Biochemical and Cytological Changes in Developing Soybean Cotyledons*

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GROWTH patterns of the pods and seeds of soybeans have been studied for at least 50 years. Garner et al. (6) found a slow increase in dry weight of young seeds immediately after flowering but a more rapid increase during the period 2 to 5 weeks after flowering. Later workers have reported similar data. Cell division is complete in the cotyledons by about two weeks after flowering (11), although mitotic figures appear in the radicle throughout the period of seed development. A slight loss in dry weight may occur during the week just before maturity (2).

The composition of mature soybean seeds has been widely studied (15). Lipids average more than 20% and proteins more than 40% of the dry weight of mature soybean seeds (12). Lipids are mostly neutral triglycerides, but non-triglycerides may amount to 5 to 10% of total lipid (4). About 80% of the fatty acid is unsaturated (3). Fatty acid synthesis presumably occurs in cells of the cotyledon (26, 29) and is associated with smaller cell particles as reported by Green (7) for animal tissues. Total fat synthesis is correlated with excess carbon dioxide production during July 12 to 18. July 15 was used as the flowering date in expressing ages of developing seeds. Later flowers were left on, but not used. Pods from tagged flowers were harvested periodically, beginning July 30. Dry weights were determined after drying the tissues in a forced draft oven for 12 hours at 85° C.

Microscopy and histochemistry—Freshly harvested immature seeds were cut in half and immediately fixed in FPA (formalin, 4%; propionic acid, 2%; ethanol, 48%; in aqueous solution) for 4 to 6 hours (22).Fixed tissues were dehydrated with a series of 50, 70, 85, 95, and 100% ethanol solutions. Dehydrated tissues were cleared in 2 changes of xylol, infiltrated with 4 changes of paraaffin, and embedded. Paraffin sections 10 to 12 μ thick were cut on a rotary microtome. Safranin-fast green stain was used for general observation of the paraffin sections under the light microscope. Proteins, nuclei, chromosomes, and lignified or cutinized cell walls are stained brilliant red by safranin. Cytoplasm and cellulose cell walls are stained green or blue-green by fast green F. One-micron sections of methacrylate-embedded specimens prepared as described below were tested for carbohydrate by the periodic acid-Schiff (PAS) reaction (20). Amylase digestion for 30 minutes at 37° C. and I2KI tests were used to locate and identify starch. The Sakaguchi test as modified by Thomas (27) was used to locate and identify the protein.

Cotyledon slices about 0.5 mm. thick were fixed at 0-2° C. and pH 7.0 for 20 to 30 minutes in a solution of 1% OsO4, 0.3% MgCl2·6H2O, and 1.5% sucrose in veronal-acetate buffer (18). The tissues were dehydrated at 0 to 2° C. in a series of 20, 35, 70, 95, and 100% ethanol solutions, each containing 1% MgCl2·6H2O (14). The specimens were in each of the lower alcohols for 20 to 30 minutes with 3 changes in each and in absolute alcohol about 1 hour with 4 changes. The specimens were infiltrated with a mixture of 3 parts n-butyl to 1 part methyl alcohol for 20 to 30 minutes with 3 changes, followed by 1 hour each with 2 and 1 parts methyl alcohol. The tissues were infiltrated with a mixture of 3 parts n-butyl to 1 part methyl alcohol about 1 hour with 4 changes. The specimens were infiltrated with a mixture of 3 parts n-butyl to 1 part methyl alcohol about 1 hour with 4 changes. 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