zeatin produced transgenic tomato plants. Five transgenic Micro-Tom and eight Red Cherry plants were obtained. These plants expressed the CAX1 (Calcium Exchanger 1) gene for calcium accumulation driven by the 35S or the *cdc2* promoter (Tables 1, 2 and 3, Fig 1 d, e, and f).

The primary plants exhibit the same phenotype as described for the transgenic tobacco reported by Hirschi (1999) (Fig 1d). Calcium deficiency symptoms in the primary plants expressing the CAX1 gene are indicated by blackening and death of the shoot tip (Fig 1e, g and j). This phenotype can be reversed by watering the plants with additional calcium for total reversion to the normal phenotype suggesting that these plants are calcium deficient (Fig 1f and h).

To ascertain whether CAX1 expression altered total calcium levels, presumably through sequestration into the vacuole, the total calcium accumulation in the leaves and fruit of transgenic and non- transgenic tomato plants was assayed. As shown in Tables 2-3, CAX1 expression in Micro-Tom plants led to an increase of 12% calcium in leaves and 5% in fruits. CAX1 expression in Red Cherry plants led to an increase of 10% in leaves and 150% in fruits. The level of calcium in the fruits did not include calcium in the seeds as these were removed and saved. This is similar to levels in transgenic tobacco vegetative tissue expressing the gene (Hirschi, 1999). The level of calcium in a fresh market tomato fruit is 1.8 mg/g dry weight without the seeds and 2.05 mg/g dry weight with the seeds included (Park and Smith, unpublished). Based on the Red Cherry data, calcium levels in the fruit could be elevated ~150% to 6 mg/g dry weight (Table 2).

New Goals and Objectives:

1. Examination of a fruit specific promoter

We recently obtained a fruit specific promoter, E8, from R. Fisher at UC Berkley, and spliced it into the CAX1 plasmid. In tobacco transgenics with this promoter we do not see the calcium deficiency symptoms (Fig. 1 c and i). We have Micro-Tom plants in culture with the fruit specific promoter on the calcium accumulation gene (Fig 1 k). Additionally, we have 60 transgenic calli from Micro-Tom and Red Cherry on regeneration media.

2. Expand studies to include commercial processing and fresh market tomatoes

The long-term goal is to develop tomato plants with enhanced calcium nutrition and plants that may have fruit more resistant to pathogen attack and blossom end rot. The short-term goal, however is to test different promoters on the CAX1 gene to express the calcium accumulation gene only in the fruit. We have seed of three commercial tomato cultivars, a fresh market, and two processing tomato cultivars. There will be maximum value and impact if such cultivars had enhanced calcium nutrition and/or pathogen and blossom end rot resistance. Primary transgenics will be generated, calcium levels determined, gene copy number, and level of foreign gene expression established. Seed from these plants will be more extensively evaluated in the R1 and R2 generation for inheritance and expression of the trait. The evaluation of fruit for pathogen resistance will be conducted in collaboration with a plant pathologist and tomato breeder. We would anticipate that this could be initiated in the third year of the project with an industry partner, and additional funding. However, with R1 and R2 generation seed and plant material, we anticipate industry interest, partnership, and funding for these evaluations.

3. Human nutrition studies

This same material will also be evaluated at the University of Texas Center for Human Nutrition in year 3 and 4 and would require additional funding.

Carrot Progress:

Somatic embryogenic callus of carrot cultivar Danvers (Dr. L Pike's breeding program) type has been initiated and cocultivated with *Agrobacterium* with the CAX1 gene driven by the 35S or cdc2 promoter. In vitro selection on kanamycin has resulted in 1000's of potentially transgenic carrot calli. Over 100 plants have been regenerated on selection media and over 30 plants are ready to be established in soil. They will be analyzed for calcium accumulation in root and vegetative tissue when the plants have fully developed.

Potato Progress:

Five different media are being tested to develop a regeneration protocol for potato cultivar Russet Norkota (Dr C. Miller's potato improvement program). Concurrently, transformation studies have been initiated and 100's of calli are growing on selection media.

Table 1. Plasmid Constructs

| Plasmid | Promoter | Construct | Note |
|---------|----------|------------|----------------------------------|
| 1 | 35S | 35S::CAX1 | constitutive expression promoter |
| 2 | cdc2 | cdc2::CAX1 | cell division cycle promoter |
| 3 | E8 | E8::CAX1 | fruit specific promoter |

Table 2. Calcium Concentrations in Tomato Fruits

| Cultivar | Control/Transformed | Calcium (mg/g dry wt)* | % increase |
|-------------------|---------------------|---------------------------|------------|
| Commercial tomato | Control | 2.1 | |
| Roma | Control | 2.9 | |
| Micro-Tom | Control | 1.9 | |
| Micro-Tom | Transgenic (35S) | 2.0 | 5% |
| Red Cherry | Control | 2.4 | |
| Red Cherry | Transgenic (cdc2) | 6.0 | 150% |

*Results shown are the mean of fruits taken from three experiments at different harvest times. These data reported on 100% dry matter basis.

Table 3. Mineral Concentrations in Leaves and Fruits of Transgenic Tomato Plants

| Cultivar | Transformant | Explant | Mineral* | | | | | | | |
|------------|--------------|---------|----------|------|------|------|----|-----|----|-----|
| | | | Ca | K | Mg | Na | Zn | Fe | Cu | Mn |
| | | | % | | | PPM | | | | |
| Micro-Tom | Control | Leaves | 4.23 | 2.39 | 0.68 | 6228 | 61 | 126 | 20 | 909 |
| | 35S::CAX1 | Leaves | 4.73 | 2.20 | 0.55 | 5803 | 53 | 104 | 11 | 885 |
| | Control | Fruits | 0.19 | 3.37 | 0.22 | 5119 | 23 | 55 | 7 | 41 |
| | 35S::CAX1 | Fruits | 0.20 | 2.40 | 0.14 | 4707 | 16 | 43 | 3 | 34 |
| Red Cherry | Control | Leaves | 3.07 | 1.87 | 0.74 | 5648 | 83 | 94 | 6 | 650 |
| | cdc2::CAX1 | Leaves | 3.39 | 2.42 | 0.61 | 5658 | 72 | 105 | 11 | 462 |
| | Control | Fruits | 0.24 | 1.93 | 0.14 | 2508 | 28 | 66 | 7 | 40 |
| | cdc2::CAX1 | Fruits | 0.60 | 3.25 | 0.26 | 2912 | 65 | 80 | 21 | 46 |

*Results shown are the mean of fruits taken from three experiments at different harvest times. These data reported on 100% dry matter basis.

