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RESEARCH ARTICLE

Studies on the Calcium Oxalate Crystals of Some Selected Aroids (Araceae) in Eastern India

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ABSTRACT
The presence or absence of micro-characters in plant system like calcium oxalate crystals has been used for understanding the evolutionary relationships of plant species. The size and appearance of calcium oxalate crystals (COC) can differ within families, genus, and species and these characteristics might be genetically controlled. We have studied the calcium oxalate crystals in the different plant parts (leaves, stems, petiole, corm, and root) of some selected species belonging to the family Araceae. The selected plants belonged to different habitats like marshy, semi aquatic, terrestrial and were mostly herbs, shrubs, and climbers. Among the selected species, two species are edible and economically important. Edibility of petiole, leaves, stems and corms depends upon the frequency and intensity of the calcium oxalate crystals. Two types of crystals were observed which were mostly species specific. The frequency of crystals is probably related with the habit, habitat and also the environmental conditions.
KEYWORDS: *Calcium Oxalate Crystals (COC), Araceae, edibility, evolutionary relationships*

INTRODUCTION

Needle shaped calcium oxalate crystals called raphides have been upon as an important indicator of aroids. The aroids are a sub-group of Araceae which is a family of mainly herbaceous plants that are known to produce raphides in abundance. When eaten raw they cause swelling of the lips, mouth & throat. Detoxification via cooking, pounding or leaching neutralizes the chemical, hence making the aroids edible but does not destroy or degrade the raphides [1,2,3,4].

Calcium oxalate crystals occur in more than 215 higher plant families, as well as the algae, lichen and fungi, in two hydration states in plants, as the monohydrate [Whewellite (CaC_2O_4, H_2O)] or dehydrate [weddellite (CaC_2O_4, $2H_2O$)][5,6].They can form in any organ or tissue within the plants, including in stems, petiole, leaves, roots & tubers, and have a variety of function including calcium storage, defense and providing structural strength [7,8]. According to Horner and Wagner 1995[6], unlike phytoliths, which vary considerably in size and shape across families, calcium oxalate crystals are generally restricted to five basic morphological types –Needle shaped raphides, rectangular or pencil shaped styloids, mace head shaped aggregates called druses, block shaped aggregates called crystal sand, variously shaped prisms.

Given that the size and appearance of these crystals can differ within and between families, and that these morphological characteristics are probably under genetic control [5]. Calcium oxalate crystals may have taxonomic potential for both botanist and taxonomist [6].

Types of raphides

Anatomical study indicates that raphide morphology and formation in specific locations within a plant are genetically controlled [6]**.** Key attributes for differentiating raphides include size, cross-section and termination morphology, all of which appear to vary to differing degrees depending on taxa of origin.

Type I Raphides

It is the most common raphide form and consists of four-sided single crystals that have two symmetrical pointed ends. (Fig.17)

Type II Raphides:

Which are also four-sided, have one pointed and one bidentate or forked end [9] This type of raphide has so far only been recorded in a few families such as the Vitaceae[10,11]. The bidentate end is formed by crystal twinning [12](Fig.17)

Type III Raphides

The third form is crystals with six to eight sides and symmetrical pointed ends. This raphide type is known to occur in the Agavaceae[13]. Typhaceae [14] and Dioscoreaceae. (Fig.17)

Type IV Raphides

The fourth raphide form comprises twinned crystals with H-shaped cross-sections and asymmetrical ends (one wedge-shaped and the other sharply pointed) [1, 15]. (Fig.17)

MATERIAL AND METHODS

Plant material

The plant specimens belonging the family Araceae. The species identification of the selected materials was determined according to standard literature. It was done in the month February to May.

Crystal Isolation and Purification

Crystals were isolated from both fresh and dry plant specimens. However, dry material was preferred to increase crystal recovery. With the purpose of avoiding potential contamination of crystalline samples by soil particles, plant stems, leaf, petiole, root, corm or storage organ were carefully washed with abundant distilled water. After removal of needles epidermis, thin sections of plant stems, leaf, petiole, root, corm or storage organ were excised and washed several times. The raphaides could be easily separated manually. Clearing technique is used to specifically locate the calcium oxalate crystals in the plant tissue. Tissue sections were macerated in water and crystals were mechanically freed with the help of dissection knives, segments were fixed in glycerine and water. After that we prepared a slide for observation. The slides were observed under light microscope (10X x 40X) [Olimpus] as well as Phase Contrast Microscope (Leica DM- 1000) for detailed analysis and obtining better picture as well as measuring the length & breadth of raphide crystal.

