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In Vivo Measurement of Phytochrome in Tomato Fruit[1]

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ABSTRACT

Presence of phytochrome in two kinds of tomatoes (*Lycopersicon esculentum* Mill.), the yellow lutescent strain and cherry tomatoes (*L. esculentum* Mill. var. *cerasiforme* cv. Red Cherry), was established by measuring the absorption difference spectra of the whole fruit after irradiation with red and with far red light. Phytochrome content was determined in yellow lutescent tomatoes and decreased gradually during the ripening period.

Piringer and Heinze (7) reported that the production of a flavonoid-type pigment in the cuticle of tomato fruit was promoted by red and inhibited by far red illumination. Production of lycopene in tomato was enhanced by red light illumination (5). Thomas and Jen (9, 10) presented evidence that suggested that biosynthesis of carotenoids in tomato was partially mediated by phytochrome.

Detection and assay of phytochrome by physical means were first reported by Butler *et al.* (3). With advancement in technology and refinement of the instrument, it became possible to detect phytochrome directly in a variety of plant tissues (8), but we do not know of any report on *in vivo* measurement of phytochrome in any fruit tissue. This report presents data on the quantitative *in vivo* measurement of phytochrome during ripening of tomato fruit.

MATERIALS AND METHODS

Tomatoes. Yellow lutescent tomatoes (*Lycopersicon esculentum* Mill.) were grown in the field (Clemson, S.C.) and greenhouse (Beltsville, Md.). Fruits were harvested at the mature white stage (4) and showed no visible evidence of Chl except for a few that had green streaks on the shoulder. Cherry tomatoes were obtained from local market.

Phytochrome Assay. A whole tomato was placed, stylar end up, in a black spongy holder and centered in the cell compartment of an in-house designed spectrophotometer (6, 11). In the cell compartment, red and far red sources of light were placed at an equal distance and at a 45° angle to the fruit. The red light source was a dichromic reflector 80-w lamp with a high rejection 656 nm interference filter. The far red light source was a similar lamp with a far red plastic filter. The absorption spectrum from 500 to 800 nm was recorded for each tomato after a 30-sec irradiation with red light and after a 30-sec irradiation with far red light. The difference curve computed from the two spectra was used to identify the phytochrome. The quantitative estimation of total phytochrome was based on the difference between the absorbance at 660 and 730 nm of the difference spectrum (ΔΔOD [660-730 nm]) (8). The irradiation and measurements were repeated four times and the average ΔΔOD (660-730 nm) was reported.

CO_2 and Ethylene. Carbon dioxide and ethylene were determined by gas chromatography using molecular sieve and alumina columns, respectively. Each fruit was incubated in the dark at 23 ± 1 C in a small respiration chamber connected to a flow board with humidified air flow at 4 to 5 ml/min.

RESULTS AND DISCUSSION

The absorption spectra from 500 to 800 nm are shown for whole fruits of unripe and ripe lutescent tomatoes in Figure 1. The unripe tomatoes were at the preclimacteric stage and considered mature physiologically. Although the unripe tomatoes showed no green color, a trace of Chl was present as indicated by a slight absorption band at 675 nm. The quantity of carotenoids in the yellow lutescent tomatoes was very low in comparison with pigment in normal red tomatoes (4).

A typical absorption difference spectrum of ripe lutescent tomatoes (Fig. 2) was obtained by averaging 20 far red minus red reversal spectra. The curve, characteristic of phytochrome, shows a maximum positive increase at 662 nm and a maximum decrease at 728 nm. We are able to show an absorption difference spectrum, characteristic of phytochrome, in red cherry tomatoes at the pink stage. The change in signal caused by interference from the irradiated Chl was so large that the quantitative change of phytochrome was difficult to measure. Interference of Chl in measurement of phytochrome has been reported (8). Because of their low Chl content, the lutescent tomatoes were ideal for our ripening study.

The quantity of reversible phytochrome, based on ΔΔOD (660-730 nm), in the ripe lutescent tomato fruit was low. The ΔΔOD (660-730 nm) of a 40-mm diameter tomato was about 1.2×10^{-3}, whereas the ΔΔOD of a 3-mm diameter etiolated cucumber seedling was about 4×10^{-3} (8).