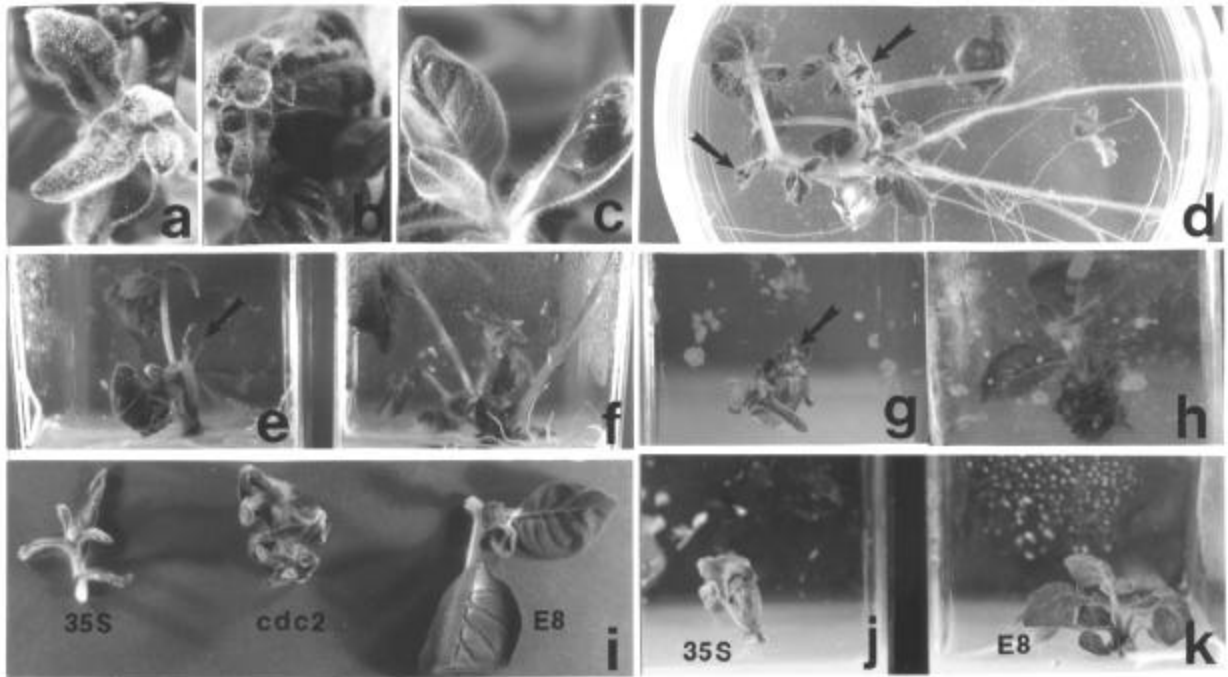


Figure 1. Phenotype of tobacco plants expressing CAX1 gene driven by the 35S promoter (a) and *cdc2* promoter (b) displayed leaf necrosis and developed terminal buds that turned black (calcium deficiency symptoms); however, plants with the fruit specific promoter (E8) show no calcium deficiency symptoms (c and i). Transgenic tomatoes, Red Cherry (d and e) and Micro-Tom (j), and tobacco (g) exhibiting calcium deficiency symptoms (arrows). The ability to suppress the growth defects of CAX1-expressing tomato (f) and tobacco (h) with exogenous Ca^{2+} strongly suggests that these plants are deficient in Ca^{2+} . Transgenic Micro-Tom plant with the fruit specific promoter (E8) shows a normal (k) shoot.

Enhancing the Nutritional Content and Quality of Melon

Investigator:

Marla Binzel, Horticultural Sciences, TAMU

Marianne Arnold, Graduate Assistant, PhD candidate

Stated Objectives and Timetables from Proposal:

Projected Completion

- | | |
|--|-----------|
| 1. Isolation and characterization of melon homologs of RIN and NOR | |
| Screening library | 6 months |
| Sequence analyses and characterization | 12 months |

Status:

i. Southern analyses with melon genomic DNA were performed using cDNA probes from tomato for both the RIN and NOR genes. The results confirmed that homologs of both RIN and NOR appear to exist in melon, and supported the rationale for cloning these genes from melon.

ii. After initial attempts at screening the melon TRIPLEX cDNA library with the tomato cDNA probes proved unsuccessful, an alternate PCR based approach was used successfully for RIN. Degenerate oligonucleotide primers were synthesized to conserved regions of the RIN gene, based on DNA sequence alignments between tomato and cucumber. These primers were used to amplify sequences from the melon TRIPLEX cDNA library via PCR. The PCR products were purified and subcloned into the vector pTOPO. A subset of clones from each reaction were sequenced and compared to the tomato sequences. Two different clones of approximately 350 and 430 bases with sequence homology to tomato RIN were obtained. These clones are being used as probes to screen the melon cDNA library in order to obtain more complete sequences of the genes. This screening is expected to be completed within three weeks. It is anticipated that we should have RIN clones isolated, sequenced and characterized by the end of the one year project period. A similar approach will be used for NOR. Degenerate oligonucleotide primers based on the tomato NOR sequence and a homologous sequence in strawberry have been designed and are currently being tested. Sequence amplification, subcloning and sequencing is expected to take about two months.

iii. A number of different RNA isolation methods were evaluated to determine which protocol would be most effective for the isolation of RNA from melon fruit samples. It was determined that a protocol from Shellie et al. (Hort Sci 1997) provide both the best quality and yield of total RNA from melon. Tissue was collected from 3 different melon genotypes over a course of fruit development. Extraction of total RNA from these samples has been initiated and should be complete within the 12 months of the project.

Stated Objectives and Timetables from Proposal:**Projected Completion**

- | | |
|---|-----------|
| 2. Optimization of transformation and regeneration protocols for melon | |
| a) Identification of optimal Agro strains, vectors, transgene promoters and co-cultivation conditions | 4 months |
| b) Optimization of regeneration media | 12 months |

Status:

i. Experiments have been performed comparing transient expression of the GUS reporter gene driven by different promoters, different Agro strains, vectors and co-cultivation conditions. An optimal set of these variables has been identified and will be used in future melon transformation experiments.

ii. A suitable regeneration medium has been identified, but experiments are continuing to determine if additional advances can be achieved. We have identified the following factors that may have an effect on regeneration and are currently testing them: the concentration of sucrose and cytokinins during germination, shoot initiation and shoot elongation, the developmental stage of the germinating seedlings and the effect of combining growth regulators and antibiotics. In addition, we are evaluating modifications in the protocol that allow us to process larger numbers of explants. We expect that by the end of the project period, we will have identified an efficient and cost effective transformation/regeneration protocol for the production of stable transformants of melon.

Breeding and Genetics

Identifying and Developing Potato Varieties with Enhanced Functional Properties for Human Health Benefits

Investigators:

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Mohamed S. AL-Saikhan, Horticultural Sciences, TAMU
Anna L. Johnson, Graduate Assistant, PhD Candidate

Objective:

To identify and quantify important antioxidant compounds in the most important potato varieties grown commercially in the US.

Total antioxidant activity and relative levels of important antioxidant compounds in 9 of the most important potato varieties grown in the U.S. – Atlantic, Ranger Russet, Red LaSoda, Russet Burbank, Russet Norkotah, Shepody, Snowden, Viking, and Yukon Gold were identified.

1. There were significant differences in total antioxidant activity in varieties evaluated. Russet Burbank and Snowden were highest in total antioxidant activity using water extraction while Atlantic was the lowest. With methanol extraction, Russet Burbank and Shepody were the highest and Snowden and Yukon Gold were the lowest. While the water extraction method is less expensive and safer, the methanol extraction method is probably preferred because it extracts compounds that are not soluble in water.
2. Although potato skin is higher in antioxidant activity than the flesh, it is not a major contributor to the overall activity. This is due to the fact that skin comprises a very small percentage of the whole tuber.
3. There were highly significant differences among varieties in the amount of caffeic acid, chlorogenic acid, ferulic acid, protocatechuic acid, tryptophan, tyrosine, and vanillic acid.
 - a. Chlorogenic acid, tryptophan, and tyrosine were the most abundant antioxidants.
 - b. Russet Burbank had the highest level for most of these compounds, followed by Russet Norkotah and Viking. Shepody and Yukon Gold were the lowest.
 - c. The level of antioxidant compounds in potato makes them a good source of antioxidants, especially considering that potato is the fourth most important crop grown worldwide and is one of the most important sources of food and nutrition. Per capita consumption of potato in the U.S. is about 145 pounds.
 - d. The differences in total antioxidant capacity and specific antioxidant compounds should provide plant breeders the opportunity to select and develop varieties with higher levels of these useful compounds.

4. In comparing antioxidant compounds from varieties grown in Texas versus Colorado, highly significant differences in lutein, lutein-epoxyde, neoxanthin, violaxanthin, zeaxanthin, and total carotenoid content for location, variety and location x variety interaction. The varieties grown in Texas had higher total carotenoid levels than the same varieties grown in Colorado.
5. The positive correlation between total carotenoids (ug/100g) obtained from spectrophotometer and Hunter colorimeter data (L*, chroma, and hue angle) validated the hypothesis that the Hunter colorimeter reading can be used to predict total carotenoid content of potato, resulting in savings of both time and money.

Publications:

AL-Saikhan, Momahed S. 2000. Antioxidans, Proteins, and Carotenoids in Potato (Solanum tuberosum, L.). Dissertation; Texas A&M University.

