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METABOLISM OF POLAR LIPIDS IN GREEN AND SENESCENT SQUASH LEAVES

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Key Word Index—*Cucurbita maxima*; Cucurbitaceae; squash leaf; galactolipids; phospholipids; turnover; senescence.

Abstract—The rates of turnover of the various moieties of the lipids of green leaf tissue and of senescent leaf tissue were determined with pulse labeling experiments.

INTRODUCTION

Although proteins have been extensively studied in relation to senescence [1–4], some emphasis has now been given to the investigation of lipids [5] since cellular membranes lose their integrity during senescence. This paper reports on the turnover of the various moieties of the polar lipids of squash leaves in relation to senescence induced by growing the plants in an N-deficient nutrient.

RESULTS AND DISCUSSION

Various polar lipids in normal and N-deficient squash leaves were labeled with acetate-[^{14}C], the hexose of UDP-galactose [^{3}H], or with choline [^{14}C] to determine the rates of synthesis and turnover. There was a continuous synthesis of glycolipids and phospholipids during the 96 hr incubation period on the labeled substrates. Pulse labeling experiments with choline [^{14}C] indicated that the half life of the choline moiety of phosphatidyl choline in both normal and N-deficient tissue was slightly longer than 24 hr under those experimental conditions. The half-lives of both the acyl and hexose moieties of monogalactosyl diglyceride were about 24 hr for normal tissue and a few hr longer for those of N-deficient tissue. The half lives of these moieties of digalactosyl diglyceride in normal and N-deficient tissues were less than 24 hr. N-deficient tissue incorporated less label into the acyl and hexose moieties of the lipids. Since the half lives of the acyl and hexose moieties of monogalactosyl diglyceride in N-deficient tissue were slightly longer than those of the same lipid in complete-grown tissue, monogalactosyl diglyceride in the N-deficient tissue was turned over, presumably, at a slower rate.

Generally, the N-deficient tissues (senescent tissues) synthesize the lipids at a slower rate but the rate of turnover is dependent upon the particular lipid.

EXPERIMENTAL

Blue Hubbard squash (*Cucurbita maxima*, Duchesne) was grown either in a complete nutrient or N-deficient nutrient for 21 to 26 days at *ca* 25°. Surface sterilized leaf discs were floated on a 0·04 M Pi buffer pH 6·8 containing 0·03 M $NaHCO_3$ and labeled substrates. To the Pi buffer 10 μCi of acetate-2-[^{14}C] (sp act, 50 mCi/mmol) and 4·1 μCi of hexose-[^{3}H]-UDP-galactose (sp act, 1300 mCi/mmol) were added in 20 ml of floating soln. The leaf discs were floated for 24, 48, or 96 hr. In one expt 3·78 μCi choline chloride-[^{14}C] (sp act, 41·3 mCi/mmol) was used and in another experiment 3·78 μCi of hexose-[^{3}H]-UDP-galactose (sp act, 1300 mCi/mmol) and 10 μCi of acetate-2-[^{14}C] (sp act, 50 mCi/mmol) were used. In these two expts the leaf discs were given a pulse of 24 hr of the label and then transferred to a sterile unlabeled buffer soln to determine the half lives of the compounds.

The lipids were extracted in $CHCl_3$–MeOH (2:1), dried after filtering and separated by column chromatography followed by TLC [6]. The radioactivity of the separated lipids was determined by liquid scintillation counting in a BBOT [(2,5-bis-2(5-*tert*-butylbenzoxazolyl) thiophene)]-Cab-O-Sil cocktail. The values were corrected for decay and crossing-over in the channels.

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