

sions of Andhra Pradesh and in isolated parts of Gujarat, Sourashtra, NI-Kar, East-UP, West-UP, East-Raj, West-Raj and Jharkhand sub-divisions. Negative values of difference image over Bihar sub-division need to be interpreted differently, since 2002 was a near-normal year in this sub-division. The negative values of difference image in the NI-Kar were caused due to residual cloud cover in the satellite images.

Objective comparisons with geospatial indicators, performed in this study indicate intense agricultural drought situation in the sowing period (up to the first fortnight of July) in both 2009 and 2002 kharif seasons. From the second fortnight of July, the recovery in the situation was faster in 2009, facilitating more crop-sowing in contrast to 2002. End-of-sowing-period crop growth anomalies confirmed more delay/reduction in crop sown area and poor crop growth in 2002 compared to 2009. Active crop-growing period integrated crop condition (NDVI)

also revealed more intense agricultural drought conditions in 2002 kharif season compared to kharif 2009. Normal or slightly less than normal agricultural performance in 2009 could be inferred in the sub-divisions of Orissa, Uttar Pradesh, Madhya Pradesh, Punjab, Haryana, Maharashtra, Karnataka and Chhattisgarh, and better than 2002 and less than normal agricultural performance over the sub-divisions of Rajasthan and Gujarat. Equally intensive agricultural drought situation in both the years in all the three sub-divisions of Andhra Pradesh was evident. Agricultural performance of Bihar during kharif 2009 could be slightly less than normal. In Jharkhand, the coarse-resolution geospatial indicators did not show sensitivity because the crop area is isolated and constitutes a small proportion of the geographic area.

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## Occurrence of non-protoplasmic mineral deposition in seed coat of *Biota orientalis* Endl. (Cupressaceae)

Biominalization of calcium crystals is of common occurrence in all photosynthetic organisms from small algae to angiosperms and giant gymnosperms<sup>1</sup>. Calcium oxalate crystals occur in plants in two principal forms, monohydrate (CaC<sub>2</sub>O<sub>4</sub>·H<sub>2</sub>O) and dihydrate (CaC<sub>2</sub>O<sub>4</sub>·2H<sub>2</sub>O). Each of these has a different crystal structure. The monohydrate form known as whewellite belongs to the monoclinic system of crystallization, and the dihydrate form known as weddellite, belongs to the tetragonal system<sup>2</sup>. The crystals have specific shapes and sizes, and are usually found in the form of prisms, druses, styloids, raphides and crystal sand<sup>3</sup>. Their occurrence and abundance in specific tissues of various plants are so constant that they are often used as a taxonomic tool<sup>4</sup>.

Calcium oxalate crystals are found in various plant parts and occur in the cell wall or vacuole within specialized cells called idioblasts. The crystals in the vacuole are formed within the intravacuolar membrane chambers (crystal chambers) that differentiate and proliferate exclusively in the vacuoles of crystal cells<sup>5,6</sup>.

In gymnosperms crystals are widely distributed generally in the wood of *Abies magnifica*<sup>7</sup>, the bark of *Abies nordmanniana*<sup>8</sup> and *Larix decidua*<sup>8,9</sup>, *Picea excelsa*<sup>8,9</sup>, leaves of *Tsuga canadensis*<sup>10</sup>, *Ginkgo biloba*<sup>11</sup> and rarely in the seed coat and the nucellus of *Picea abies*<sup>12</sup>.

In the present paper occurrence of non-protoplasmic crystal inclusions in the form of solitary crystals, druses and crystal sands in the seed-coat cell layers of *Biota orientalis* Endl., a monotypic genus of Cupressaceae, has been reported and described, and its chemical nature determined. The solitary crystals are of prismatic or styloid type, polyhedral in shape. Druses are mainly found in star or rosette form. All forms of crystals are monohydrate calcium oxalate in nature, confirmed by X-ray diffraction (XRD) pattern and energy dispersive X-ray (EDX) analysis.

