California Avocado Society 1970-71 Yearbook 54: 85-90

THE RESPONSE OF AVOCADO PERICARP TISSUE TO TEMPERATURE AND LIGHT IN VITRO

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Some responses of excised avocado tissue taken from the fleshy fruit pericarp and grown under controlled conditions on agar nutrient media have been described (3,4,5,6,7). Growth of tissue disks consists of increase in size and in fresh and dry weight. The initiation and continuation of cell division results morphologically in the major contribution to size and weight increase in such materials. Normally such tissues maintained in vitro continue to grow and develop as an amorphous tissue callus with no particular form or surface character. Internal tissue differentiation is sometimes observed, however, which results in the formation of sclerids, elongated parenchymatous cells, and lignification of some of these cell types (4). Differentiation of typical and apparently functional roots has been observed in some cultures (6). The present report presents a summary statement regarding some additional effects of light intensity and temperature conditions on the growth response of avocado disks associated with many of the phenomena described above.

Materials and Methods

The tissue used in the experiments consisted of the fleshy pericarp wall of nearly mature Hass avocado fruit. The fruits were collected while still firm in texture, generally when the first indication of color appeared on the skin. Some fruits were nearly fully developed in respect to size but were still green in color. The fruit was scrubbed with soap and water and immersed in a 15% Chlorox solution for 30 minutes. Subsequent procedures were conducted in a sterile tissue transfer room. The fruit was cut in half and slices 1 mm. thick cut on a stainless steel cutting table. A stainless steel punch was used to cut disks 8 mm. in diameter. These disks were then placed one each in 6 dram screw-top vials on 10 ml. of nutrient culture medium in agar. The medium used is Nitsch's (2) with the substitution of FeEDTA for iron citrate. The addition is made of Indole-3-acetic acid at 10 ppm concentration. All materials and equipment were autoclaved prior to the tissue planting procedure. Some of the vials were then wrapped in aluminum foil to provide a condition of complete darkness. The vials held in the light were merely placed in groups about 6 inches from inflorescent lamps which provided a light intensity of approximately 600 f.c. under the given conditions. The vials with the tissue disks were placed in temperature and light controlled growth chambers for a period of approximately three weeks at which time differential growth between treatments was detected by weight determinations. The individual disks were weighed on a Roller micro balance.

Observations

Growth and development of the disk tissues is not noted by casual observation until about four or five days after tissue planting. The first indication of growth response is a proliferation of tissue on the upper disk surface. This is evidenced by a crystalline appearance of the surface cells when viewed under the low power microscope. Actually, internal evidence of cell division has been observed within 24 hours of planting in some cases, but the general gross morphological appearance of surface proliferation is somewhat delayed in time. Continued cell division and enlargement result in increase in disk weight. Preliminary studies have indicated that reliable and differential observations can be made on responses after the tissues have been held in the growth chambers for approximately three weeks. Growth continues after this period, but daily increments are small and generally less dramatic than the total growth observed at the three week period.

Data for three typical experiments are given in Figures 1, 2 and 3. While variation in results at a given temperature can be detected, the indicated general trends and the responses were demonstrated in repeated experiments.

