CITRUS TISSUE CULTURE AS A MEANS OF STUDYING THE METABOLISM OF CAROTENOIDS AND CHLOROPHYLL[1]

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ABSTRACT

Tissue culture technique offers a means of studying pigment changes in relatively sterile, long time conditions in citrus fruit tissue. Grapefruit albedo tissue was capable of new growth and synthesizing chlorophyll and carotenoids. The synthesis of chlorophyll was light dependent, but the pigment was not produced immediately upon exposure to light. It needed about 5 days to be seen visually and spectrophotometrically. Carotenoids were shown to be present when chlorophyll was present. The decomposition of chlorophyll was slow in the absence of light but was accelerated when growth regulators (2,4-D and kinetin) were removed from the nutrient culture. Even though chlorophyll was destroyed, only 50% was lost in 2 weeks.

Some protein inhibitors caused complete inhibition of growth, protein synthesis, and chlorophyll synthesis at 1000 ppm.

INTRODUCTION

In the past, the study of the flavedo of citrus fruits has been limited to the use of whole fruits. This limited the application of chemicals that may be utilized to control a particular plant process. There was often doubt as to whether the chemicals actually reached the area of the fruit under investigation. Color changes in citrus were quite slow, taking a matter of weeks for noticeable changes to occur. Attempts were made to remove sterile discs from the peel of citrus, but this resulted in contamination by a fungus growing beneath the waxy cuticle of the peel which resisted sterilization. Although other plant parts have been cultured, citrus tissue has resisted the efforts of workers to establish good growth. Kordan (2, 3) was able to obtain limited growth of lemon vesicles in cultures. Recently, Tucker (6) established nutrient parameters under which citrus tissue would grow and could be transferred repeatedly. He was also able to show that cultures of fruit parts other than the flavedo were capable of producing chlorophyll and carotenoid pigments.

This study was made to extend the work of Tucker and to develop a means of studying the metabolism of fruit pigments.

METHODS

Culture Medium: The medium used was that of Tucker (6) which contained a full complement of all the essential elements plus 2,4-D, kinetin, thiamin-HCI, nicotinic acid, pyridoxine-HCI, myo-inositol, glycine, and sucrose. Agar was added at 1% to form a solid media. Ten ml of solution were placed in 25 x 100 mm test tubes and sterilized in an autoclave at 15 lbs pressure for 20 minutes.

Tissue Preparation: Whole fruit or quarters were initially sterilized in a 1 to 20 dilution of chlorox for 15 minutes. After surface sterilization, plugs of flavedo and albedo were removed near the equator of the fruit with a cork borer, 1 cm in diameter. The plugs were sliced into discs about 1 to 1.5 mm thick; only the white albedo was used. These discs weighed approximately 50 mg. Enough discs were accumulated to fill all the culture tubes necessary for an experiment. The discs were chosen at random to be placed in culture tubes. In these experiments, grapefruit tissue was used because of the large quantity of tissue available.

Growth Conditions: The cultures were grown at 90° F in darkness or under artificial illumination—fluorescent and incandescent—for 12-hour photoperiods at 450 foot candles.

Chlorophyll Determination: Individual discs were ground in a mortar with pestle to give a 5 ml final volume of 80% acetone. The mixture was clarified by centrifuging in an International table model centrifuge. Spectra or absorbance at 663 and 645 $m\mu$ were obtained on a Beckman DK2 spectrophotometer. Chlorophyll content was calculated by the method of Arnon (1).

Protein Determination: Individual discs were

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ground and extracted with 3 ml of 0.1M. Tris buffer pH 7.0 in a mortar with pestle. The solution was drained into a 10 ml volumetric flask. This was repeated 2 times, and the extract brought to volume. The extract was centrifuged and samples for protein determinations were removed and analyzed by a modified Lowry method (5).

Development of Pigments in Tissue: Culture flasks were planted with tissue from immature fruit (2 to 3 cm in diameter) and from mature fruit. One-half of each set of culture flasks was placed in the dark and the other half in the light. After 7, 9, and 14 days, 2 discs from each treatment were removed and analyzed for chlorophyll. On the fourteenth day, the tissue extract was saponified and analyzed for the presence of carotenoids.

Synthesis and Decomposition of Chlorophyll: A single disc of albedo from immature fruits (2 to 3 cm in diameter) was placed in a culture flask. After 11 days in the light, the remaining discs (after previous sampling) were divided and one-half was transferred to a new flask containing a complete nutrient culture. The remaining discs were placed on a growth-regulator-deficient culture. After the transfer, all of the cultures were placed in the dark. At random intervals, discs were analyzed for fresh weight, chlorophyll, and proteins. Each determination represented 4 replications.