The changes in phytochrome concentration and other physiological activities during the ripening of yellow lutescent tomatoes are shown in Figure 3. With ripening, the phytochrome content decreased to about one-third of the amount in mature white fruit. The decline in the phytochrome content was sharpest near the climacteric peak of the respiratory pattern, when the ethylene production was increasing sharply. The phytochrome content of some fruit decreased gradually and did not have a sharp drop at the time of the climacteric rise. These fruits required a longer time to ripen than the others, so they probably were physiologically immature when harvested.

[1] This study was undertaken when J.J.J. was at the Agricultural Marketing Research Institute, ARS, USDA, Beltsville, Md. on a sabbatical program. Technical contribution No. 1404 of the South Carolina Agricultural Experiment Station.

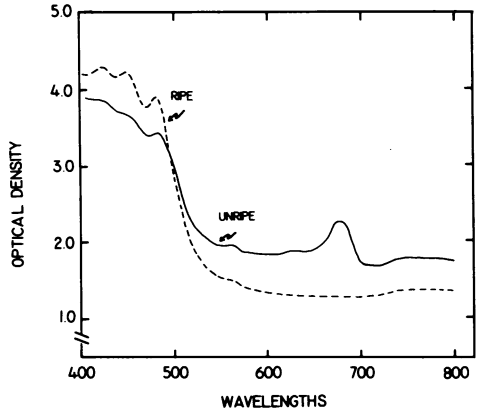

FIG. 1. Absorption spectra of ripe and unripe lutescent tomatoes.

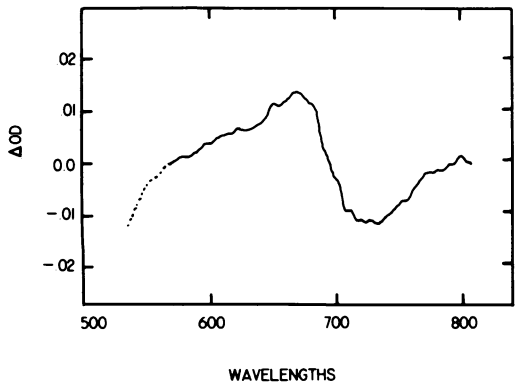

FIG. 2. Difference spectrum of ripe lutescent tomato.

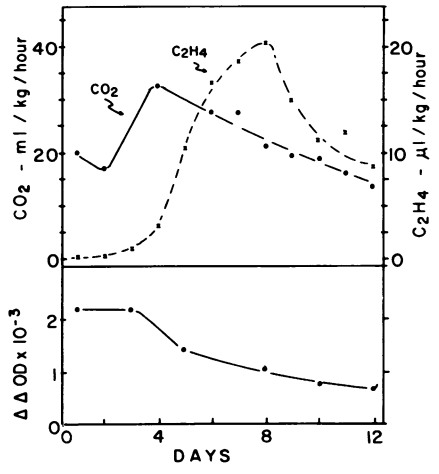

FIG. 3. Rate of CO_2 and ethylene production (top) and quantity of reversible phytochrome (bottom) in ripening lutescent tomatoes.

In a preliminary experiment, red light was found to hasten the occurrence of the climacteric rise and ethylene production in normal red tomatoes (unpublished data). Thomas and Jen (9) reported that the synthesis of lycopene was initiated at the preclimateric minimum and was hastened by red light and suppressed by far red light. Apparently, phytochrome is closely related to several phenomena associated with ripening. As noted by Borthwick (2), the primary action of Pfr on various physiological phenomena is probably through changes of membrane permeability of cell and cell organelles. Biale (1) postulated recently that changes in cell membrane permeability might conceivably account for the wide variety of both anabolic and catabolic processes associated with ripening.

The cause of phytochrome loss during ripening of the yellow lutescent tomatoes was not known. Ethephon-treated sample fruit had a shorter ripening period and lost phytochrome faster than nontreated fruit. The loss of phytochrome during ripening was not caused by repeated irradiation with red and far red light because fruits held for 2 weeks in the dark lost the same amount of phytochrome as those shown in Figure 3. Perhaps the fruits entered a phase of senescence during ripening and phytochrome was no longer needed in the various physiological processes.

It was assumed that the quantity of reversible phytochrome was related linearly to the ΔΔOD as measured under our experimental conditions. This relationship could be affected by several factors including distribution of phytochrome, optical properties of the tissue, and problems of stray light from the instrument. These factors are virtually inaccessible at the present state of the art, and were not evaluated (8). Nevertheless, the existence of phytochrome in tomato fruit tissue was unequivocally proven by the *in vivo* measurement of the difference spectrum shown in this report.

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