Calcium sulphite crystals in plants*

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An illustrated account of calcium sulphite crystals found in nine plants, viz., *Amaranthus viridis* L., *Beta vulgaris* L., *Beta vulgaris* var. *cicla* L., *Beta vulgaris* L. var. *rapa* Dum, *Chenopodium album* L., *Croton sparsiflorus Morong*, *Lycopersicum esculentum* Mill, *Portulaca oleracea* L. and *Trigonella foenum-graecum* L. is presented. Calcium-sulphite crystals have been isolated in pure state. Morphology, affinities and distinguishing features of these crystals with other crystals found in plants have been discussed. Authentic methods for ascertaining the chemical nature of plant crystals have been evaluated.

Key-words - Angiosperms, dicotyledons, morphology, crystals, Calcium sulphite.

INTRODUCTION

Recent studies by Bharadwaj (1988, 1989, 1991) have revealed that the chemical nature of some plant crystals has not been correctly recorded earlier. Calcium sulphite crystals of many plants have been identified as calcium oxalate because of the similarity between the two. They were mostly identified by light microscope or x-ray diffraction studies which are not efficient for ascertaining their correct chemical nature. Revision of the chemical nature of plant crystals studied by such methods has hence become a necessity. Franceschi and Horner (1980) in their review, recorded calcium oxalate crystals identified on the basis of light microscope, SEM, TEM, IR spectoscopy and x-ray diffraction studies. Chemical evidence is lacking in these studies. Arnott and Pautard (1970) recorded various chemical salts identified in plants, viz., calcium sulphate, phosphate, oxalate, silicate, carbonate, citrate, tartrate and malate.

Bharadwaj (1980, 1989) discovered four more new crystal kinds, viz., calcium sulphite, calcium thiosulphate, calcium lactate and ferrous oxalate from plants. Chemical nature of these crystals was ascertained by SEM and chemical tests. Bharadwaj (1989) also confirmed that crystals from sugar beet leaves, earlier identified by x-ray diffraction studies by Al Rais et. al. (1971) as calcium oxalate, did not consist of calcium oxalate, but consisted of five other chemical salts.

Crystals of *Lycopersium esculentum* fruits, and *Trgonella foenum-graecum* leaves and stems, earlier identified by Kapoor and Mitra (1976) as calcium oxalate, were also found by Bharadwaj (1991) to be composed of calcium sulphite, calcium thiosulphate and calcium carbonate, and not calcium oxalate.

The present paper is a synthesis of the studies on the nature of calcium sulphite crystals found in nine plants, viz., *Amaranthus viridis* L., *Beta vulgaris* L. (red beet), *Beta vulgaris* var. *cicla L.* (leaf beet), *Beta vulgaris* var. *rapa* Dum (sugar beet), *Chenopodium album* L., *Croton sparsiflorus* Morong, *Lycopersicum esculentum* Mill, *Portulaca oleracea* L. and *Trigonella foenum graecum* L. (Bharadwaj 1988, 1989, 1991).

MATERIAL, METHODS AND TERMINOLOGY

Botanical methods

Isolation of crystals - Bharadwaj (1988) isolated crystals from leaves and stems in pure state. This method has been improved which involves the following steps :

1. Washing the plant material thoroughly with filtered water (filtered through a bacterial filter or filter paper) in order to remove all soil particles which could contaminate the crystals.
2. Homogenising the plant material in a blender with some distilled water to expel the crystals out of the plant tissues.

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3. Filteration of the blended material through two sieves.
(i). A sieve having aperture size of 200-300 µm to separate the fibrous material from the cytoplasmic suspension.
(ii) After discarding the fibrous material, the cytoplasmic +uspension is refiltered through the second sieve, having aperture size of 100-150 µm, to separate minute fragments of leaves seen in the cytoplasmic suspension.

4. Siphoning out the cytoplasmic suspension, after allowing it to stand for 10-30 minutes, to discard minute cell organelles suspended in it. (The crystals are heavier and settle at the bottom of the container). This process is repeated 7-8 times, after adding filtered water each time till most of the chloroplasts and other cell organelles are discarded.

5. Dissolving the remnant chloroplasts and cell organelles, present amongst and crystals by keeping them in a 360% aqueous solution of chloral hydrate for 24 hours.