Phytochemicals in Stone Fruit and the Development of Healthier Cultivars for the Niche Market

Investigators:

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Bolivar Cevallos, Graduate Assistant, MS Candidate

1. Survey the levels of phytochemicals in a range of peach and plum genotypes.

Since the funding began in October, these measurements were taken on fruit samples collected in August and frozen at -20°C . Eight red-flesh peaches and 13 red-fleshed plums collected from plots at the USDA-ARS Prunus collection at Byron, GA were assayed for total phenolics and anthocyanin content. The anthocyanin content of the peaches ranged from 3.4 to 22.0 mg/100g tissue and that of the plums ranged from 19.4 to 101.7 mg/100g tissue. The total phenolics content for peaches ranged from 99 to 261 mg chlorogenic acid/ 100 g tissue and the phenolic content of plums ranged from 158 to 245 mg chlorogenic acid/ 100 g tissue. This survey will be expanded and repeated with new samples collected this coming year.

2. Characterize the specific anthocyanins, carotenoids, and phenolics in selected peach and plum genotypes.

Work is progressing with this on extracts obtained from the survey work. The laboratory has recently been equipped with a new HPLC for this chemical characterization.

3. Characterize the biological properties (anti-oxidant, anti-microbial, anti-cancer) of the phytochemicals.

The anti-oxidant activity of the extracts for 8 peaches and 13 plums has been determined. The anti-oxidant activity ranged from 440 to 1780 ug equivalent Trolox/ g tissue for peaches and from 1250 to 3240 ug equivalent Trolox/ g tissue for the plums. Correlation analysis indicated that the anthocyanin content but not the phenolic content was well correlated with the anti-oxidant activity. Additional amounts of extracts are being prepared for the assays for anti-microbial and anti-cancer activity.

In our proposal we indicated that the initial survey would be done within 4-5 months from the beginning of the project and that the characterization steps would follow in the last 6-months of the project. According to this schedule we are progressing well. Thus far we have done the initial characterization with previously collected fruit samples. Once these fruit again (June to August), additional samples will be collected to confirm these findings and to supply more samples for the characterization work.

Chemical analyses of functional compounds in ethnic vegetables

Investigators:

Kil Sun Yoo, Vegetable & Fruit Improvement Center, TAMU

Dianna Liu, Vegetable & Fruit Improvement Center, TAMU

Nine leafy and root vegetables were brought from J&D Produce and tested for vitamin C, carotene, flavonoid, and anthocyanin content. They included Swiss chard (green and red), collard, beet, kohlrabi (red and green), turnip, kale, and dandelion. The results are presented in Table 1. After this initial study, more detailed and various varieties will be tested on other chemical compounds for promoting health benefits of these vegetables.

Table 1. Content of vitamin C, carotene, lycopene, quercetin, and anthocyanin in nine different vegetables.

| Name | Vitamin C (ppm) | Carotene (ppm) | Lycopene (ppm) | Quercetin (ppm) | Anthocyanin (ppm) |
|-------------------|--------------------|-------------------|-------------------|--------------------|----------------------|
| Collards | 1525 | 21.7 | - | 1.1 | - |
| Beet | 18 | 0.3 | - | 0 | - |
| Red Swiss Chard | 213 | 25.0 | - | 0.5 | - |
| Green Swiss Chard | 324 | 15.9 | - | 0.5 | - |
| Dandelion | 47 | 14.8 | - | 1 | - |
| Green Kohlrabi | 1432 | 0.3 | - | 0.03 | - |
| Red Kohlrabi | 1610 | 0.2 | - | 0.02 | 21 |
| Kale | 2000 | 24.0 | - | 2.7 | - |

1. Vitamin C was measured by extracting with 3% citric acid and HPLC method.

2. Carotene was measured by spectrometer after extracting with hexane.

3. An HPLC analysis did not show lycopene peak from the samples.

4. Quercetin was measured after hydrolysis by beta-glucosidase.

5. Anthocyanin was measured after extracting with methanol, water, and acetic acid mixture.

* The red pigment in beet and Swiss chard is betanin, not anthocyanin.

Carrot analysis: About 300 lines of carrot were tested for carotene, anthocyanins, sugars, and terpenoid content for developing new cultivars. Carotene content varied between 100 and 180 ppm. Anthocyanin, uniquely existing in 'BetaSweet' maroon carrot, was contained between 400 and 900 ppm among our breeding lines. Sugar content ranged between 6 and 8%. Terpenoids are responsible for oily and harsh flavor in carrots and we screened at level less than 10 ppm of total terpenoids.

Commercial baby-style carrot varieties grown from California and Wisconsin were also tested for their quality evaluation. They contained slightly less amounts of carotene, sugars, and terpenoids, probably because they were harvested earlier than ordinary fresh market type.

Onion analysis: I have tested more than 6,000 individual onion bulbs from our breeding lines and commercial cultivars. Some of our advanced lines showed extremely mild pungency (about 2.0 unit pyruvic acid content) after 3 or 4 generation screening for low pungency. Our data clearly indicated that breeding for low or high pungency onion is practically possible and progressing by using the automated pyruvic acid analysis system, which enables us to process 500 samples per day.

Publications:

Yoo, K.S. and L.M. Pike. 2000. Determination of background pyruvic acid concentrations in onions, *Allium* species, and other vegetables. *Scientia Horticulturae* (in press).

Lazcano, C.A., K.S. Yoo, and L.M. Pike. 2000. A method for measuring anthocyanins after removing carotenes in purple colored carrots. *Scientia Horticulturae* (in press).

Onion haploid production for hybrid onions

Investigators:

Kil Sun Yoo, Vegetable & Fruit Improvement Center, TAMU

Sung Gil Kim, Graduate Assistant, PhD candidate

We have harvested 8 dihaploid lines that we produced 2 years ago and evaluated the uniformity of the bulbs. Four lines were yellow and the other four were red colored.. Bulb shape and color were extremely uniform but size was varying slightly. We are fairly confident that these dihaploid lines are not segregating and can be used as a new open-pollinated cultivar or as a parent of hybrid cultivars. We will continue to test their uniformity in the field conditions.

For regeneration of haploid plants from various genetic background, we have cultured more than 100,000 flowers from more than 140 lines and produced around 190 haploid plants. Another 50 haploid plants were regenerated from an F-1 hybrid plant to study color genetics in onion. We have demonstrated that yellow and red onion plants could be produced from the mother plants without pollination. These individual plants are expected to be completely homozygous in their genetic make-up and , thus, non-segregating. Thirty-six test crosses were made between male-sterile plants and existing dihaploid plants to test combining ability and bulb quality.

Using these materials, we will try to identify molecular marker inked to the restore gene of male sterility. For this purpose, two F-2 populations, 250 plants of T-3 and 360 of T-5, are growing for BSA (Bulk segregant analysis). Currently we are testing various probes for this marker. The red color gene is partially dominant and quantitative but its genetic is not clearly understood. We are also planning to study on the inheritance of red color in onion bulbs using the segregating populations.

Evaluating genetic variation for beta-carotene and ascorbic acid in cantaloupe to breed improved cultivars

Investigator:

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JinSuk Lee, Graduate Assistant, MS Candidate

Jonathan Sinclair, Graduate Assistant, PhD Candidate

Five objectives were set for the period of May, 2000 through May, 2001:

1. Create families with approximately twenty diverse melon varieties to study the segregation of genes controlling beta-carotene and vitamin C development in melons.
2. Plant parents and progeny in the Fall, 2000 to produce fruit for analysis of beta-carotene and ascorbic acid.
3. Harvest fruit in the Fall and perform nutrient analyses. Analyze data and determine best sources of genes for high beta-carotene and ascorbic acid.
4. Plant parents and progeny in two locations in the Spring, 2001 to produce fruit for nutrient analyses.
5. Harvest fruit in the Spring and perform nutrient analyses. Analyze data and select parents for crossing to increase levels of beta-carotene and ascorbic acid in TAES breeding lines. Begin crossing in greenhouse.

The first objective was completed by January 2001. Plants of 20 lines were intercrossed to produce more than 50 families. All crosses were carried out in the greenhouse. Seed was cleaned, labeled and sorted for the field tests.

