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Calcium Oxalate Crystals in the Roots of Sweet Potato[1]

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Abstract. **Druse crystals observed with light and scanning electron microscopy in root tissue of *Ipomoea batatas* (L.) Lam. cv. Jewel were identified as calcium oxalate monohydrate by use of energy-dispersive X-ray analysis and X-ray diffraction techniques. Crystals occurred primarily in the parenchyma cells of the edible root.**

Druse crystals of calcium oxalate have been described in leaf and stem tissues of sweet potatoes (11, 12, 18), but not in the root (edible portion). The occurrence and theoretical function of crystalline calcium oxalate in plants was summarized by Arnott and Pautard (3), Christiansen and Foy (8), and Gallaher (10). The morphology of calcium oxalate druse crystals in plants was observed by light microscopy (18), scanning electron microscopy (13), and by transmission electron microscopy (7).

The composition of crystalline calcium oxalate has typically been investigated by chemical procedures (1, 7, 16), by optical properties of the crystals (9) and by unit cell measurements calculated from X-ray diffraction data (2, 15, 17, 19). Al-Rais

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et al. (2) concluded that most crystals in flowering plants growing under normal conditions were almost entirely calcium oxalate from X-ray diffraction and chemical tests. Terblanche et al. (17) investigated calcium-containing crystals in apple pedicels by X-ray diffraction and found that the crystals were not calcium oxalate, which was contrary to the findings of Liegel (14) and Wieneke and Fuhr (20). Arnott and Pautard (3) suggest that conflicting and confusing results in studies of biological calcium oxalate crystal inclusions is due to the complicated crystal habits of the oxalates, the occurrence of mixtures of salts in biological materials, and the ability of the tetragonal crystal lattice form to accommodate variable amounts of water.

The present study was to determine the chemical composition of the druse crystals found in 'Jewel' sweet potato root tissue. Our objectives were to: 1) confirm or deny that the crystal inclusions in the root parenchyma cells were calcium oxalate, and 2) identify any other compounds.

Materials and Methods

Tissue blocks (5 mm cubes) from 30 fresh, mature 'Jewel' sweet potato roots were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 24 hr at 4°C. The fixed tissue was embedded for light microscopy in glycol methacrylate according to Bennett et al. (5). Transverse sections 10 μm in thickness were cut on a Porter-Blum MT-1 ultramicrotome, stained with 1% toluidine blue, and photographed using a Wild photomicroscope. Fixed tissue for scanning electron microscopy was rinsed overnight in 5.6% sucrose (for maintaining osmolarity) in 0.1 M sodium cacodylate buffer, pH 7.2, at 4°C. Then, the tissue was dehydrated in ethanol. Freon 113 was used as an intermediate fluid for critical-point drying in a Bomar SPC-50EX. The tissue was then gold-coated in a Polaron E 5000 Diode Sputtering System. Samples were observed and photographed at 20 Kev with an ETEC Autoscan microscope. The cation component of the crystals was identified in a JEOL JSM-2 SEM fitted with an EDAX 707 energy-dispersive X-ray analyzer.

The bulk sample of crystals for the X-ray diffraction pattern was obtained from 750 g of fresh, unpeeled 'Jewel' sweet potatoes washed with water and ground in a Fitz-Mill Model D. During grinding, an equal volume of distilled water was added to the roots, and the mixture was filtered through unbleached muslin and Pelon to remove large aggregates of unbroken cells and fibrous material. The filtrate was then stored overnight at 5°C to allow the crystals, starch grains, and cell fragments to settle. The suspension was then siphoned off, and the remaining solid mixture was washed and resuspended in distilled water. The settling and siphoning procedures were then repeated.

The final washed solid mixture was air-dried in a dehumidified, 5°C room for 2 days, and yielded a cake of material 50 mm in thickness. Then, 10 g from the bottom 5 mm of the dried, solid mixture, which contained the crystals, starch, and cell fragments, were slurried in 25 ml of distilled water. The slurry was added in 1 ml portions to 50 ml of 95°C distilled water and a drop of Tenase (Miles Laboratories, Inc., Elkhart, IN), a high-temperature α-amylase to break down the starch. The process was repeated until crystals could be seen in the bottom of the beaker. The solution containing the crystals and cell fragments was cooled and again filtered through unbleached muslin and Pelon to remove additional cell fragments. The crystal-containing filtrate was centrifuged (Sorvall RC-2) at 12,500×g for 15 min. The pellet was resuspended in 2 ml of water and freeze-dried (Virtis model 10-147 MR-BA). The freeze-dried crystals were crushed with a mortar and pestle, placed on a galss rod, and inserted into a Debye-Scherrer X-ray powder diffraction camera (114.59 mm diam). The camera was mounted on a Philips electronic X-ray apparatus, and the crystals exposed to nickel-filtered Cu K-α radiation at 35 KV and 20 mA. The film was exposed for 6 hr and developed. The d-values were measured on a General Electric X-ray Corporation Fluoroline.