6. Finally, the chloral hydrate is washed off by distilled water. The washed crystals which are free of contamination from other cell organelles are centrifuged, dehydrated through alcohol (30-100%), and dried at room temperature or in an oven at 50°C.

Chemical methods

I. *Preparation of chemical compounds-* Crystals of seven chemical compounds, viz., calcium oxalate, calcium sulphite, calcium sulphate (gypsum), calcium thiosulphate, calcium carbonate, ferrous oxalate and calcium lactate were prepared in the laboratory for comparison with scanning electron micrographs of plant crystals.

II. *Chemical tests*Specific chemical tests were selected by Bharadwaj (1988, 1989) from Welcher (1917), Furman (1939) and Girl et al., (1976) for confirmation of the presence of cations and anions, viz., Ca^{++}, Mg^{++}, Fe^{++} CO_3, PO_4, $C_2O_4^{--}$, S^{--}, SO_4^{--}, SO_3^{--}, S_2O_3 and $CH_3CHOHCOO^-$.

Terminology

Terminology has been adopted after Read (1984) and Bharadwaj (1988, 1989).

OBSERVATIONS

Chemical compounds

Chemical calcium sulphite exhibits a number of affinities with chemical calcium oxalate. An emended diagnosis (based on SEM morphology) of the two compounds prepared in the laboratory is given below for comparison with plant crystals and their identification.

1. *Calcium oxalate* ($CaC_2O_4 . 2H_2O$) - Individual crystals rarely irregular, mostly tetragonal or orthorhombic with 2 terminal faces and 4 side faces, or less often cubical; occasionally crystals more or less triangular with 3 side faces; crystals with 5 and 6 side faces rare; curved; white in colour height often less than half the width, sometimes side faces straight edges sharp or rounded; surface smooth. Crystals formed as druses in the form of aggregates of 4-numerous individuals. Druses spherical, ovoid or irregulr in shape, measuring 1-27 um in their greatest diameter, isolated crystals rare.

2. *Calcium sulphite* ($CaSO_3 2H_2O$) - Individual crystals variously shaped; often tetragonal or orthorhombic with 2 terminal faces and 4 side faces, or cubical; sometimes triangular with 3 side faces or rarely 5 or 6 sided. Usually, irregular; other common types being pyramidal, curved or with shallow or deep depressions or channels on one face, box or boat shaped, thin-papery sheet like, tubular with thin or thick walls, spherical with a small pore in the centre, irregular with 1 or more perforations, funnel-like or horn-shaped; other rare types being dendroid and feather-like, white, 0.4 to 23 µm in their greatest diameter; surface smooth.

Diagnostic features of calcium sulphate (gypsum), calcium thiosulphate, calcium carbonate, ferrous oxalate and calcium lactate have been given by Bharadwaj (1988, 1989).

Calcium sulphite crystals in plants - Calcium sulphite occurs in plants as solitary crystals or as druses or rosettes in which numerous individual crystals aggregate together. Solitary crystals isolated from plant tissues measure from 0.1 to 140 µm in their greatest diameter. The druses and rosettes, isolated, measure from 5-80 um in their greatest diameter.

Solitary crystals of calcium sulphite are abundant in leaves and roots of sugar beet, leaf beet and red beet (Pl. 1, figs 1-3). They are variously shaped, cubical, orthorhombic, tetragonal or irregular, often possessing a shallow or deep

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PLATE 1

Abbreviations

b: box shaped crystal; bt: boat shaped crystal; c: curved crystal; ch: crystal showing channel on one face; cb: calcium carbonate crystal; d: crystal showing depression on one face; dr: a druse crystal; p: crystal showing a pore; py: pyramidal crystal; ro: a rosette; rg: crystal possessing ridges and grooves; sl: crystal showing a slit on one side; t: tubular crystal.

Crystal in Pl.2. fig.4 has not been treated with chloral hydrate. Plates 1-3: Scanning electron micrographs of plant crystals.

1-3. Solitary crystals of calçium sulphite from sugar beet, leaf beet (leaves) and red beet (roots) respectively. 4. Solitary, irregular crystals of calcium sulphite from *Trigonella foenum-graecum* leaves. Two calcium carbonate crystals (cb) are also seen.