The second and third objectives were not successfully completed in the Fall, 2000. All lines were transplanted to the field in late August. Vines were healthy until record cold temperatures in October destroyed small fruit and slowed down vine growth. As new fruit begin to set in November, constant light rain caused a severe outbreak of *Myrothecium roridum* which caused fruit and stem cankers on 90% of the lines. Only one family and 5 parents managed to produce acceptable fruit for analyses. All fruits were harvested at full slip stage for analysis. A positive result was that TXC 2015 and 'Doublon,' which have been used extensively in the breeding program for disease resistance, had significantly higher beta-carotene levels than three popular commercial hybrids. In addition, all progeny from the cross between 'Doublon' and another breeding line with lighter color flesh, had beta-carotene levels similar to 'Doublon'. This suggests that dominant genes are likely involved in beta-carotene production in this line, and selection for high levels in families derived from it should be productive. Beta-carotene and total soluble solids values in Table 1 reflect averages over three replications.

Table 1. Variation in beta-carotene and sugar content of several melon lines.

| Entry | Beta-carotene ($\mu\text{g/g}$) | Total Soluble Solids |
|----------------|-----------------------------------|----------------------|
| Doublon | 24.8 | 11.0 |
| TP45 x Doublon | 24.0 | 12.5 |
| Primo | 19.0 | 11.0 |
| Caravelle | 18.6 | 11.5 |
| Hymark | 16.0 | 9.5 |
| TXC 2015 | 25.7 | 11.0 |

Plans to accomplish the fourth and fifth objectives are currently being organized. Seed from twenty melon lines and progeny are being prepared for planting in late February, 2001. Harvest and analyses will take place in May and June. At that time, determinations on which parents will be the best sources of genes for high beta-carotene and ascorbic acid can be made. These can then be placed in the greenhouse for crossing to lines with other useful traits. 'Doublon' and 'TXC 2015' are already being crossed in the GH with lines carrying valuable disease resistance genes. In addition, TXC 2015 will be extensively trialed around south Texas for yield and quality with several commercial growers this Spring, 2001.

Because both 'Doublon,' and 'TXC 2015' have dark orange flesh, it may be possible to select for high beta-carotene based on this characteristic. The extensive data to be collected in May and June will help to determine if this trend is true across many genotypes.

Improving seed germination, fruit quality and lycopene content of seedless watermelon

Investigators:

Daniel I. Leskovar, Texas Agricultural Experiment Station, Uvalde

Hae Jeen Bang, Graduate Assistant, PhD candidate

Objectives

1. To improve seed germination and transplant uniformity of triploid watermelon genotypes
2. To increase plant growth, fruit quality and lycopene content under deficit irrigation

Seed germination

Scanning electron micrographs (SEM) of dry and imbibed seeds of triploid (3n) Tri X 313 and Tri X Sunrise, and diploid (2n) Sunsweet cvs. were taken to characterize structural components. The endotesta layer, the seed cavity surrounding the embryo and the cotyledons arrangement inside the seed cavity were three distinctive characteristics present in 3n and absent in 2n (see SEM micrographs). The air space or seed cavity was also greater and more variable in 3n. Seeds of 2n Allsweet and SugarLee, and 3n Tri X 313 and Tri X Sunrise were imbibed and the seed moisture increase was recorded until 125 h. Imbibition during the first h proceeded rapidly with an approximate gain weight of 50%. There was no correlation between weight gain and germination level of high and low vigor lots of Tri X 313; however, there was an inverse correlation in the 2n SugarLee, and 3n Tri X Sunrise with high and medium seed vigor. Overall, water uptake was greatest for 3n than 2n cultivars. Total seed protein content was analyzed by the BioRad micro-protein assay using BSA standards. Triploid seeds had higher level of proteins compared to 2n seeds, but not correlation was established with germination levels. All 2n and 3n cultivars contained raffinose and sucrose as the main sugars in the dry stage. After imbibition, raffinose was not present in 2n Allsweet, and 3n cvs. Tri X 313 and Tri X Sunrise high germination lot, but present in SugarLee, Tri X 313 and Tri X Sunrise low germination lots.

Solid matrix priming (SMP), incorporates extra-fine, hydrophilic particles into the seed coat to facilitate uniform water uptake into the seed and to promote seed germination. SMP was initially used in 3n Tri X 313 seeds by keeping the mixture of seed : Micro-Cel E : water for 3 days at 25°C. Seed germination test was conducted using thermogradient table at 18 °C and 25°C. SMP with hydrogen peroxide (H₂O₂) was effective to promote early germination rate. H₂O₂ alone decreased early germination rate compared to SMP+ KNO₃. H₂O₂ treatment also caused seedling vitrification. Even though intact seeds showed higher germination rate and normal seedlings than SMP treatments, there was a high fungi contamination with *Rhizopus* sp., *Fusarium* sp., and *Aspergillus* sp., common seed colonizers of many crop seeds. Because these fungi species inhibit germination and seedling growth, it was necessary to modify the initial plans in order to first control the fungi development. Currently, several fungicides used as seed treatments are under investigation.

In preliminary tests we used mefenoxam (Apron), difenoconazole (Dividend), captan, 2,6-Dichloro-4-nitroaniline (Botran), Thiram: tetramethylthiuran disulfide and fludioxonil (Maxim). Difenoconazole, captan+Botran, Thiram+Botran were effective in preventing fungi growth, but because of high concentration of captan and thiram, seed germination was poor, causing abnormal cotyledon and root growth. Once the most effective fungicide and rate is selected, it will be incorporated into the SMP treatments.

Fruit quality and lycopene response to irrigation

A lycopene assay developed by Sadler et al. (1990) was tested and used in watermelon fruit samples collected during 2000 harvest and kept frozen at -80°C . Based on preliminary assays, lycopene content averaged $36.5 \text{ ug} \cdot \text{g}^{-1}$ across 8 cultivars: Allsweet, 32 ; Star N-Stripes, 42.7; SugarLee, 27.1; Sugar Time, 20.8; SF 710, 55.4; SF 900, 51.2; and SS 5244, 27.0, and XIT $35.7 \text{ ug} \cdot \text{g}^{-1}$. During this spring, watermelon transplants were grown in trays in a commercial greenhouse, Donna, TX. Four-week old transplants were mechanically established in the field using a subsurface drip system and plastic mulch on 9 April 2001. Diploid cvs. are: SF 710, 8036, Sugar Lee and Allsweet. Triploid cvs. are: SS 5244, 7036, 8706, Sugar Time and Tri X Sunrise. Three irrigation treatments are being imposed based on evapotranspiration (ET) rates (1.0ET, 0.75ET and 0.50ET). Plant growth is proceeding normal and fruit set started 40 d after planting. We expect to start harvesting at the end of June, time at which fruit quality parameters and lycopene content will be determined.

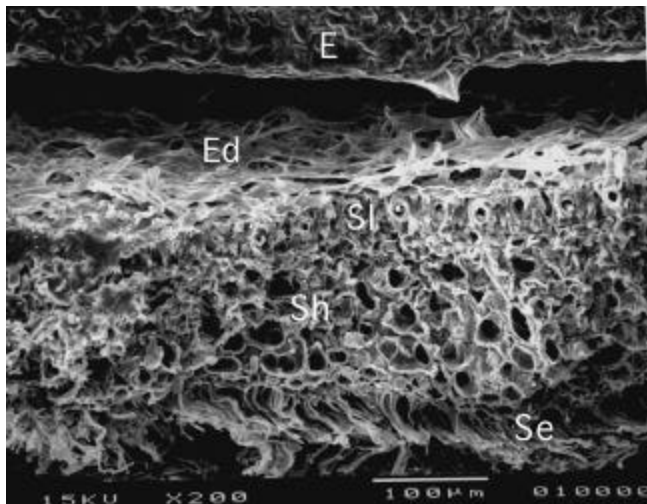


Figure 1. Cross section of a dry Sunsweet diploid seed at 200X magnification.