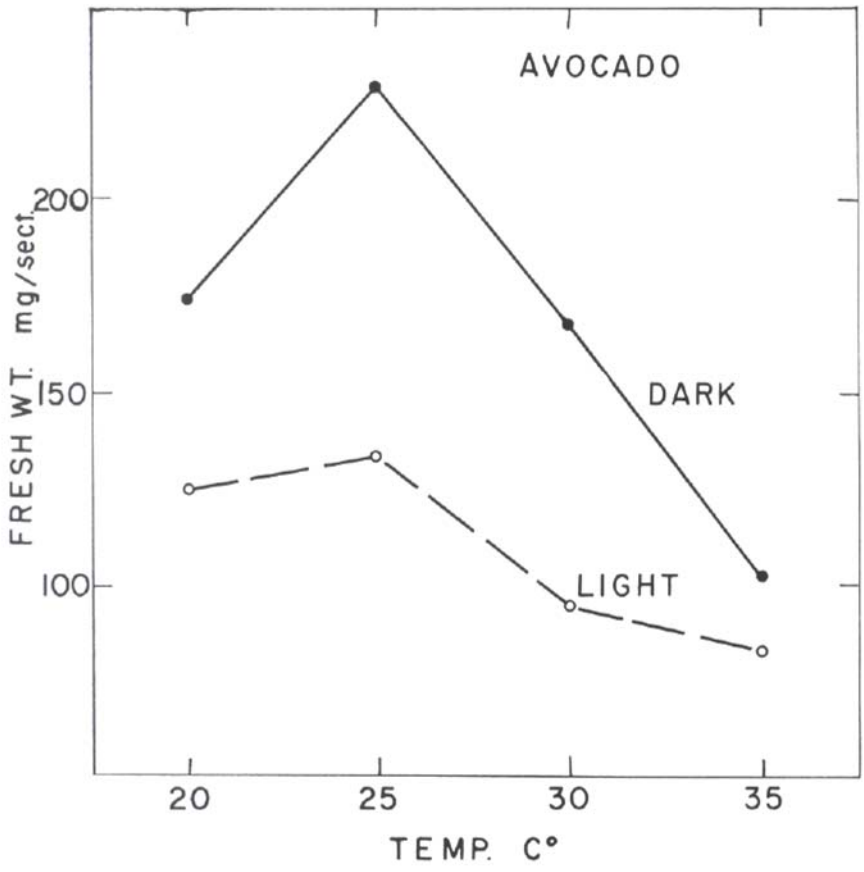

Figure 1. Fresh weight increase of avocado pericarp tissue maintained for three weeks in vitro in dark or light (600 f.c.) at various constant temperatures (20°-35°C.). Original weight—74 mg.

The optimum temperature for growth of avocado tissue in vitro is approximately 25°C. This has been determined from casual observation in many experiments and is confirmed by the present data (Figure 1). The precise optimum temperature probably is slightly less than 25° but this has not yet been ascertained experimentally. Empirical data from many experiments indicate a favorable growth response under constant temperature conditions between 20° and 25°C. Lower and higher temperatures result in significantly less total growth. There is some preliminary evidence that fluctuating temperatures may possibly be favorable toward increase in total growth compared to a constant temperature. The data presented in Figure 1 indicates clearly the favorable 25°C. temperature and a reduction in total growth at the higher temperatures (30° and 35°C.).

A rather significant effect of light on the reduction of tissue growth is noted in nearly all cases. Tissues kept in complete darkness attain considerably larger size compared with those grown in constant light at 600 f.c., particularly at 25°C. These differences between dark and light exposed tissues are less evident at lower (20°C.) and higher (35°C.) temperatures.

The significance of the more favorable growth response in the absence of light is not clear. High light intensity has been shown to have deleterious effects on several aspects of plant life as it impairs photosynthesis by breakdown of the chlorophyll (9) and as related to high temperature (1).

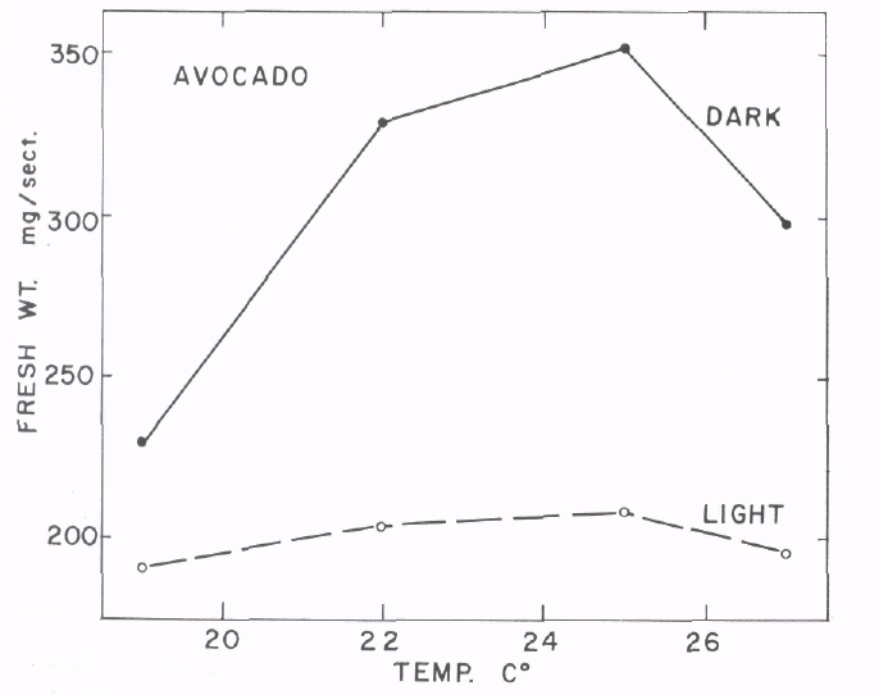

Figure 2. Fresh weight increase of avocado pericarp tissue maintained for three weeks in dark or light (600 f.c.) at various temperaures (19-27°C.). Original weight—96 mg.