5. Solitary irregular crystals of calcium sulphite from *L. esculentum* fruits, seen sticking to a coalesced mass of calcium thiosulphate. A calcium carbonate crystal (cb) is also seen.

6-8. Crystals from sugar beet leaves.

6. A calcium sulphite rosette. (ro)

7. A calcium sulphite druse (dr)

8. A cylindrical crystal of calcium sulphite showing a slit (sl) on one side.

9. A calcium sulphite crystal showing a rectangular depression (d) on one face. A calcium carbonate crystal (cb) is also seen.

10, 11. Druses from *A. viridis* leaves. 10. A druse of calcium sulphite showing two prominent boat shaped crystals (bt) and a tubular crystal (t).

11. A calcium sulphite druse showing two pyramidal crystals (py). Part of another druse showing a large box shaped crystal (b).

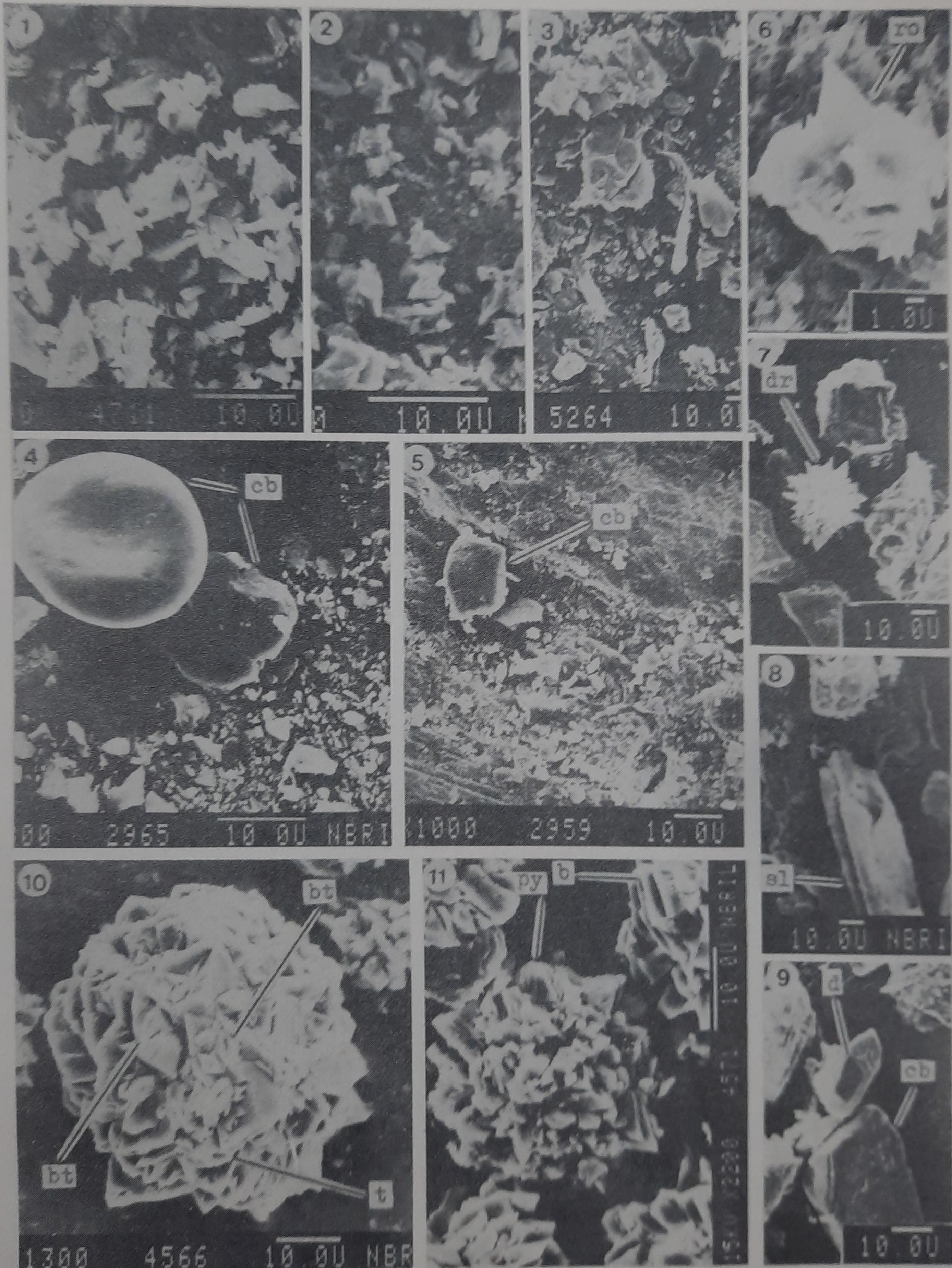
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PLATE 1