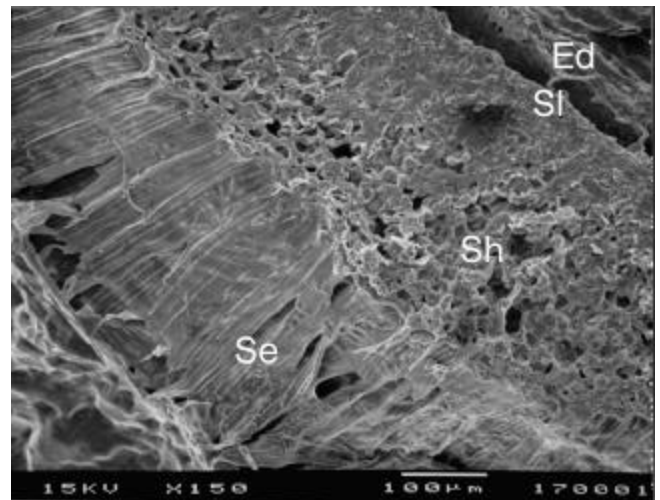


Figure 2. Cross section of a dry Tri X 313 low germination log triploid seed at 200X magnification.

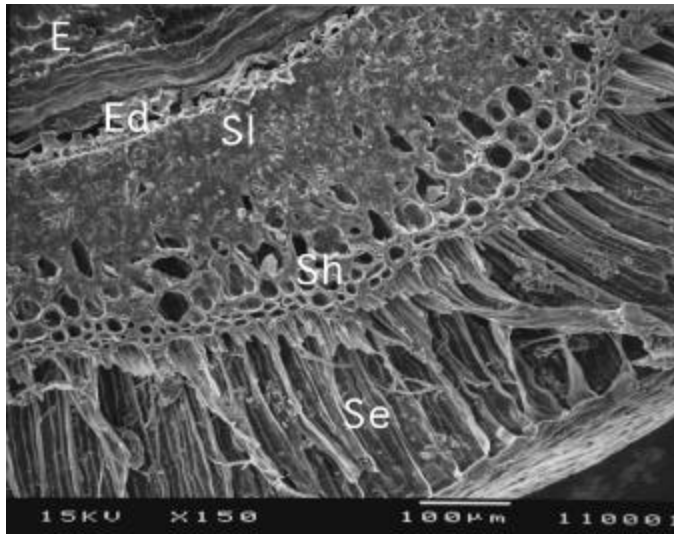


Figure 3. Cross section of an imbibed Sunsweet diploid seed at 150X magnification.

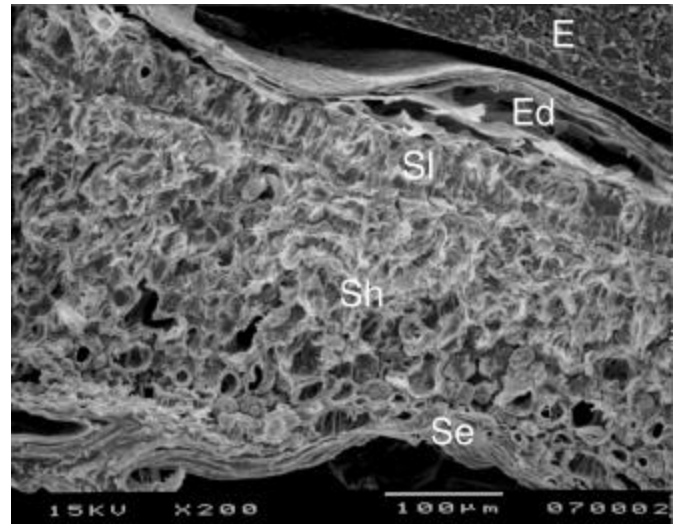


Figure 4. Cross section of an imbibed Tri X 313 low germination lot triploid seed at 200X magnification.

Cross sections of a diploid Sunsweet and a triploid Tri X 313 low watermelon seed by JEOL T-330A at 15Kv. E=embryo, Ed = endotesta, Sl = Sclerotic layer, Sh = seed hypodermis, Se = seed epidermis. The triploid endotesta (Ed) layer appears to be thicker and more dense than the diploid layer (Figs. 1 and 2). The difference between a dry and imbibed seed is also noticeable with the expansion of the seed epidermis(Se) of both the diploid and triploid seeds (Figs. 3 and 4).

Post-harvest

Irradiation Effect on Grapefruit Phytochemicals

Investigator:

Dr. Bhimanagouda Patil, TAMU-Kingsville Citrus Center

Objectives: The objectives are 1) to determine the effect of irradiation on carotenoids, flavonoids, and vitamin C; 2) to understand the effect of irradiation on consumer perception.

An orchard with 'Rio Red' grapefruit on sour orange rootstock planted at 8.4 and 4.5 m spacing was identified from the Texas A&M University Citrus Center South Farm. Approximately 200 fruits were harvested at each harvest (replication) time with three replication and run in commercial packing line, washed and waxed.

Irradiation treatment: Subsamples of 16 fruit/treatment/replication were treated with different doses of irradiation in USDA facility in Mission and treatments were as follows: control, 70 Gy, 200 Gy, 400 Gy, 700 Gy, with a dose of 0.716 Gy/second. Control (non-irradiated) samples were transported along with the other samples to avoid environmental effect. Fruit were stored for 4 weeks at 10 C and one week at 24 C with relative humidity 90-95%. Fruit from each treatment were extracted for phytochemical levels within 24 hours after irradiation and at the end of storage (five weeks).

Laboratory analysis: Subsamples of fruit were evaluated for different fruit quality attributes such as fruit color, peel thickness, firmness and analyzed for naringin, narirutin and total flavonones, soluble solids, acidity, % juice.

Flavonoid analysis. Samples were analyzed for flavonone content by reverse phase HPLC with some modifications of the Berhow (2000) method. An aliquot of juice sample was diluted at 1:1 ratio with dimethylsulfoxide, centrifuged and filtered through 0.45- μ m nylon filters and 20 μ l of this solution was injected into HPLC system. The same HPLC system explained the LG section was used and a Adsorbosil C-18 column (250 X 4.6mm, 5 μ m) was eluted with a linear gradient starting with 10% acetonitrile 5 mM phosphoric acid and ending at 26% acetonitrile in acetonitrile 5 mM phosphoric acid for 36 min. The narirutin and naringin peak were detected at 280 nm with retention times of 25 and 27 min, respectively. The flavonones were identified by confirmation of their respective spectra with authentic standard and retention times.

Quantification. External standards were used to quantify the compounds. Peak areas were normalized to the external standard and standard curve was fitted by linear regression (peak areas vs. concentration in μ g.g⁻¹). Total flavonone was calculated by combining naringin and narirutin concentrations. The long-term variability of flavonones in the laboratory was low (coefficient of variation 6%).

Sensory evaluation. Fruits from each treatment were evaluated for consumer acceptability by 20-25 average panelists (TAMUK Citrus Center employees) to determine the flavor and external appearance of the fruits. Flavor, external appearance, and overall ratings were evaluated from a scale with 1 being extremely disliked and 10 being the extremely liked (hedonic scale).

Results and discussion

Although doubts have been raised about the prospect for gamma irradiation exposures as a post harvest treatment for fresh produce (Maxie et al., 1971), the Food and Drug Administration has approved the treatment of fruits and vegetables with gamma irradiation up to 1 kGy (USDHHS, 1986). Presently, at least 21 countries irradiate different foods on a commercial scale.

Results from Table 1-3 indicate the effect of irradiation on naringin, narirutin, and total flavonoids concentrations in grapefruit immediately after treatment and after 5 weeks of storage (4 weeks at 10 C at 90-95% relative humidity and one week at 24 C at 90-95% relative humidity). Fruits received 70 Gray irradiation treatment recorded higher concentrations of flavonones at the end of storage compared to initial concentration in three different tests. Previous researchers reported that irradiation treatment enhanced the content of some phenolic acids and flavonoids in mango fruits during prolonged low temperature storage (Lacroix et al., 1990). Increase in flavonoids concentrations due to irradiation can be attributed to enhanced phenylalanine ammonia-lyase (PAL) activity (Oufedjikh, 1996 and 2000).

The present study also showed a slight increase in naringin concentration compared to control fruits. Faragher et al., (1983) reported similar results previously in apple. The authors attributed the increase in naringin to higher PAL activity in fruits stored at 10 C than fruits stored at 24 C.