RESULT

At first, we observed the shape, size and position of raphides of the selected plant materials under light microscope (10X x 40X) as well as Trinocolor microscope Leica (DM-1000). Considering measurement of the size, shape and the result of the experiment. Experiment reveals that the raphides varies between species to species like druses [Fig-12], needle shaped, H-shaped. We are observed in the leaves, stem, petiole, storage organ or corm and root. Simultaneously few are small to large. The number of raphides also varied because occurring in different environmental conditions. In case of *Alocasia indica,* we are also observed the idioblast arranged in a ring [Fig-16].

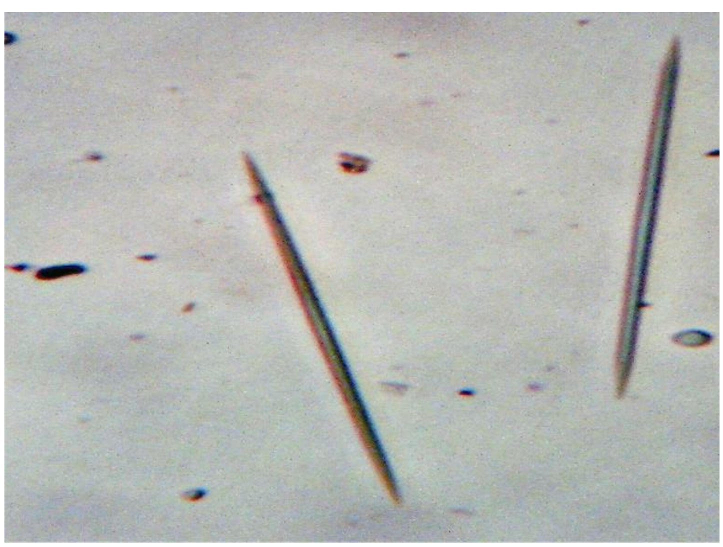

Fig.1 - Calcium oxalate crystal (COC) of *Lasia heterophylla* from petiole.

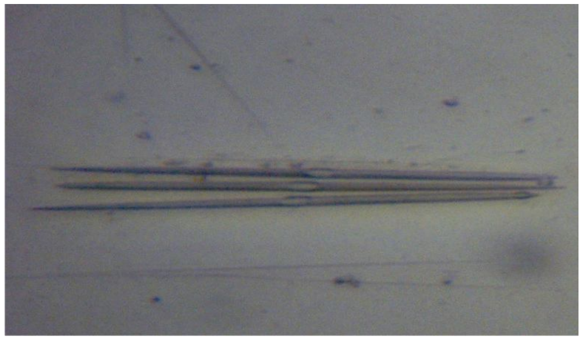

Fig.2 - calcium oxalate crystal of *Colocasia* (black) from storage organ.

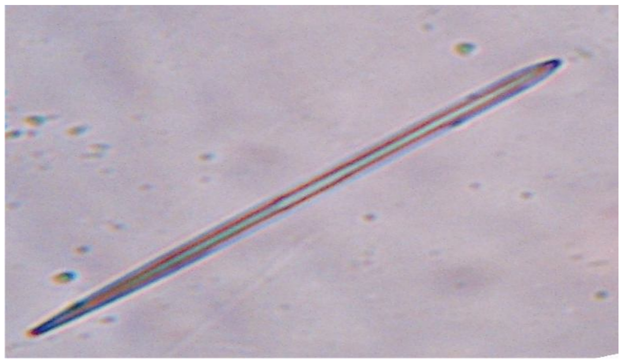

Fig.3 - calcium oxalate crystal of *Caladium schomburgkii* from root.