Fresh, mature seeds of *B. orientalis* Endl. were collected from Nainital (29°N, 79°28'E) and Ranikhet (34°N, 81°02'E), Uttaranchal, North India during the end of November 2008. Cones attached to branches were collected and seeds

isolated. Fresh seeds (Figure 1a) were taken for anatomical study and the remainder preserved for maceration and mineralogical observation. The seeds were soaked overnight for softening, and transverse sections of seed coats were made. Maceration of the dry seed coats was done by overnight treatment with conc. nitric acid, washed thoroughly several times to make it acid-free and centrifugation to recover the crystals. Both the thin sections and scattered isolated crystals were observed, studied and photographed under SEM (Leica S440).

EDX analysis (INCA, Oxford Instruments, UK) was done for elemental analysis of the crystals present in the seed coat, following the usual technique. The accelerating voltage used during the analysis was 15 kV. XRD scans for all the samples were taken using the X'pert Pro XRD machine, which is a fully computerized instrument with CuK $\alpha$  radiation. The XRD pattern of each sample was matched with the standard ICDD database installed in the machine. After suitable matching with the standard data,

the computer revealed results of mineral assemblage semi-qualitatively present in the sample.

Under light microscope, transverse sections of the seed coat reveal the occurrence of distinct and conspicuous crystals inside the cell lumen (Figure

1 b). Under SEM the crystals appear as a bunch or druses. Scattered solitary crystals have also been recovered by maceration of seed coat (Figure 1 d and e). Druses (Figure 1 g, i and j) are mainly found in star or rosette form (Figure 1 i and j) measuring  $25\ \mu\text{m} \times 35\ \mu\text{m}$  and as

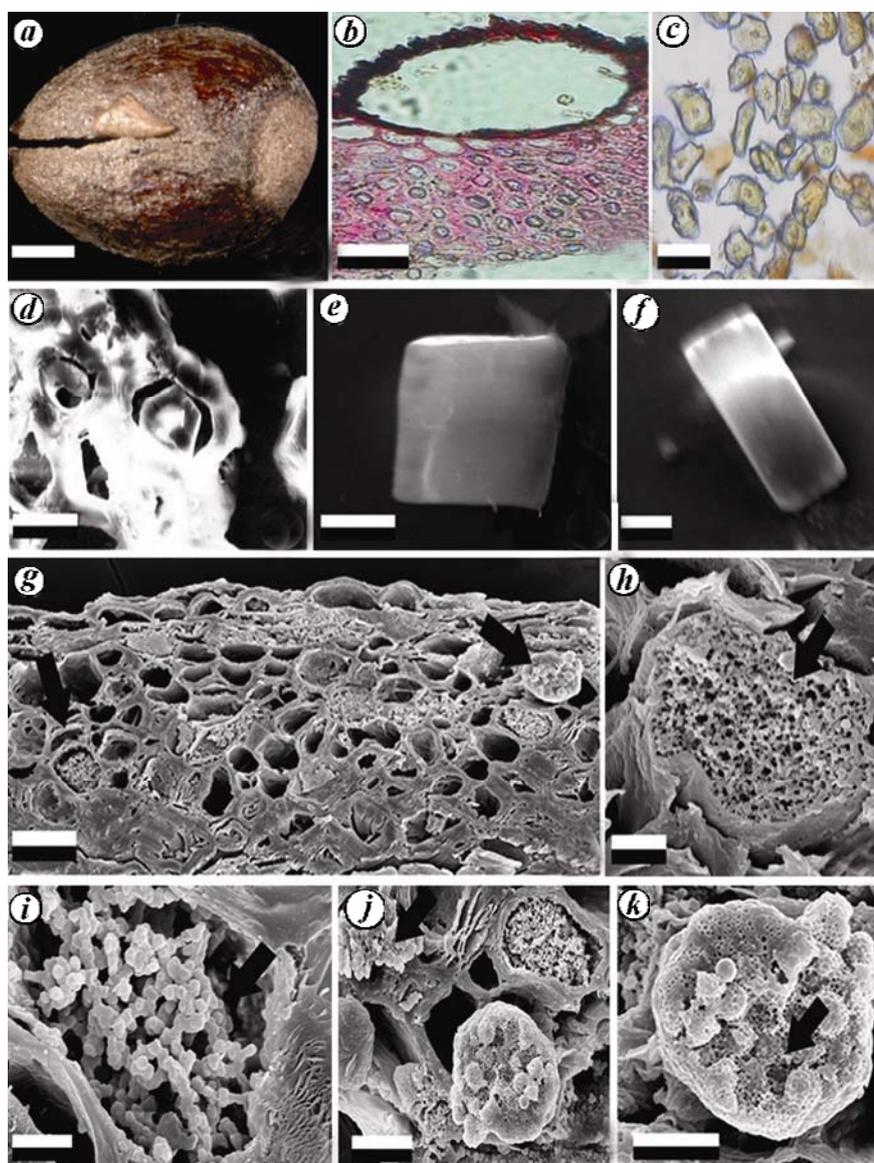
crystal sands (Figure 1 h) ranging in size from 2 to  $4\ \mu\text{m}$ . The solitary crystals (Figure 1 b and c) have a prismatic (Figure 1 d) form measuring  $30\ \mu\text{m} \times 35\ \mu\text{m}$ , cuboidal (Figure 1 e) measuring  $25\ \mu\text{m} \times 30\ \mu\text{m}$ ; styloid type (Figure 1 f), ranging in length from 30 to  $35\ \mu\text{m}$  and in breadth from 15 to  $18\ \mu\text{m}$ , or polyhedral in shape (Figure 1 b), measuring  $20\ \mu\text{m} \times 25\ \mu\text{m}$ . Crystal containing cells vary in size from 60 to  $75\ \mu\text{m}$ .