Accumulation of phenolic compounds varies strongly in relation to the physiological state of fruit and time of harvest and is a result of equilibrium between biosynthesis and further metabolism including turnover and catabolism. PAL (EC 4.3.1.5) catalyses the first connected step in the biosynthesis of a diverse range of phenyl propanoid derived secondary products in plants, such as flavonoids, isoflavonoids, coumarins and lignins (Hanson and Havi, 1979; Jones, 1984). Variations observed in the concentrations of flavonoids in different tests at similar irradiation treatments can be attributed to the differences in time of harvest.

The differential response of naringin and narirutin to the same level of irradiation treatment in the present study could be attributed to degradation of flavonoids due to irradiation. Previous results showed a significant correlation ($r = -0.91$) with totality of methoxy groups (OCH_3) linked to flavone skeleton and degradation is an exponential function coupled to the number of $-\text{OCH}_3$ groups (Oufedjikh et al., 1998).

Plans for next quarter

Samples for analyzing remaining phytochemicals such as lycopene, beta-carotene, and vitamins C were extracted. Analysis of these phytochemicals is under progress.

Table 1. Effect of gamma irradiation on naringin concentrations^z in 'Rio Red' grapefruit.

| Treatment | Test I | | Test II | | Test III | |
|-----------|-----------|-----------|----------|----------|----------|----------|
| | Initial | Final | Initial | Final | Initial | Final |
| Control | 804.7 b | 1181.2 b | 1168.7 b | 810.8 c | 1148.5 b | 1360.6 a |
| 70 Gy | 1003.2 b | 1353.0 a | 815.2 b | 891.8 c | 1144.5 b | 1365.5 a |
| 200 Gy | 1497.0 a | 1127.5 b | 1245.7 a | 1136.3 b | 1440.7 a | 1347.2 a |
| 400 Gy | 963.8 b | 1071.4 b | 925.5 b | 1097.9 b | 1251.5 b | 1347.2 a |
| 700 Gy | 1291.9 ab | 1228.9 ab | 908.0 b | 1286.3 a | 1169.2 b | 1072.6 a |

^y Means separation within columns by LSD, $P \leq 0.05$

^z Values are means for 18 or 30 fruits treatment or test, respectively

Table 2. Effect of gamma irradiation on narirutin concentrations^z in 'Rio Red' grapefruit.

| Treatment | Test I | | Test II | | Test III | |
|-----------|----------|-----------|----------|---------|----------|----------|
| | Initial | Final | Initial | Final | Initial | Final |
| Control | 139.92 b | 245.50 ab | 125.4 b | 150.6 b | 140.1 b | 207.0 ab |
| 70 Gy | 220.77 a | 277.43 a | 126.5 a | 144.0 b | 141.2 b | 225.3 a |
| 200 Gy | 216.57 a | 240.52 b | 137.1 ab | 161.7 b | 230.3 a | 160.3 b |
| 400 Gy | 241.47 a | 265.26 ab | 156.8 ab | 150.6 b | 241.2 a | 252.6 a |
| 700 Gy | 231.61 a | 265.12 ab | 170.0 a | 240.0 a | 167.2 b | 231.2 a |

^y Means separation within columns by LSD, $P \leq 0.05$

^z Values are means for 18 or 30 fruits treatment or test, respectively

Table 3. Effect of gamma irradiation on total flavonone concentrations^z in 'Rio Red' grapefruit.

| Treatment | Test I | | Test II | | Test III | |
|-----------|-----------|-----------|-----------|----------|-----------|----------|
| | Initial | Final | Initial | Final | Initial | Final |
| Control | 944.7 c | 1426.7 b | 1294.0 ab | 961.5 c | 1288.6 c | 1567.6 a |
| 70 Gy | 1224.0 bc | 1630.4 a | 941.7 c | 1035.5 c | 1285.7 c | 1590.8 a |
| 200 Gy | 1713.5 a | 1368.0 b | 1382.9 a | 1297.9 b | 1671.0 a | 1269.5 a |
| 400 Gy | 1205.3 bc | 1336.7 b | 1082.3 bc | 1248.5 b | 1492.7 b | 1599.7 a |
| 700 Gy | 1523.6 ab | 1494.0 ab | 1078.1 bc | 1526.4 a | 1336.4 bc | 1303.8 a |

^y Means separation within columns by LSD, $P \leq 0.05$

^z Values are means for 18 or 30 fruits treatment or test, respectively

Fruit and Vegetable Packaging Design to Preserve Health Benefits and Quality

Investigators

Luis Cisneros-Zevallos, Horticultural Sciences, Texas A&M University

Luis Reyes, Graduate Assistant, MS Candidate

Objectives

Develop appropriate post-harvest handling procedures for maintaining the nutrient integrity and sensory quality of whole and fresh-cut fruits and vegetables during storage.

Progress Narrative - This progress report includes work done with spinach. We are actually working with maroon carrots and other vegetables as well.

Title of study: *Respiration Rates of Spinach and the Effect of Storage Temperature*

Sampling carbon dioxide and oxygen gases in closed jars, allows determination of respiration rates of fruits and vegetables at different oxygen compositions necessary for appropriate packaging design. This study looked at the effect storage temperature on the oxygen depletion and quality characteristics of spinach for packaging design.

Materials and Methods

Bossanova spinach cultivar grown in California was obtained from a local retail store. Similar spinach leaf size was used and the stems were cut, leaving only .5 inches of stem. The spinach leaves were placed in a salad spinner for one minute to remove surface moisture and 17 grams placed in one-pint respirometer jars (500ml). The jars were placed at 0°, 5°, 10°, 15°, and 20° C. Respiration rates, based on CO₂ production and O₂ uptake, were determined by measuring the concentration of gases in the respirometer jars for a short period of time. Three replicates for each temperature were tested for respiration rate. Oxygen and Carbon Dioxide were measured using a Gow Mac gas chromatograph series 500.

Following the respiration test, an oxygen depletion test was conducted. Using the same type of respirometer jars, the jars were placed at 2°, 5°, 10°, 15°, and 20° C. Three replicates for each temperature were tested for oxygen depletion and carbon dioxide production. All jars were measured after the first four hours and then every twelve hours for 10°, 15°, and 20° C, and every 24 hours for 2° and 5° Celsius until oxygen was depleted.

Results

Respiration rates decreased as the temperature of storage was reduced. (Fig. 1) Respiration rate of spinach stored at 20°C was an average value of 115 ml/kg-hr and for 15°C the average rate was 90 ml/kg-hr. Spinach stored at 5°C had a respiration rate of 11.41 ml/kg-hr. Lower temperatures reduce respiration rate and in turn deters degradation of the spinach.

The oxygen uptake occurred at a faster rate at 20°C than at 15°C. Figure 2 shows the uptake of oxygen versus carbon dioxide production over a period of ten days. B-

tween day six and seven the carbon dioxide production increased dramatically, due to the depletion of oxygen and anaerobic respiration of the spinach occurring at the lower oxygen limit (*LOL*). The spinach stored at 15°C followed a very similar pattern. Figure 3 shows the uptake of oxygen also over a ten-day period. The oxygen was depleted around the seventh day in the jars stored at 15°C. At this point the spinach entered anaerobic respiration and carbon dioxide production increased substantially indicating the lower oxygen limit was reached. Up until day seven the spinach was in very good condition with no visual signs of quality loss. However, after the spinach entered anaerobic respiration, the quality of spinach drastically decreased. There were signs of wilting; growing increasingly worse for the spinach stored at 20°C, as well as notable color changes.

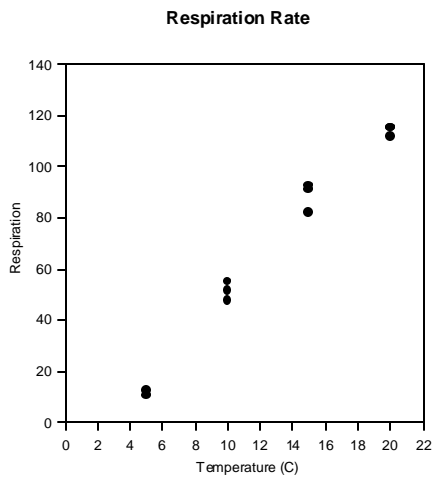


Figure 1. Respiration rate at different storage temperatures.