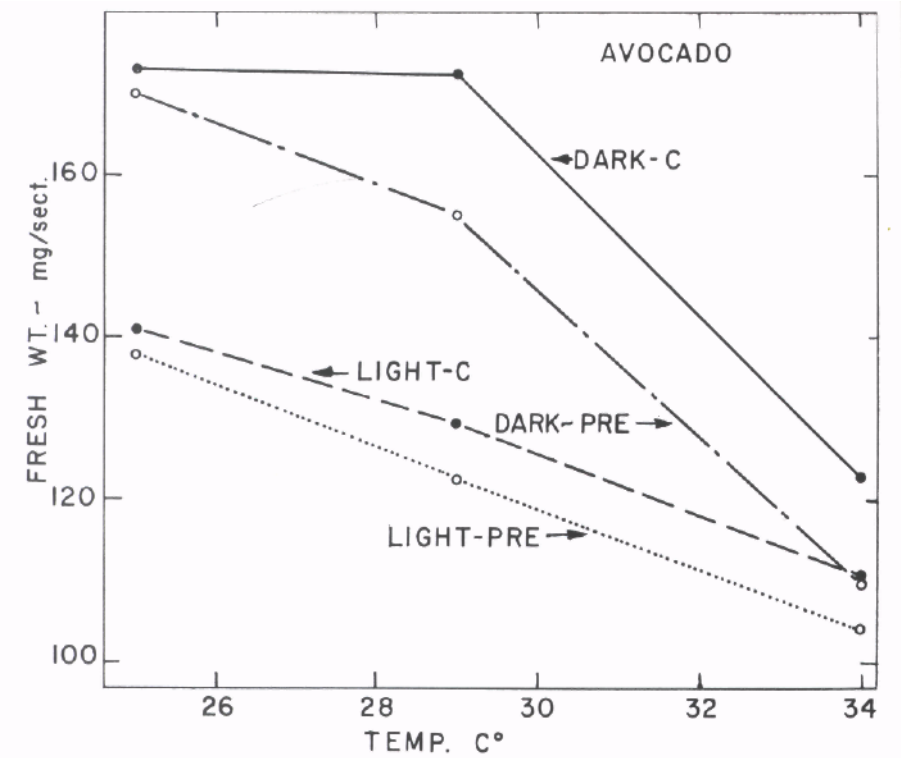

Figure 3. Comparison of avocado pericarp tissues placed directly (C) and maintained in dark or light (600 f.c.) at various temperatures (25°-34°C.) with tissue pretreated (PRE) at 50°C. for 15 minutes prior to placement in dark or light (600 f.c.) at constant temperatures for three weeks. Original weight — 84 mg.

The consistent inhibition of growth at higher temperatures provided by the comparatively low light intensity 600 f.c. was observed in several experiments and indicated by the examples given in Figures 1. and 2. This trend is further validated by results seen in Figure 3, which is a variation in the general experimental procedure. This experiment represented by Figure 3. consisted of two parts. The first series of tissues were prepared, planted and placed directly at the three given temperatures in light or dark which is the normal procedure (controls). A second set of tissues was pretreated by exposing the freshly planted disks in the vials to a temperature of 50°C. for a period of 15 minutes and then placing the vials at the three temperature and two light conditions. The 50°C. pre-exposure was attained by partial submersion of the vials in a constant temperature hot water bath.

The results shown in Figure 3. indicate that maintaining tissue disks at various temperatures in darkness resulted in better growth than those tissues in the light regardless of the thermal shock provided by the pretreatment at 50°C. Consistent with other experimental data, thermal shock as a pretreatment condition for some investigations does affect the ultimate total growth of the disk. Exposure to temperatures below 52-54° for short: periods, to 15 minutes, may not seriously reduce subsequent growth. Exposures to temperatures of 52-54°C. and above for even short periods of 4-5 minutes may inhibit or prevent all subsequent tissue growth.

The relationship of the above observations to the problems of fruit tissue development in vitro may be indicated by the fact that daily net growth of an avocado fruit on the tree appears to occur mostly during the night period (8). Diurnal fluctation in fruit size has been demonstrated. The fruit measured in diameter at noon is less than the fruit measured in early morning (7:00 A.M.) of the given day. Thus the fruit is actually reduced in size when transpiration induced by the higher temperatures of the day results in a water stress throughout the entire plant causing a reduction in size of all plant parts including the fruit. This reduction in size is regained during the night when water stress becomes less and moisture is redistributed through the various plant parts. The same fruit measured the next morning actually is slightly larger compared to the previous morning. This daily increase results from the processes of cell division and cell enlargement which apparently are most effective and active during the dark period. The present experimental interpretations indicate that dark conditions are favorable to the several processes involved in cell division and net growth increase.

The general implications derived from the data in these representative experiments is that excised avocado pericarp tissue maintained in vitro and in the dark will show greater growth as measured by increase in fresh weight at all experimental constant temperatures between 20° and 35°C. (68-95°F.). The optimum tissue growth is attained at approximately 25°C. (77°F.).

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