Qualitative data from EDX analysis indicate that the crystals contain elements like Ca, Si, C, and O (Figure 2). The quantitative data showing the relative amount of minerals present in the seed coat from EDX analysis are given in Table 1. Based on EDX analysis the crystals are suggested to be calcium oxalate in nature.

XRD study shows that the minerals present in the crystals of *B. orientalis* seed coat have a characteristic pattern containing a set of  $d$  (interplanar spacing) values along with their relative intensities ( $I/I_0$ ). With the help of standard ASTM charts and JCPDS data, the relative abundance of minerals present in *B. orientalis* seed coat indicates Ca-oxalate nature in monohydrate form.

Seed coat of *B. orientalis* reveals the occurrence of solitary and druse type of crystals. The crystals, especially raphides, are frequently surrounded by thin sheaths known as membranes or pellicles, which assume the same outlines as those of the crystals<sup>13</sup>. In the present material druses have been found to be covered by a similar membranous covering or pellicle, which is porous in nature (Figure 1 j and k).

Both EDX analysis and XRD pattern indicate calcium oxalate nature of the crystals. In *Biota* occurrence of crystals may be the manifestation of an effort to maintain an ionic equilibrium under Ca-stress condition. The crystal idioblasts may function as a means of removing the oxalate in *Biota*, which may otherwise accumulate in toxic quantities. In many plants oxalate is metabolized slowly or not metabolized at all, and this is considered to be an end-product of metabolism. Idioblast formation is dependent on the availability of both Ca and oxalate. Under Ca stress conditions, however, crystals may be reabsorbed indicating a storage function for the idioblasts for Ca. In addition, it has also been suggested that the crystals serve as a mechanical support or a protective device against