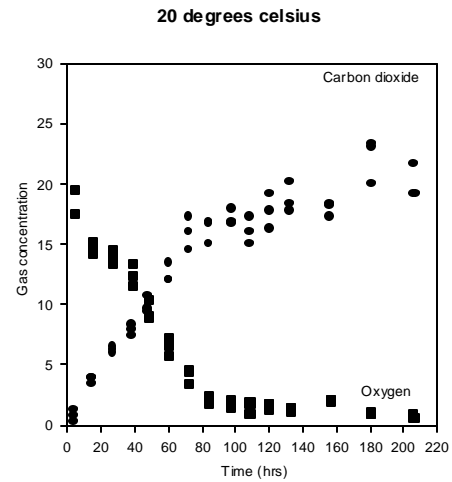


Figure 2. Oxygen depletion at 20°C.

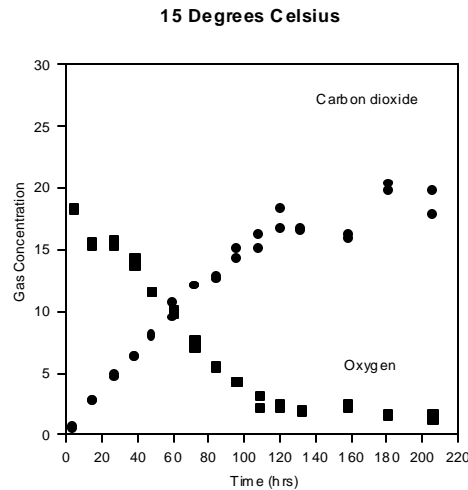


Figure 3. Oxygen depletion at 15°C.

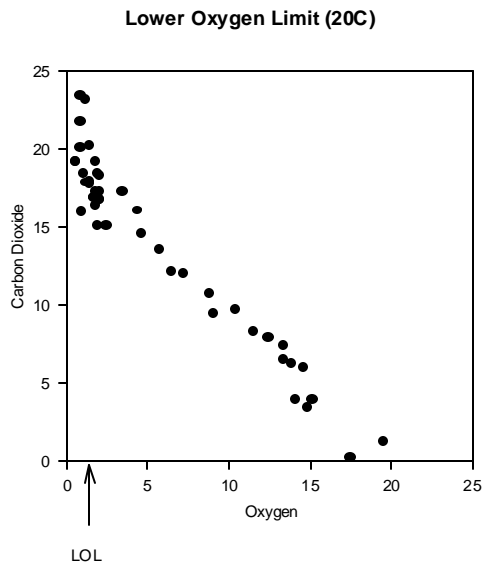


Figure 4. Lower Oxygen limit at 20°C.

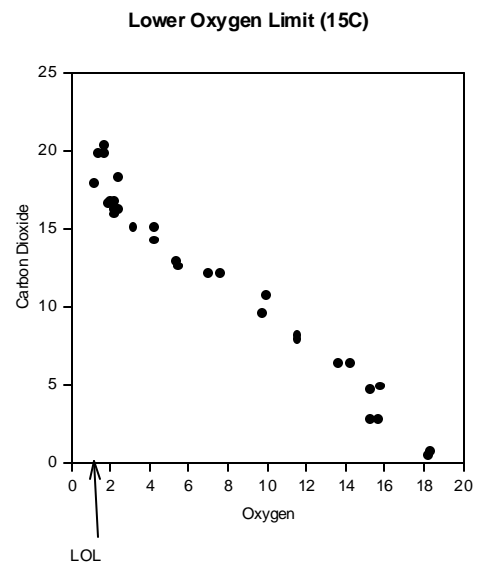


Figure 5. Lower Oxygen limit at 15°C.

Similar results were obtained at 10, 5 and 0C. The lower oxygen limit in our study ranged from 1 to 2%. This value can be used as a "oxygen safety margin" for packaging design. Studies with other spinach varieties and maroon carrots are in progress with similar approach

From our results we confirm that knowledge of aerobic respiration rates of spinach tissue is essential for the design of an ideal package, which extends the shelf life of the product. The determination of the lower oxygen limit is very important when designing the appropriate modified atmosphere package (e.g., for spinach). Depending on the permeable surface and gas permeability of the packaging material and the rates of oxygen consumption and carbon dioxide evolution of the produce, the atmospheric composition around the produce is modified. As can be seen from the results, spinach is a delicate tissue that respire very quickly. Therefore, in order to increase the shelf life of the spinach a package must be designed, which will prevent the oxygen uptake beyond the lower oxygen limit (*LOL*). The spinach stored at 20°C had a lower oxygen limit at approximately 2% O₂. Once the oxygen uptake continued below 2% O₂, the tissue went into anaerobic respiration and the carbon dioxide production drastically increased. The spinach stored at 15°C had a slightly lower *LOL* at around 1.5% O₂. To preserve the quality of fresh spinach for longer periods of time, it is best to lower the storage temperature to reduce the respiration rate and the rate at which the spinach tissue approaches the lower oxygen limit (*LOL*).

Publications -- list those publications ready for submission including the journal.

1. Packaging design for fresh-cut Spinach. 2001. To be submitted to the Journal of Food Science.

Evaluation of Watermelon Cultivars Produced under East Texas Cultural Practices and Growing Conditions for Lycopene and Other Antioxidant Concentrations

Investigators:

D. R. Earhart, Texas Agricultural Experiment Station, Overton
Luis Cisneros-Zevallos, Dept. of Horticultural Sciences, TAMU
M. L. Baker, Texas Agricultural Extension Service, Overton
F. J. Dainello, Texas Agricultural Extension Service, TAMU
Dave Bender, Texas Agricultural Experiment Station, Lubbock
John Drawe, Texas Agricultural Experiment Station, Weslaco
Julio Loaiza, Dept. of Horticultural Sciences, TAMU

Objective:

The overall objective of this study is to determine the effects of growing location on accumulation of phytochemicals with health benefit properties in watermelon fruits. Selected red and yellow flesh hybrids and a red flesh seedless watermelon fruits adapted to three distinct Texas climates, Overton, Weslaco and Lubbock, will be tested for carotenoids, vitamin C, phenolic compounds and antioxidant activity.

It is expected that pre-harvest factors will have an influence on the synthesis of secondary metabolites in fresh watermelons that previous work has shown to have health benefit properties. This information could be used for variety selection and identifying growing location for enhancement of antioxidant compounds in watermelon fruit.

Progress narrative:

Transplants of two hybrid (Royal Sweet, Summer Gold) and one seedless (Gem Dandy) watermelon varieties grown in a greenhouse have been field planted in Weslaco, Overton, and Lubbock in replicated trials. The plots will be harvested in June, mid July and beginning of August, respectively. An appropriate number of fruit from each replicate at each location will be transported to Dr. Cisneros in College Station for analysis. Fruits will be stored at 0C until use for quality attribute evaluations, while tissue samples will be stored at -40C for phytochemical analysis.

a) Characterization of phytochemicals and quality attributes:

Watermelon varieties will be analyzed for quality attributes and levels and types of phytochemicals present. Phytochemicals to be studied are, phenolic compounds, carotenoids, vitamin C, sugars and acids. Determination of total phenolic content will be done by the Folin-Ciocalteu phenol reagent assay (Hyodo et al., 1978; Talcot and Howard, 1999). Identification, quantification of individual phenolics will be done by HPLC method (Ke and Saltveit, 1988; Lee et al., 1995) coupled to a PDA detector. Total carotenoids will be determined using a spectrophotometric method (Talcot and Howard, 1999). Sugar analysis (Howard et al., 1996), specific carotenoids (including Lycopene) and ascorbic acid determination (Howard and Hernandez, 1998) will be performed using HPLC methods. Additionally, total acidity, soluble solids, pH, density and dry matter will be evaluated.

b) Characterization of antioxidant activity.

