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Starch Synthesis in Excised Lemon Fruit Tissue Growing in vitro

Abstract. *Significant quantities of starch accumulation were evident in excised mature lemon fruit tissue growing in vitro on a nutrient solution of mineral salts and sucrose. Positive evidence for the presence of starch in the growing cells was obtained with iodine staining and polarizing microscopy. Starch was observed in all growing cultures regardless of age.*

Vesicle stalks freshly excised from mature yellow lemon fruits [*Citrus limon* (L.) Burmann, variety Eureka] squashed in dilute iodine solution showed no signs of starch-containing bodies. This is consistent with the observation that most of the starch has disappeared from mature fruits except in seed tissue and occasional grains in the outer layers of the yellow peel (*1*) and in masses of small cells in or near the center of each juice vesicle (sac portion) (*2*).

Although lemon fruits belong to the class of plant organs which are without starch reserve at maturity (*3*), excised vesicle stalks from mature Eureka lemons are apparently capable of indefinite starch synthesis when grown in vitro on a nutrient solution consisting of mineral salts and sucrose. Starch synthesis has been shown by iodine staining and polarizing microscopy. Hence the genetic capacity for starch synthesis is not irreversibly altered in this tissue during growth and maturation of the fruit in vivo.

Vesicle stalks were excised from mature Eureka lemon fruits and planted in vitro as described previously (*4, 5*). The cultures were maintained in constant darkness at room temperature. After 2 to 3 weeks' growth in vitro, proliferating stalks were examined with a Mineralite shortwave ultraviolet hand lamp (253 mμ), and all invariably showed the characteristic yellow-green fluorescence (*6*). Living proliferating material was squashed in sterile nutrient solution and examined with bright field and polarizing microscopy, respectively. Relatively large numbers of round or slightly acentric bodies were present in the solution external to the squashed cells as well as in intact cells. When examined between crossed polarizers, these bodies exhibited birefringence and the polarization cross characteristic of starch granules (Fig. 1). The symmetry of the starch granules in the proliferating fruit tissue under polarized light was similar to the symmetry of starch granules of waxy and normal maize (*7*, Figs. *8–11*; *8*, Fig. 3; *9*, Fig. 40). The addition of a dilute aqueous iodine solution (0.3 percent I_2 in 1.5 percent KI) to the squash preparations resulted in a deep blue coloration of the acentric bodies—that is, the bodies gave a positive color reaction for starch. The birefringence and polarization cross were still clearly evident in the blue, iodine-stained bodies. The unstained and iodine-stained bodies, respectively, were optically positive between crossed polarizers.

Proliferating fluorescent stalks, when dropped into the dilute iodine solution, manifested an extensive blue coloration soon after immersion. Hand sections as well as squash preparations of the iodine-treated proliferating tissue revealed relatively large numbers of the blue-stained bodies under bright field microscopy. These blue-stained bodies were anisotropic; they showed the polarization cross characteristic of starch granules and were optically positive between crossed polarizers. Subcultures of proliferating lemon fruit tissue (3½ years old) which fluoresced yellow-green under ultraviolet light also gave the same specific color reaction with iodine and the same birefringent bodies. These bodies, like those in the 3-week-old stalks, showed the polarization cross and were also optically positive. Hence the innate capacity to synthesize starch does not decrease with increasing age of the growing culture. Cultures which no longer manifested visible signs of cellular growth and which did not fluoresce under ultraviolet light showed very few bodies that stained blue with iodine, thus indicating an apparent cessation of starch synthesis in quiescent or dying tissue. The specificity of the blue coloration with iodine for starch in the growing cells is shown by its complete restriction to specific bodies which are birefringent and which show the distinct polarization cross characteristic of starch granules (*8–10*).

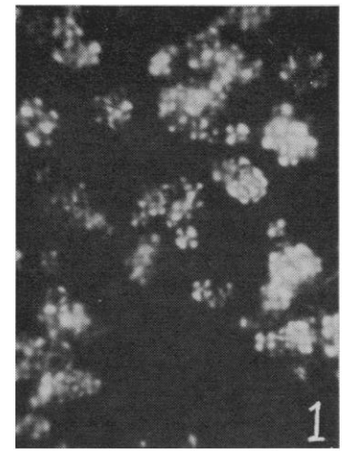

Fig. 1. Starch granules in an unstained section of excised lemon fruit tissue proliferating in vitro as observed between crossed polarizers. (× 750)

The ability of excised lemon fruit tissue to synthesize starch on a medium of minerals and sucrose affords a possible tool for studying the transformation of sucrose, the main transport sugar in plants (*8*), into starch under relatively simple and precisely standardized into vitro conditions (*5; 11*).

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