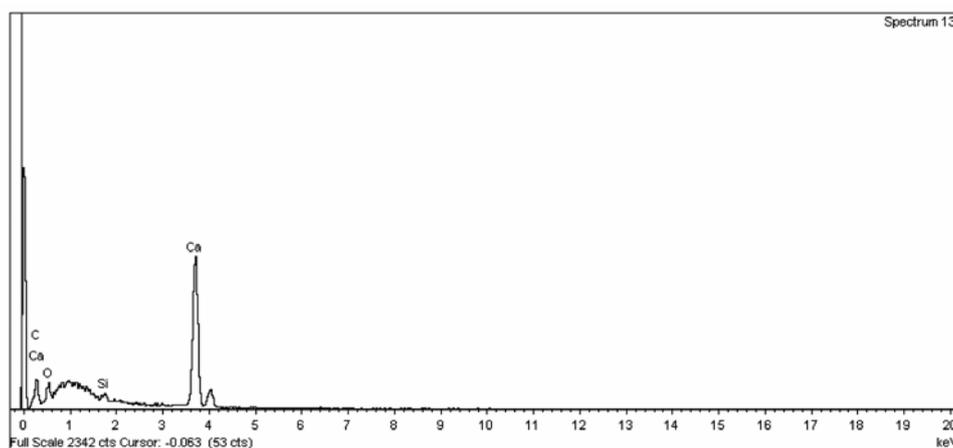


**Figure 1.** a, Entire seed of *Biota orientalis*. Scale bar = 2 mm. b, Transverse section of seed coat showing profuse polyhedral shaped crystals in the cell layers. Scale bar =  $40\ \mu\text{m}$ . c, Macerated seed coat showing isolated cells each containing a solitary crystal under compound light microscope. Scale bar =  $40\ \mu\text{m}$ . d, A prismatic crystal in the transverse section of seed coat layer under scanning electron microscope (SEM). Scale bar =  $40\ \mu\text{m}$ . e, Solitary cuboidal crystal under SEM from maceration of dry seed coat. Scale bar =  $10\ \mu\text{m}$ . f, A styloid type of crystal under SEM from maceration of dry seed coat. Scale bar =  $10\ \mu\text{m}$ . g, Transverse section of mature seed coat showing cells containing druses under SEM. Scale bar =  $40\ \mu\text{m}$ . Arrows indicate different types of druses. h, A single cell of seed coat in transverse section enlarged showing thick depositions of crystalline materials in the form of crystal sand under SEM. Scale bar =  $9\ \mu\text{m}$ . Arrow indicates crystal sand. i, Druses magnified under SEM. Scale bar =  $6\ \mu\text{m}$ . Arrow indicates cell lumen containing globular crystal druses. j, A bunch of druses and a bunch of sheathed crystals covered by a pellicle under SEM. Scale bar =  $40\ \mu\text{m}$ . Arrow indicates druse and pellicle surrounding the druses. k, Crystals showing porous structure of pellicle. Scale bar =  $60\ \mu\text{m}$ . Arrow indicates pores of pellicle surrounding the crystals.

## SCIENTIFIC CORRESPONDENCE

**Table 1.** Quantitative data on the occurrence of elements present in the seed coat of *Biota orientalis* from energy dispersive X-ray analysis

Element	App concentration	Intensity concentration	Weight (%)	Weight (%) Sigma	Atomic (%)	Compound (%)	Formula	Number of ions
C K	2.74	0.9131	12.96	1.11	21.01	47.50	CO <sub>2</sub>	2.76
Si K	0.17	0.8726	0.85	0.22	0.59	1.82	SiO <sub>2</sub>	0.08
Ca K	8.60	1.0294	36.22	0.94	17.59	50.67	CaO	2.31
O			49.97	1.23	60.80			8.00
<b>Cation sum</b>								<b>5.16</b>



**Figure 2.** Graphical representation of qualitative data from energy dispersive X-ray analysis showing the occurrence of different elements in the seed coat of *Biota orientalis* Endl.

foraging animals<sup>3,14,15</sup>. Seeds of *Biota* have significant medicinal and herbal value. They contain an essential oil consisting of borneol, bornyl acetate, thujone, camphor and sesquiterpenes<sup>16,17</sup>. The seed is used as aperient, lenitive and sedative. It is also used internally in the treatment of palpitations, insomnia, nervous disorders and constipation in the elderly people<sup>18,19</sup>. Due to its therapeutic importance, correct identification of the species is necessary. In addition to physiological, ecological and mechanical functions, the occurrence of specific types and forms of crystals in the seed coat of *B. orientalis* may have a taxonomic value and could be used as a reliable associative tool in proper identification of the species.

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