depression or channel on one face. Other less common types are horn-shaped, funnel-like or curved. Rare rosettes, which are aggregates of variously shaped crystals (Pl. 1, fig. 6-ro) and druses of horn-shaped crystals (Pl. 1, fig. 7-dr), have been isolated from sugar beet leaves. A cylindrical crystal, possessing a longitudinal slit on one side has also been isolated from sugar beet leaves (Pl.1, fig.8-sl).

Calcium sulphite crystals of *T. foenum-graecum* leaves (Pl. 1, fig 4), and *L. esculentum* fruits (Pl.1, fig.5) are usually irregular. Crystals with depressions on one face are rarely seen in *T. foenum-graecum* (Pl.1, fig.9-d).

Druses of calcium sulphite are abundant in *A. viridis*, *Chenopodium album*, and *P. oleracea* leaves, but solitarity crystals have not been seen in these plants.

In *A. viridis*, occasional box-shaped (Pl.1, fig. 11-b), pyramidal (Pl.1,fig.11-py), curved (Pl.2 fig.1-C), boat-shaped (Pl.1,fig.10-bt), and tubular crystals (Pl.1, fig.10-t) are seen amongst the more frequent cubical, tetragonal, orthorhombic and irregular crystals of the druses. Crystals possessing depressions and channels on one face are also frequent. An isolated druse from *A. viridis leaves*, in which most of the crystals are orthorhombic and tetragonal (Pl.2, fig.1), closely resembles a calcium oxalate druse. However, a few curved crystals (c) and some possessing depressions (d) on one face, attached to it, distinguish it as a calcium sulphite druse.

In druses of *Chenopodium album*, the more common crystal types are; triangular, cubical, tetragonal, orthorhombic and irregular. Pyramidal crystals may possess a pore at the apex; curved crystals, tubular crystals, and crystals possessing depressions or channels on one face are also quite frequent (Pl. 2, figs 2,3). Pyramidal and triangular crystals sometimes possess fine ridges and grooves on the surface (Pl.2, fig.4-rg).

Druses of *P. oleracea* usually consist of irregular, orthorhombic and tetragonal crystals. In some druses, majority of the crystals are tetragonal while in others orthorhombic. Box-shaped crystals and crystals possessing channels and depressions on one face are occasional (Pl.3, fig.3, b, ch). Two druses in which majority of the crystals are orthorhombic (Pl.3, fig.2). resemble calcium oxalate druses, but one of them can be recognized as calcium sulphite due to the presence of a few crystals possessing depressions (d) on one face. In the other druse, one irregular crystal, possessing a pore (p) in the centre, confirms it to be calcium sulphite. Solitary crystals of calcium sulphite are absent in *Croton sparsiflorus* also. Druses of calcium sulphite are less common in this plant. They are aggregates of irregular cubical, tetragonal to orthorhombic crystals. Some of them possess shallow depressions on one face (Pl.3, fig.1-d). Rosettes of calcium sulphite are abundant in *Croton sparsiflorus* (Pl.2, fig.6). These are aggregates of curved crystals (Pl.2, figs.5,7) having varied degree of curvature. The crystals aggregate in such fashion that the rosettes appear like flowers.

DISCUSSION

Distinguishing features of various crystals

As evident from the diagnosis, a marked similarity exists between many kinds of individual crystals of chemical calcium sulphite and calcium oxalate. However, curved and irregular crystals are very rare in calcium oxalate whereas they are very frequent in calcium sulphite.

Calcium sulphite differs from calcium oxalate due to the presence of other types of variously shaped crystals like boat-shaped, pyramidal, tubular etc.