Results and Discussion

Druse crystals, 10-50 μm diam, were found primarily in the parenchyma of all sweet potato root tissue examined (Figs. 1-2). X-ray analysis revealed that single crystals were high in calcium. The K-α and K-β peaks corresponded to the X-ray values for calcium when superimposed on the experimental peaks (Fig. 3). After we determined that the crystals contained calcium, we analyzed for calcium oxalate or other calcium salts.

The d-spacings obtained from X-ray powder diffraction patterns are the distances, in angstroms, between the planes in a crystalline structure. The experimentally determined d-values and estimated relative intensities were compared with the known d-values and corresponding intensities for a crystal (e.g. calcium oxalate) of known chemical composition. The d-values from the X-ray powder diffraction pattern (Table 1) corresponded to the major known d-values for calcium oxalate monohydrate (whewellite). No other calcium salts or other dehydrated forms of calcium oxalate were present. The two

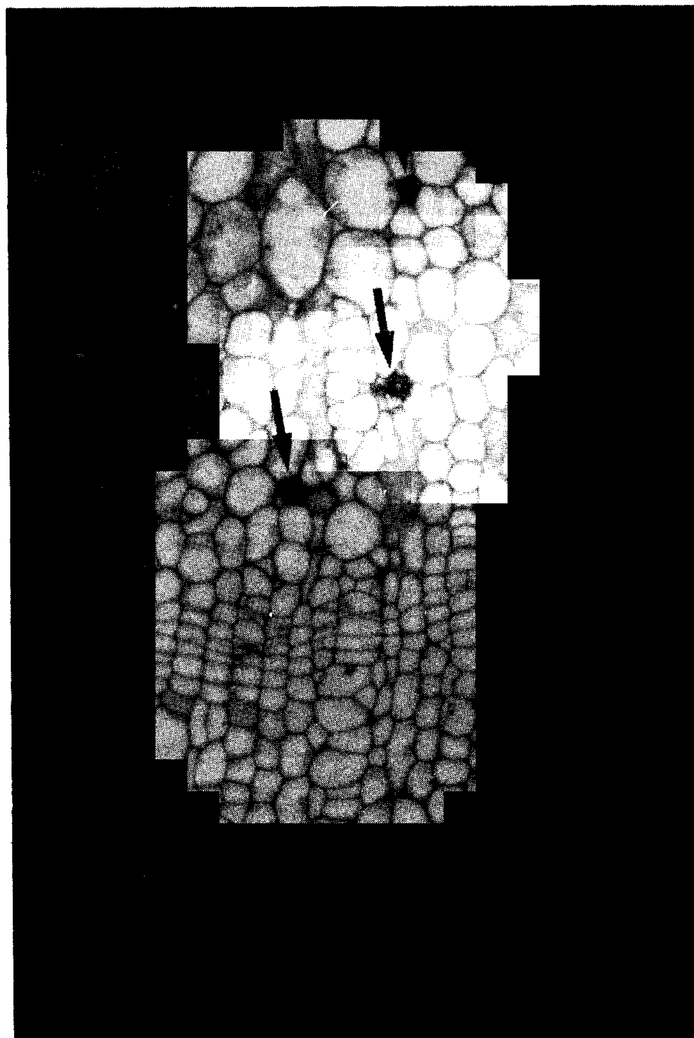

Fig. 1. Transverse section of 'Jewel' sweet potato root tissue with calcium oxalate crystals (arrows), ×130.

