

**Occurrence, Histochemistry, Ultrastructure and Crystalline
Structure of Calcium Oxalate Crystals
in the Leaf of *Ipomoea batatas***

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ABSTRACT

In situ distribution, histochemical identification, ultrastructural configuration and wavelength dispersive x-ray spectrometric analysis of intracellular crystals of calcium oxalate in the leaf of sweet potato (*Ipomoea batatas*) were studied by scanning and transmission electron microscopy, EPMA and x-ray diffraction. Leaf segments were cleared in the mixture of sodium hydroxide and chloral hydrate, and observed with light microscope for *in situ* occurrence. Calcium oxalate crystals were isolated by incubation of segmented leaf tissue in the enzyme mixture of macerozyme, cellulase and pectinase. Isolated protoplasts were mild agitated to be bursted by adding of water. Separated crystals from leaf protoplasts were purified by sucrose density gradient centrifugation.

Histochemical identification of the crystals were carried out with silver nitrate-rubeanic acid methods to investigate the hydrate form of calcium oxalate. Scanning electron microscopic ultrastructure, wavelength dispersive x-ray spectroscopy, electron probe microanalysis, and x-ray diffraction with transmission electron microscope have been carried out to identify the topography and ionic configuration of the crystals.

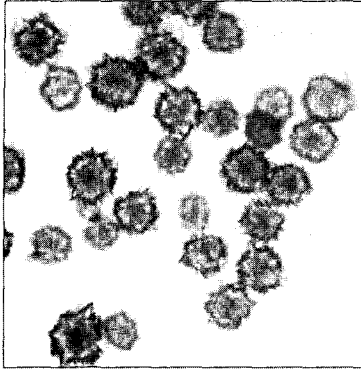


Fig.1. In situ visualization of calcium oxalate crystals in the cleared leaf of *Ipomoea batatas*.

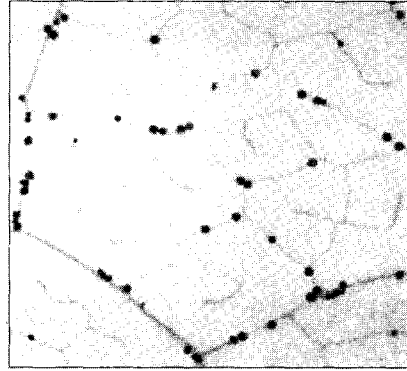


Fig. 2. Isolation of calcium oxalate crystals using sucrose density gradient centrifugation.

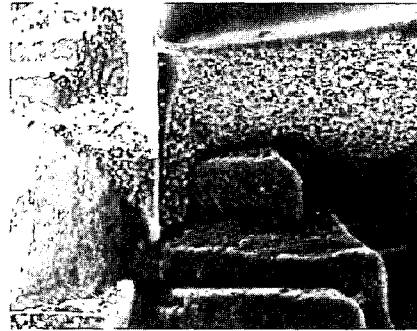
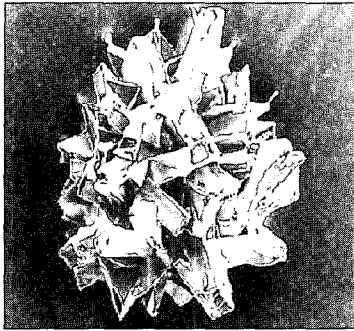


Fig.3. Scanning electron micrographs of the isolated crystals.

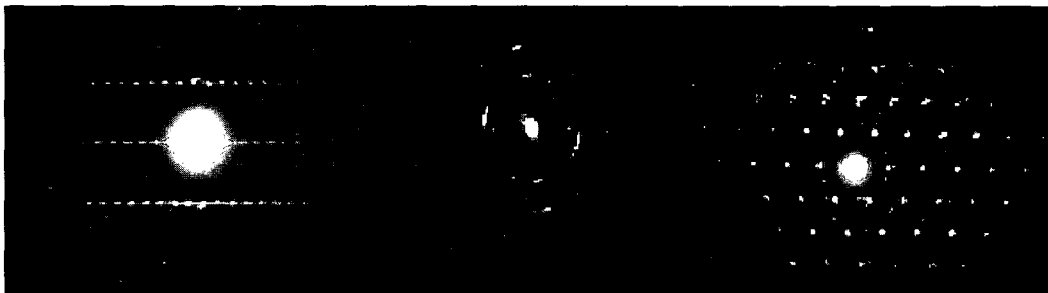


Fig.4. X-ray diffraction pattern of calcium oxalate crystals.