Results

Development of Pigments in Tissue: In a period after bloom, albedo tissue of immature and mature grapefruit was placed on complete nutrient culture. The cultures were divided between light and dark conditions. Chlorophyll was not evident, visually or analytically, until 5 days after exposure to light. The data in Figure 1 show the spectral curves of extracts of 2 individual discs of mature and immature tissue grown in the light for 7 days showing the absorbance due to chlorophyll. The tissue from the immature fruit produced chlorophyll to a much greater degree than tissue from mature fruit when grown in the light. There was a slight indication of chlorophyll in the tissue from mature fruits. In 9 days, an increase in chlorophyll was noted in both sets of tissue in the light but only a very small amount in the tissue from mature fruit. Similar spectral curves of chlorophyll were noted at 14 days. At this

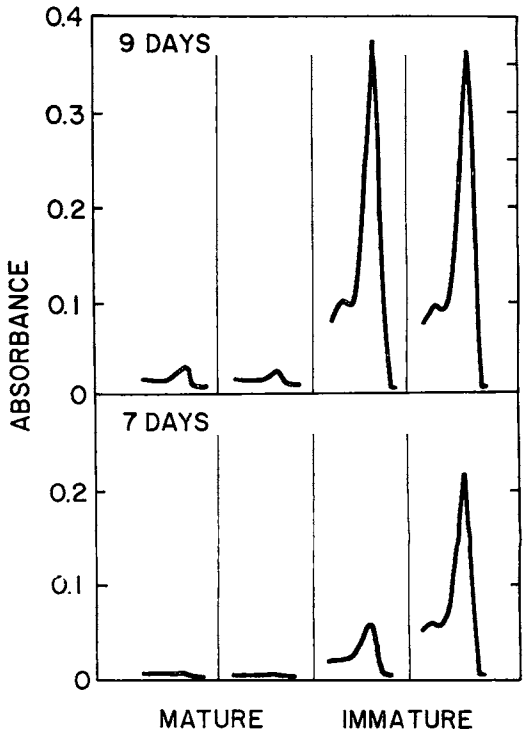

Fig. 1.—Absorbance spectra between 600 and 700 mu of duplicate extracts of mature and immature grapefruit albedo discs grown in the light showing the presence or absence of the chlorophyll absorbance peak at 663 mu.

time, the chlorophyll pigments were removed by saponification and the absorption spectra in the region of carotenoid absorption were obtained (Fig. 2). The spectra of extracts from immature tissue grown in the light were similar to those obtained for carotenoids. Carotenoids spectra were shown to be present in the extracts from the immature tissue but were not shown to be present in the mature tissue. Some carotenoids may have been present, but not in sufficient quantity to be detected by the analytical procedure used. The tissue grown in the dark did not give any indication of pigments or chlorophyll precursors.

When discs of grapefruit were placed on complete culture media, new growth did occur. New growth occurring in tissue from mature fruit developed within the tissue as small clumps or nodules around the vascular tissue in the disc. The original mature tissue appeared to be pushed out in front of the new growth. The growth occurring on tissue from immature fruit appeared on the whole surface. This would indicate

that the albedo of immature fruit have the ability to synthesize chlorophyll, but as the fruit ages, the albedo cells lose this ability.

Synthesis and Decomposition of Chlorophyll: In an attempt to study the decomposition of chlorophyll, tissue was allowed to grow in the light to develop chlorophyll. The tissue was then divided into 2 groups, 1) transferred to a complete nutrient culture and 2) transferred to a complete nutrient culture but lacking growth regulators (2, 4-D) and kinetin). After the transfer, the tissue was placed in the dark. Figure 3 shows the changes in chlorophyll and protein content and the total growth. All of these factors showed an increase until transferred to the dark. After 2 days in the dark, the growth of the tissue without growth regulators ceased while the growth of tissue on complete nutrient continued.

Chlorophyll and protein synthesis increased until the tissue was removed from the light. In the dark, protein and chlorophyll content of the tissue growing in the absence of growth regulators decreased. Although the chlorophyll decreased, it only decreased about 50% after 2 weeks compared to approximately 10% reduction occurring in the tissue in the presence of growth regulators. Protein synthesis in tissue growing in the presence of growth regulators increased for 7 days in the dark but leveled off for the next 7 days.

These results indicated that the chlorophyll was quite resistant to destruction and the turnover rate was very low. Without growth regulators, which seem to retard the destruction of chlorophyll, the pigment was destroyed to some degree, but in the presence of growth regulators, the chlorophyll pigment was quite resistant to degradation.

Effect of Antimetabolites: Protein inhibitors, chloroamphenicol, ethionine, actidione, and hydroxy urea at 1000 ppm completely inhibited growth, chlorophyll synthesis, and protein synthesis.