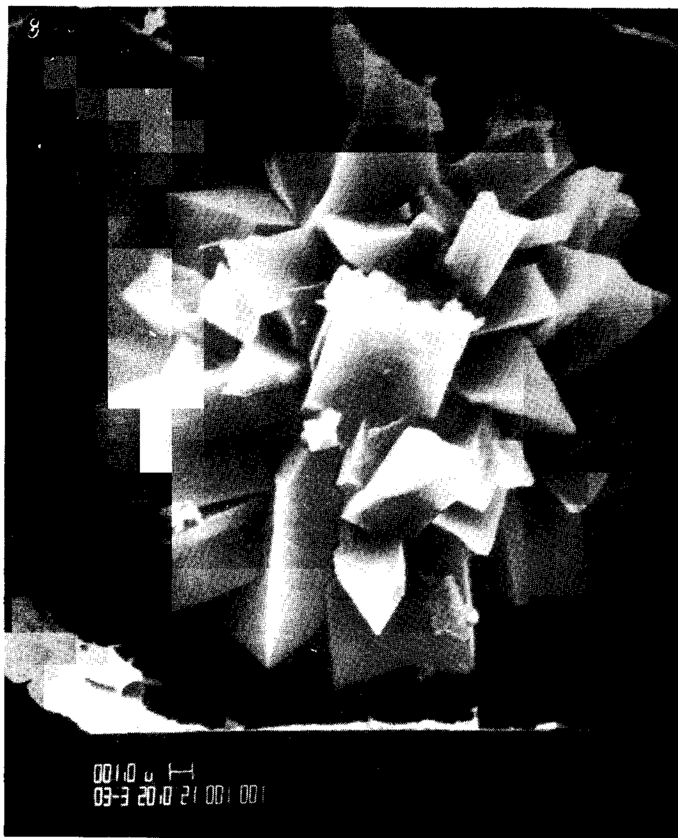

Fig. 2. Scanning electron micrograph of a single calcium oxalate druse crystal, ×3000.

d-values, 4.291 Å and 3.339 Å, not corresponding to calcium oxalate monohydrate correspond to silicon dioxide (SiO_2, powder diffraction file no. 5-490; 6). Since silicon dioxide (quartz) is a major component of sand, these two d-values can be explained by residual sand trapped in the skin of the unpeeled sweet potatoes.

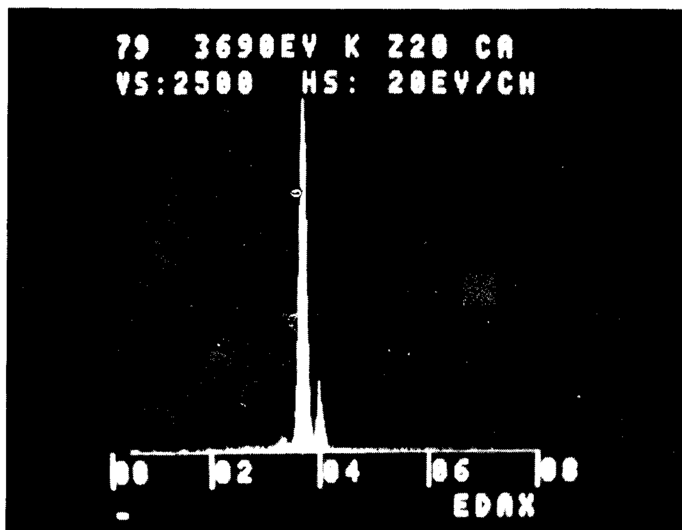

Fig. 3. K-α and K-β peaks of calcium detected in crystal by energy-dispersive X-ray analysis. Note superimposed white lines over peaks indicating known X-ray quantum energy values for calcium.

Table 1. X-ray powder diffraction data (d-values in Å and relative intensities obtained for crystals extracted from sweet potato root tissue compared with corresponding known d-values and intensities for $CaC_2O_4 \cdot H_2O$).

Crystals in root tissue		$CaC_2O_4 \cdot H_2O^z$	
I^y	d	I	d
Strong	5.985	100	5.93
Very weak	4.291^x		
Strong	3.648	70	3.65
Weak	3.339^x		
Strong	2.979	45	2.966
Very weak	2.922	10	2.915
Very weak	2.853	10	2.840
Weak	2.498	18	2.494
Strong	2.353	30	2.355
Very weak	2.259	6	2.254
Very weak	2.214	6	2.210
Very weak	2.129	2	2.130
Very weak	2.080	2	2.089
Very weak	1.975	10	1.978
Very weak	1.897	6	1.890
Very weak	1.846	6	1.846

[z]According to Powder Diffraction File no. 20-231 (6).

[y]Visual estimated intensities.

[x]d-values corresponding to silicon dioxide (SiO_2, file no. 5-490) according to Powder Diffraction File (6).

Al-Rais et al. (2) reported that among the plant species they examined, calcium oxalate monohydrate was associated only with raphide crystals. They, however, did not study sweet potatoes. Thus, our findings indicate that in some plant material druse or conglomerate crystals may also be composed of calcium oxalate monohydrate.