For the evaluation of antioxidant activity of watermelon fruits, extracts will be allowed to react with a stable radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in a methanol solution (Brand-Williams et al., 1995). This will provide an easy and rapid way to evaluate the anti-radical activities of antioxidants. Two standard curves will be prepared, one with Ascorbic Acid (vitamin C) and one with Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a powerful antioxidant), to use as a reference with the extracts. We will also evaluate the antioxidant activity of blueberries to establish a comparison with our samples.

c) Time table.

| Research work | | | Time (month) | | |
|---|--------|---------------|------------------|--------------|---------|
| | June | July | August | September | October |
| a)Phytochemicals and quality Weslaco Overton Lubbock | XXXXXX | XXXXXX XXX | XXXXXX XXXXXX | XX XXXXXX | XX |
| b) Antioxidant activity Weslaco Overton Lubbock | XXXXXX | XXX | XXXX XXXXXX | XXX | |

Publications:

After completing the study, papers and abstracts will be prepared and submitted for publication.

Feeding Study

Assessment of the ability of quercetin to inhibit colon carcinogenesis

Investigators:

Nancy D. Turner, Animal Sciences, Texas A&M University

Joanne R. Lupton, Human Nutrition and Cell Biology, Texas A&M University

Robert S. Chapkin, Human Nutrition and Cell Biology, Texas A&M University

Stephen S. Safe, Veterinary Physiology & Pharmacology, Texas A&M University

Objectives: The experiment is designed to demonstrate whether dietary quercetin is able to: (1) inhibit the formation of DNA adducts and reduce the number and multiplicity of aberrant crypt foci, and (2) alter proliferation and apoptosis through inhibition of PI 3-kinase.

Procedures: Sprague Dawley rats will be received in the LARR animal facility at 28 d of age, and will be acclimated to the facility and to being handled for 1 week. Then the rats will be provided with experimental diets containing either no quercetin or 0.5% quercetin, which they will consume for the remainder of the study. After consuming the assigned diets for 1 week, the rats will then be given the first subcutaneous injection of azoxymethane (15 mg/kg body weight) or saline. Seven days later they will receive the second carcinogen or saline injection.

Five rats assigned to each diet*injection group (n = 20) will be terminated following the second carcinogen injection (12 h after the injection). The remaining rats (n = 40) will continue to receive the experimental diets for 3 more weeks before they are terminated. At termination, the colon will be removed, cleaned, and opened longitudinally. The right half will be used for the aberrant crypt foci assay. The left half will be used for immunohistochemical assays on fixed tissues and determination of PI 3-kinase protein content and activity from scraped mucosa. One centimeter from the proximal and distal colon will be fixed in 4% paraformaldehyde. Samples from the proximal and distal colon containing aberrant crypt foci will also be fixed for immunohistochemical analyses. We will determine DNA damage, proliferation and apoptosis on the fixed tissues. Samples of the liver will also be collected to determine DNA damage.

Progress: Final experimental design considerations have been made and the diet ingredients ordered. We are in the final stages of coordinating and scheduling the use of the negative pressure animal facilities required to conduct this work. Because of the extensive use of this facility by other long-term projects, we were unable to gain access to the facility until spring 2001. Once we have the final schedules established with the animal facility, animals will be ordered and the experiment initiated.

Timetable: The in vivo portion of this experiment will be completed within 4 months of starting the project. Immunohistochemical assays and statistical analysis will require 6 months for completion. Within 4 months of completing the project, a paper will be prepared and submitted for publication.

Health Promoting Properties of Citrus Limonoids

Investigators:

Edward G. Miller, Baylor College of Dentistry, Dallas

Bhimanagouda S. Patil, TAMU-Kingsville Citrus Center

Samuel E. Taylor, Baylor College of Dentistry, Dallas

Charles W. Berry, Baylor College of Dentistry, Dallas

Recent events will delay by approximately 2-3 months the start of the feeding trial and the work with microorganisms. As indicated in the original proposal commercial interest in the health-promoting properties of the citrus limonoids is starting to develop. Most of the companies are located in Asia and Europe; however, two large international companies have their home offices in the United States. One of these companies has recently decided to set up a pilot plant to produce large quantities of mixed limonoid glucosides from by-products from one of their juice processing plants. Because of their interest in the eventual development of functional foods containing mixed limonoid glucosides, they have agreed to supply the chemicals to us free of charge for our research. This will give us access to kg quantities of mixed limonoid glucosides and will provide a mixture that is essentially free of contaminants. The first shipment is scheduled to arrive this spring (April or May). Since sources for the aglycones have already been secured (Dr. Shin Hasegawa, Western Regional Research Center, ARS, USDA, Albany, CA), we will be able to start the one-month feeding trial and the microbiological assays at that time.

To accelerate the HPLC analyses especially the blood assays, several preliminary experiments will be done in February and March. These experiments will help to standardize the procedure for preparing the samples. Similar studies have already been done with the growth media for the microorganisms. This work was part of a recent presentation given at the International Society of Citriculture Meeting held December 3-8, 2000 in Orlando, FL. The title of our presentation was "Limonin 17- β -D-glucopyranoside: Effects on Microorganisms Commonly Found in the Lower GI Tract". Overall these preliminary studies will help to speed up the HPLC assays, which will be done this summer and early fall. The data will also analyzed at this time. It is expected that all of the work will be completed on schedule by the end of October 2001.

Education

Can Education and Hands-on Learning affect Children's Attitudes and Behaviors Regarding Fruits and Vegetables?

Investigators:

Jayne Zajicek, Horticultural Sciences, Texas A&M University

Roxanne Boyer, Graduate Assistant, MS Candidate

Sharon Koch, Graduate Assistant, MS Candidate

Carolyn Robinson, Graduate Assistant, MS Candidate

Objective 1: Evaluate if students develop a more positive attitude about fruits and vegetables and better nutritional behaviors after participating in a garden-based curriculum program.

Nutrition has a major impact on the growth and development of children and therefore plays an important role in their lives. One part of proper nutrition is the consumption of five fruits and vegetables a day. Children currently eat an average of 3.4 servings of fruit and vegetables a day. Education is needed to increase the consumption of fruits and vegetables. Two research projects investigating the effect of nutrition education and garden activities on children's nutritional knowledge, attitudes, and eating behaviors have been completed.

The first study incorporated the garden program - *Nutrition in the Garden* – which was designed to help teachers integrate nutrition education into their classroom using a hands-on tool, the garden. The objectives of this research project were to: 1) develop a garden activity guide to help teachers integrate nutrition education, specifically as it relates to fruits and vegetables, into their curricula, 2) evaluate whether students developed more positive attitudes towards fruits and vegetables by participating in the garden program, and 3) evaluate whether students developed better nutritional behavior by eating more fruits and vegetables after participating in the garden program. Students' nutritional attitudes regarding fruits and vegetables were measured with a fruit and vegetable preference questionnaire divided into three sections targeting vegetables, fruits, and fruit and vegetable snacks. Students' nutritional behaviors regarding fruits and vegetables were evaluated through 24-hour recall journals. After gardening, students' attitudes towards vegetables became significantly more positive. In contrast, no differences were detected in attitudes towards fruits. Students also had more positive attitudes towards fruit and vegetable snacks after gardening, with female students and younger students having the greatest improvement in snack attitude scores. Even though school gardening improved students' attitudes towards vegetables, fruit and vegetable consumption of students did not significantly improve due to gardening. Overall, the average daily fruit and vegetable consumption of the students participating in the *Nutrition in the Garden* study was 2.0 servings a day. This falls short of the estimated national average for daily fruit and vegetable consumption for this age group (3.4 servings) and extremely short of the nationally recommended 5 servings a day.

A second study was completed to evaluate whether a nutritional curriculum guide, including garden activities, could improve children's nutritional knowledge, attitudes, and eating behaviors. A curriculum guide "Health and Nutrition from the Garden" was developed for *Better Living for Texans* County Agents to use in workshops with children. A modified version of the pretest/posttest instrument used in the first study was used to determine students' attitudes toward fruits and vegetables. A questionnaire was developed to determine knowledge gained, and five interview questions were asked to help determine changes in eating behavior. Results indicate differences in children's nutritional knowledge and eating behaviors prior to participation in the nutritional activities due to age. Younger students had less knowledge, poorer eating behaviors, and lower fruit and vegetable preference scores than older students. These differences were no longer statistically significant after completing the nutritional curriculum.

Objective 2: Educate and train undergraduate and graduate students in all facets of this proposal.

The above two studies were designed, conducted, and analyzed by two Master of Science students.