Druses are unknown in chemical calcium sulphite whereas chemical calcium oxalate precipitates as spherical, ovoid, or irregular druses, and hence to distinguish between chemical calcium oxalate and sulphite is rather free from constraint.

However, druses of calcium sulphite occur in plants. A marked similarity exists between calcium sulphite and calcium oxalate druses of plants when majority of the crystals in the former are tetragonal, orthorhombic and cubical. In such cases, a careful study is required to distinguish between the two. Nevertheless, because of the presence, of a few of the following crystal types which are altogether absent in calcium oxalate druses the two can be distinguished, viz., boat-shaped, box-shaped, horn-shaped, tubular, funnel-like, pyramidal spherical with a pore in the centre or with shallow or deep depressions, or channels on one face. Calcium sulphite druses which possess numerous

PLATE 2

1. A calcium sulphite druse from *A. viridis* showing two curved crystals (c) and two crystals showing depressions (d) on one face.

2-4. Druses from *Chenopodium album* leaves.

2. A calcium sulphite druse showing a prominent tubular crystal (t) and a pyramidal crystal showing a pore (p) at the apex.

3. A calcium sulphite druse showing a tubular crystal (t) and a pyramidal crystal showing three pores (p) at the apex.

4. A calcium sulphite crystal showing one pyramidal and one triangular crystal showing fine ridges and grooves on the surface. (rg).

5-7. Crystals from *Croton sparsiflorus* leaves.

5. A calcium sulphite rosette.

6. Numerous calcium sulphite rosettes.

7. A calcium sulphite rosette.

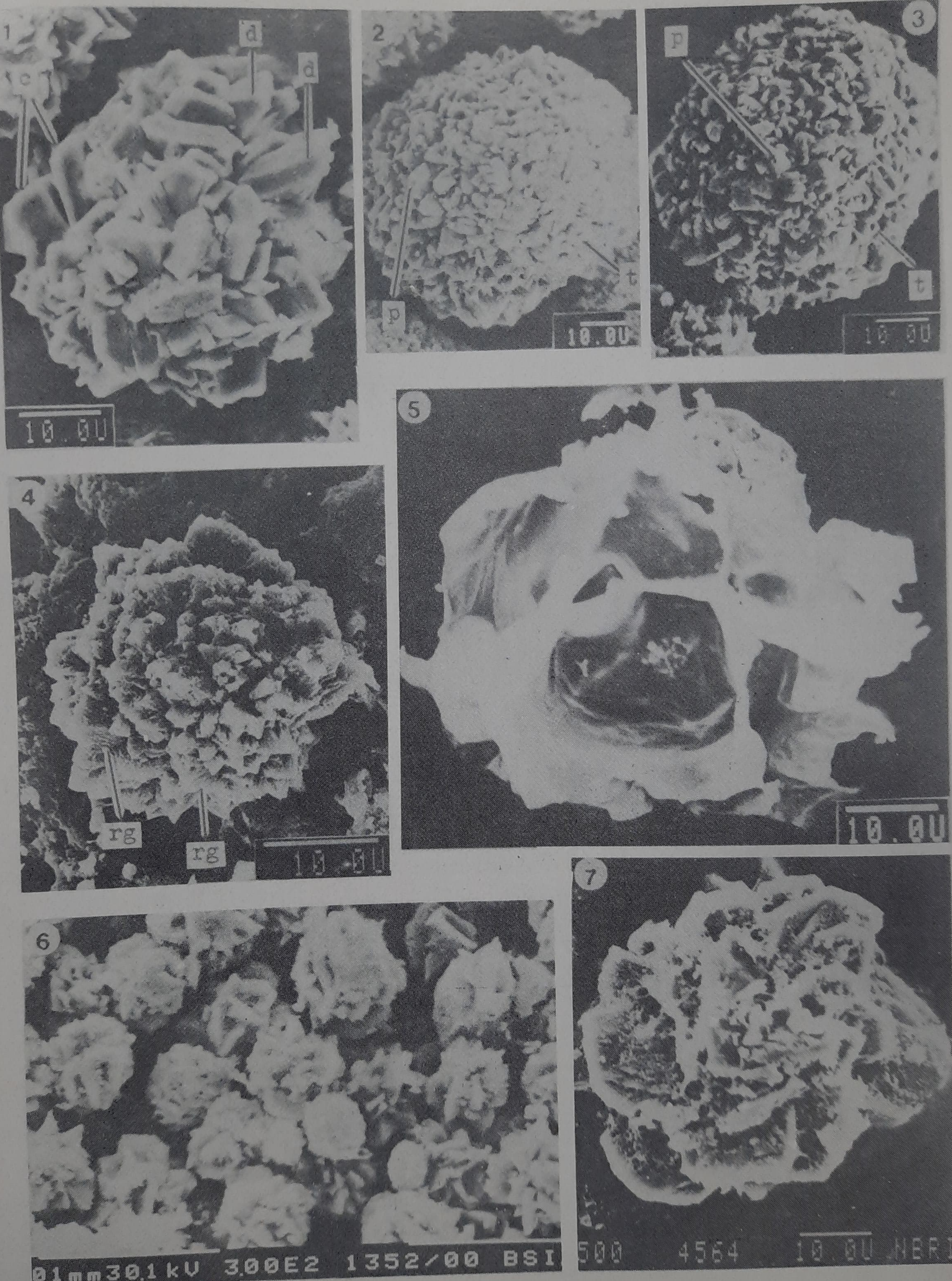
1
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PLATE 2