According to Christiansen and Foy (8), the formation of calcium oxalate crystals as the result of a mechanism within the plant to isolate surplus calcium is supported by increased crystal formation with increased calcium supply. However, Arnott and Pautard (3) suggest that such a calcium disposal system is not needed by the plant since the guttation process (discharge of water from the plant interior to the plant exterior through the leaves) may serve to dispose of excess calcium in the xylem stream. A recent study by Balerdi (4) has investigated the growth response of sweet potatoes to various calcium salts applied to different soils. The increased calcium increased top weight, root yield, and calcium content of both tops and roots. The form of this increased calcium content of both tops and roots should be investigated. This would help resolve the differences between the 2 theories of disposal of excess calcium. Increased calcium content in the form of increased calcium oxalate crystals would support the calcium disposal theory of calcium oxalate crystals. Increased calcium content in the form of increased calcium ions in the xylem stream and not in the form of increased crystals would support the disposal of excess calcium by guttation. Our investigation demonstrates that calcium is deposited as calcium oxalate crystals in sweet potato roots but does not resolve the differences between the 2 theories of disposal of excess calcium.

Literature Cited

1. Abaza, R. H., J. T. Blake, and E. J. Fisher. 1968. Oxalate determination: analytical problems encountered with certain plant species. *J. Assoc. Offic. Agr. Chem.* 51:963-967.
2. Al-Rais, A. H., A. Myers, and L. Watson. 1971. The isolation and properties of oxalate crystals from plants. *Ann. Bot.* 35:1213-1218.
3. Arnott, H. J. and F. G. E. Pautard. 1970. Calcification in plants. p. 375-446. *In* H. Schraer (ed.) Biological calcification, cellular and molecular aspects. Appleton-Century-Crofts, New York.

4. Balerdi, F. F. 1972. The response by sweet potatoes to various calcium salts applied to Providence silt loam and Stough very fine sandy loam soils. *Diss. Abstr. Intern.* B, 32, 12, I:6765.
5. Bennett, H. S., A. D. Wyrick, S. W. Lee, and J. H. McNeil, Jr. 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technol.* 51:71-97.
6. Berry, L. G. (ed.). 1976. Powder diffraction file. Joint Committee on Powder Diffraction Standards USA. Swarthmore, Penn.
7. Buttrose, M. S. and J. N. A. Lott. 1978. Calcium oxalate druse crystals and other inclusions in seed protein bodies: eucalyptus and jojoba. *Can. J. Bot.* 56:2083-2091.
8. Christiansen, M. N. and C. D. Foy. 1979. Fate and function of calcium in tissue. *Commun. Soil Science & Plant Anal.* 10:427-442.
9. Frey, A. 1927. Calcium oxalat-monohydrat und trihydrat. p. 81-118. *In* K. Linsbauer (ed.) Handbuch der Pflazenanatomie, Vol. 3. Gebruder Bornstraeger, Berlin.
10. Gallaher, R. N. 1975. The occurrence of calcium in plant tissue as crystals of calcium oxalate. *Commun. Soil Science & Plant Anal.* 6:315-330.
11. Groth, B. H. A. 1911. The sweet potato. *Contr. Bot. Lab. Univ. Penn.* 4:1-104.
12. Hayward, H. E. 1938. The structure of economic plants. MacMillan, New York. p. 485-513.
13. Horner, Jr., H. T. and V. R. Franceschi. 1978. Calcium oxalate crystal formation in the air spaces of the stem of *Myriophyllum. Scan. Elec. Micros.* II:69-76.
14. Liegel, W. 1970. Calcium oxalat-abscheidungen in fruchstielen einiger apfelvarietaten. *Angew Botanik* XI.IX:223-232.
15. Philipsborn, H. Von. 1952. Uber calcium oxalat in pflanzenzellen. *Protoplasma* 41:415-424.
16. Silver, V. L. and J. L. Price. 1969. Demonstration of calcium oxalate crystals in plant tissues by the Pizzolato ($AgNO_3$-H_2O_2) method. *Stain Technol.* 44:257-259.
17. Terblanche, J. H., L. G. Wooldridge, I. Hesebeck, and M. Joubert. 1979. The redistribution and immobilization of calcium in apple trees with special reference to bitter pit. *Commun. Soil Science & Plant Anal.* 10:195-215.
18. Umemoto, K. 1974. Morphological character of crystalline inorganic components present in plants: calcium oxalate crystals in the leaves of sweet potato grown in natural light and in darkness. *Chem. Pharm. Bul.* 22:1968-1974.
19. Walter-Levy, L. and R. Strauss. 1962. Sur la repartition des hydrates de l'oxalate de calcium chez les regetaux. *C. R. Soc. Biol.* (Paris) 254:1671-1673.
20. Wieneke, J. and F. Fuhr. 1973. Mikroautoradiographischer nachweis von Ca-kristallablagerungen in apfelstiel- und fruchtgewebe. *Angew. Botanik.* 47:107-112.

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