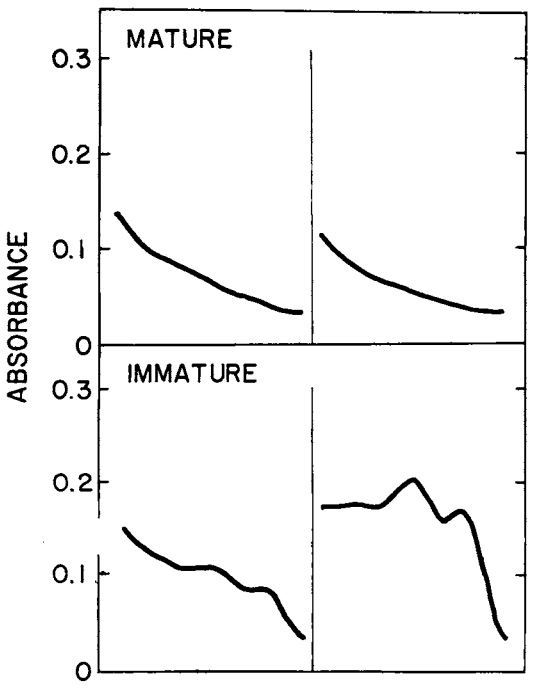

Fig. 2.—Absorbance spectra between 400 and 600 mu of duplicate extracts of mature and immature grapefruit albedo discs grown in the light showing the presence or absence of the carotenoid peak in the region of 440 mu.

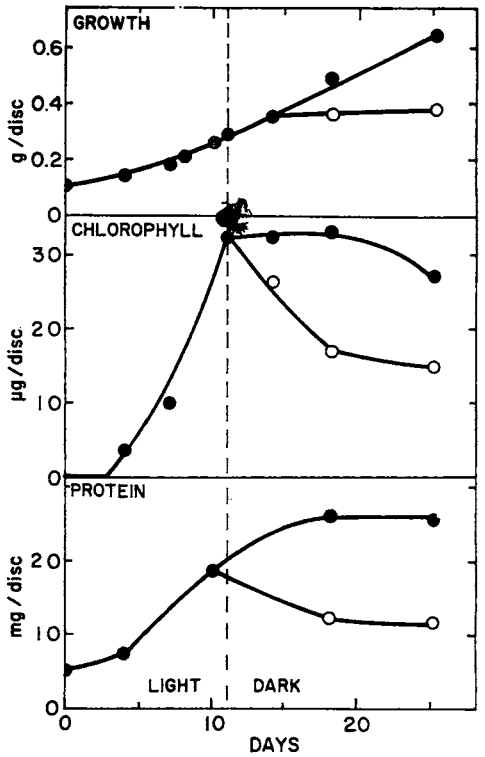

Fig. 3.—Growth, chlorophyll content, and protein content of grapefruit albedo tissue grown in the light with complete nutrient media and then transferred to media without growth regulators ●, and with growth regulators O and placed in the dark.

Discussion and Conclusions

Tissue culture technique offers a means of studying pigment changes in relative sterile, long time conditions to observe the changes in citrus fruit tissue. It was interesting that albedo tissue from immature grapefruit could synthesize chlorophyll when placed in the proper conditions but seemed to lose this ability as the fruit became older. The synthesis of chlorophyll was quite slow when the tissue was placed in the light, and the destruction was also slow. With these observations, it can be concluded that the turnover rate of chlorophyll was very low. The growth of the tissue was dependent on the presence of the growth regulators, 2,4-D and kinetin. Also these regulators appeared to protect chlorophyll from catabolic activities in the absence of light. When the regulators were removed from the nutrient solution, chlorophyll was destroyed at a slow rate. This slow destructive rate of chlorophyll in this tissue was similar to the slow degreening rate of whole fruit in the dark (4).

Carotenoid synthesis appeared to be associated with the synthesis of chlorophyll. Carotenoids are located in the choroplasts in close association with the chlorophyll molecule. It is possible that the delayed appearance of chlorophyll in albedo tissue may be associated with development of chloroplasts. If chloroplasts are not present in the tissue, then they must develop before there is a site for chlorophyll and carotenoid synthesis.

Since protein inhibitors have been shown to prevent the synthesis of chlorophyll and proteins, they provide an interesting series of compounds to study the means of affecting rate of chlorophyll decomposition in citrus fruits.

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NITROGEN CONTENT OF FLORIDA ORANGE JUICE AND ORANGE JUICE CONCENTRATE[1]

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Abstract

Total nitrogen content of orange juice samples obtained throughout the season from 3 processing plants in Florida showed a definite increase as fruit matured. The late-season fruit is much higher in nitrogen content than the early and midseason varieties. Significant differences were found among samples obtained on different days of each month, and the coefficient of variations of samples from different loads received on the same day by a single processing plant varied between 5 to 13% for different months.

The nitrogen content of commercially packed 42° Brix frozen concentrated orange juice was found to vary in a rather narrow range with a coefficient of variation of 4%.

Introduction

A study of the chemical composition of citrus juices produced in Florida is necessary in order to establish distinctive characteristics of the natural juices of the fruit. One of these chemical characteristics is the total nitrogen content which is derived from all the nitrogenous constituents in the juice. About 10% of the soluble solids of citrus juices has been reported to contain nitrogen (8). The main nitrogen containing compounds of the juice are the amino acids, proteins, amines, and amides, but soluble amino acids are

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