crystals of these types can be easily recognized.

Other chemical types occurring in plants can be identified and distinguished from calcium sulphite and oxalate crystals with ease.

In the SEM micrographs of *P. oleracea* the 3-5 sided, elongated crystals of ferrous oxalate (Pl.3, fig.8-f), seen attached to calcium oxalate druses, do not impose any difficulty in recognition.

Crystalline masses of calcium thiosulphate (Pl.3, fig.5) can also be easily marked by their spherical, oval or irregular shape, and surface which is smooth, alveolate, warted or uneven.

Prominently different from other types are calcium carbonate crystals which can be easily spotted out due to their blocky nature and stony appearance (Pl.3, fig.7). Some of the calcium carbonate crystals are lamellated. Occasionally they possess sloping side faces.

The tabular, much longer than broad crystals of gypsum are distinct from all other types and can also be spotted out at first sight (Pl.3, fig.6-g).

The unique and large crystals of calcium lactate, composed of irregular units of different sizes and shapes, possessing a smooth or perforated surface are distinct from all other types (Pl.3, fig.9).

Methods for identification of plant crystals

Crystals of every chemical compound have a characteristic morphology, and hence it is a clue to their chemical nature. Amongst the optical methods, scanning electron and transmission electron microscope studies which reveal the detailed morphology of chemical compounds and plant crystals, are the most assuring methods for their identification. Light microscopes have a low power of resolution, and are inefficient for identification of plant crystals. X-ray diffraction studies of plant crystals have not given correct results. As the isolated plant crystals are usually mixtures of two or more chemical compounds. IR spectroscopy may not be suitable for their identification: Among chemical methods, Atomic Absorption and Flame Photometeric studies are not suitable for detecting the full chemical composition. They can detect cations only, and not anions. Evidence for the chemical nature of plant crystals may be obtained from authentic chemical tests of the qualitative methods which can detect cations and anions both. Chemical tests are exceedingly trustworthy.

Chemical tests for detection of SO_3 ions - Two of the reliable tests for detection of SO_3 ions, viz., barium water test and $KMnO_4$ (oxidation and reduction) tests, have been rejected for the following reasons: barium water is fatal for human beings and the $KMnO_4$ test is given by C_2O_4 ions also. The most assuring test in which SO_2 released in the reaction reduces the acidified $K_2Cr_2O_7$ to green $Cr_2(SO_4)_3$ has been selected for detection of SO_3 ions. This test is not given by C_2O_4 ions or any other ions.

However, during systematic analysis for SO_3, SO_4, and S_2O_3 ions, the Ba^{++} ions present in the filtrate containing SO_3 ions, have proved to be interfering ions. When a $K_2Cr_2O_7$ solution, acidified with dilute H_2SO_4 is added to the filtrate containing SO_2 ions, the $K_2Cr_2O_7$ solution decolourises and very small amounts of green $Cr_2(SO_4)_3$ is formed. In this case, all SO_4 ions formed in the reaction are not available for the formation of $Cr_2(SO_4)_3$. The Ba^{++}ions interfere, and a white precipitate of $BaSO_4$ is also obtained in the reaction. The white Ba-SO_4 masks the green colour of $Cr_2(SO_4)_3$. Bharadwaj (1988) removed Ba^{++}ions from the filtrate containing SO_3 ions by adding an aqueous solution of NaOH to it. The white precipitate of $Ba(OH)_2$ obtained was discarded and the filtrate containing SO_3 ions which was free from Ba^{++}ions gave a clear green colour of $Cr_2(SO_4)_3$ on addition of acidified $K_2Cr_2O_7$.

