NONAQUEOUS ISOLATION OF CHLOROPLASTS FROM SOYBEAN LEAVES

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In the past, chloroplasts have been isolated in several aqueous media. Although these chloroplast preparations had several advantages destarching of plastids before isolation and leaching of metabolites during isolation are major sources of error; especially if the chloroplast preparation is used for a metabolite determination study. Thus, development of a nonaqueous chloroplast isolation procedure was attempted in this study.

Seeds of an indeterminant soybean variety were grown under green-house conditions. At active pod filling time leaves were removed. After removal of large veins, they were frozen in Freon 12, freeze dried, pulverized and sifted. This tissue was ground in a mixture of 5:5 v/v glycerol and poly ethylene glycol (PEG) (molecular weight 300) and centrifuged at 2000xg for 20 min at 15 C. The supernatant filtered through 10 u mesh was centrifuged at 13,000xg for 1 hr at 15 C, and the pellet was washed twice by suspension in the glycerol/PEG mixture and by centrifugation as above for 30 min.

The pallet from the above procedure contained about 50% of chloroplasts present in the original sample. Washing of pellet at high speed centrifugation removed most of the cytosol but electron microscopic examination showed some cytoplasmic contamination. With respect to starch granule size, the isolation procedure yielded a population of chloroplasts not greatly different from those in the starting material. Chlorophyll extraction was also not so severe as with aqueous media.

11th Dec. 1987 (Friday) 02.30 p.m. - 02.45 p.m.