$3\,Na_2SO_3 + K_2Cr_2O_7 + 4\,H_2SO_4 = K_2SO_4 + 3\,Na_2SO_4 + Cr_2(SO_4)_3 + 4\,H_2O$.

Medicinal property of calcium sulphite

Calcium sulphite when present with calcium thiosulphate may be of medicinal value. When an inorganic acid is added to a solution of mixture containing both SO_3 and S_2O_3ions, sulphur is released in the reaction:

$Ca\,SO_3 + Ca\,S_2O_3 + HCl = 2\,Ca\,Cl_2 + 2\,SO_2 + S + 2H_2O$. All the nine plants mentioned in this paper contain calcium sulphite as well as calcium thiosulphate crystals.

PLATE 3

1. A druse of calcium sulphite from *Croton sparsiflorus* showing a crystal with a shallow depression (d) on one face.

2, 3. Druses from *P. oleracea* leaves.

2. Two calcium sulphite druses: one showing an irregular crystal with a pore (p) in the centre, and another one showing three crystals possessing prominent depressions (d) on one face.

3. A calcium sulphite druse showing a box shaped crystal (b), and a crystal possessing a channel (ch) on one face.

4. A calcium oxalate druse, from *A. viridis*, showing orthorhombic and tetragonal crystals.

5. A crystalline mass of calcium thiosulphate, from sugar beet leaves, showing an uneven surface.

6. A tabular crystal of gypsum (g) lying amidst a calcium sulphite crystal, isolated from sugar beet leaves.

7. Blocky crystals of calcium carbonate, isolated from leaf beet roots.

8. An elongated, four sided crystal of ferrous oxalate (f) attached to a calcium oxalate druse rom *Portulaca oleracea*.

9. A calcium lactate crystal (from sugar beet leaves) composed of irregular units of different size and shapes, showing smooth or perforated surface.

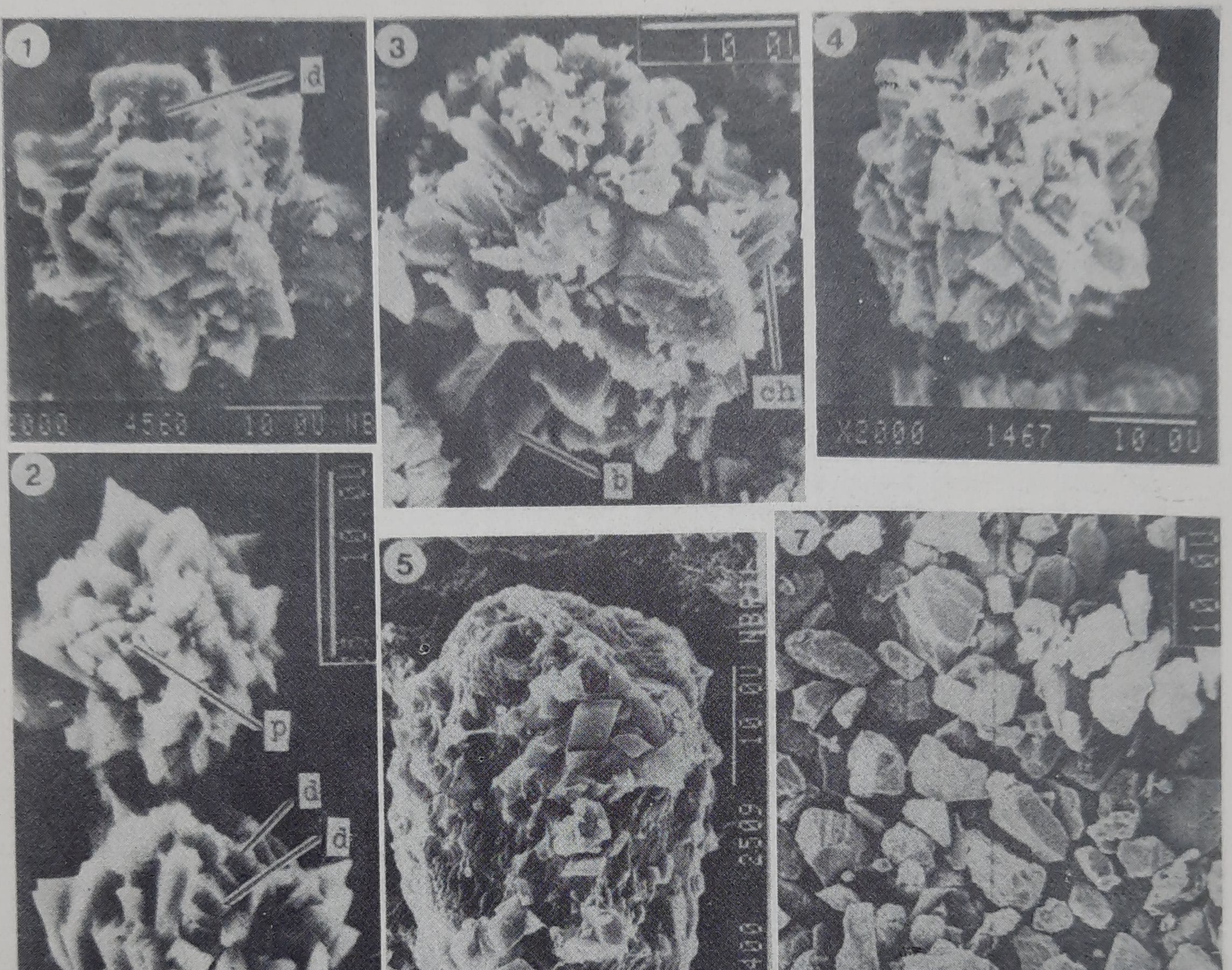
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PLATE 3

Taxonomic significance of plant crystals

Morphology and chemical nature of crystal kinds present in plants can be used in determining the phyletic positions within the higher categories, like order and families as well as lower categories, like genus and species.

Presence of three chemical kinds of crystals, viz., calcium oxalate druses, calcium sulphite druses and calcium thiosulphate masses, reported by Bharadwaj (1988) in *A. viridis* (Amaranthaceae), *Chenopodium album* (Chenopodiaceae) and *P. oleracea* (Portulacaceae), belonging to the order Centrospermae, indicates a close relationship between the three families.

The genus *Beta*, also classified in the Chenopodiaceae, differs from *Chenopodium album* in having calcium carbonate crystals which are absent in the latter. Bharadwaj (1989) reported the presence of abundant calcium carbonate crystals in leaves as well as roots of *Beta vulgaris* (red beet), *Beta vulgaris* var. *cicla* (leaf beat) and *Beta vulgaris* var. *rapa* (sugar beet). This major difference deserves separation of the genus *Beta* from Chenopodiaceae.

Four major similarities between the crystals of the three varieties of *Beta* described are the presence of 1. Large calcium thiosulphate masses of similar size, shape and surface features, 2. Gypsum crystals, 3. Solitary and variously shaped crystals of calcium sulphite, and 4. Calcium carbonate crystals. This indicates a close genetic relationship between the 3 beets, justifying their classification in the genus *Beta*. However, sugar beet differs from red beet and leaf beet in having large calcium lactate crystals in leaves, and absence of calcium oxalate crystals which are present in red beet and leaf beet leaves.

This striking difference suggests some difference in the genetic constitution of sugar beet from leaf beet and red beet. On the basis of this difference sugar beet could be classified in a species, separate from *vulgaris*.

Scott (1941) had reported the presence of calcium oxalate crystals in *Ricinus communis*, a member of the Euphorbiaceae. However, Bharadwaj (1988) confirmed the absence of calcium oxalate crystals in *Croton sparsiflorus*, belonging to the same family. Only two crystal kinds, viz., calcium sulphite rosettes and druses, and calcium thiosulphate masses have been reported in the leaves of *Croton sparsiflorus*.

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