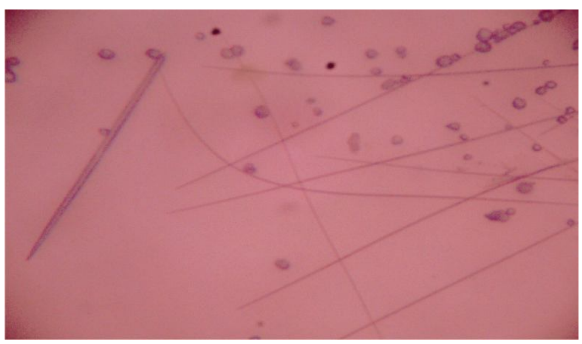

Fig.4 - calcium oxalate crystal of *Colocasia* (black spotted) from leaf.

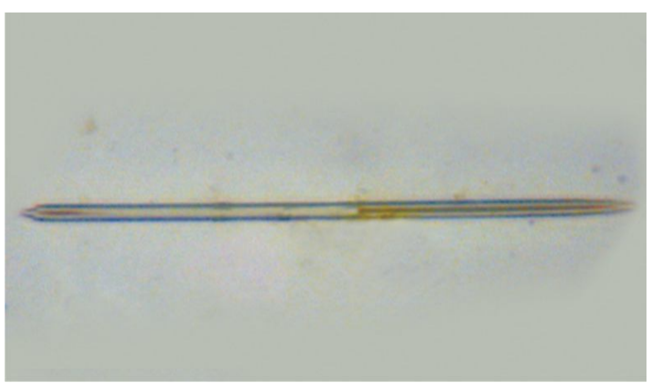

Fig.5 - calcium oxalate crystal of *Caladium schomburgkii* from root.

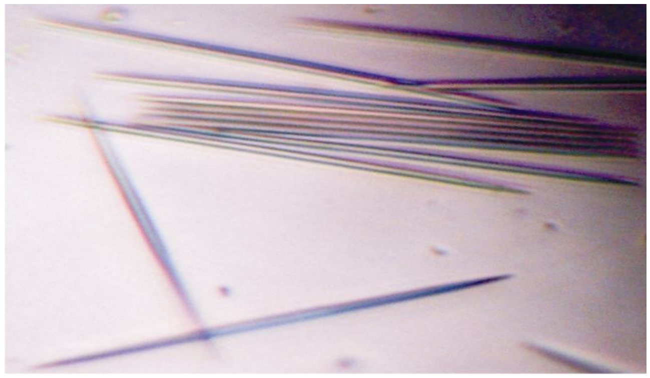

Fig.6- calcium oxalate crystal of *Lasia heterophylla* from root.

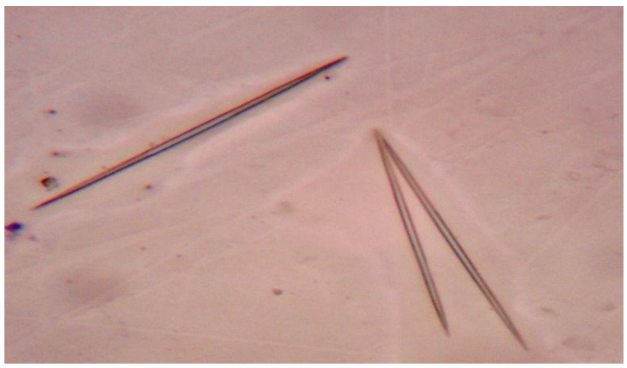

Fig.7 - calcium oxalate crystal of *Epipremnum pinnatum* from petiole.

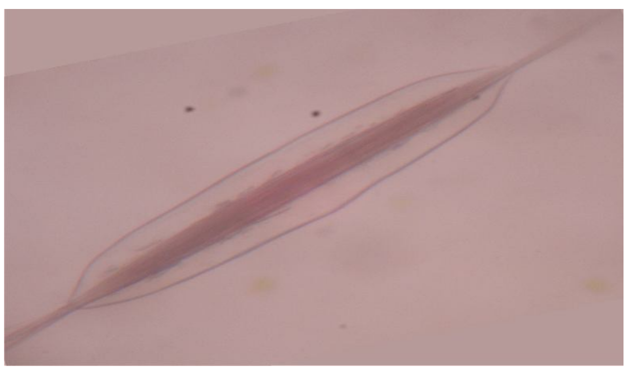

Fig.8 – Idioblast of *Colocasia* (black spotted) from leaf. COC are partially ejected through the papillae at this end.

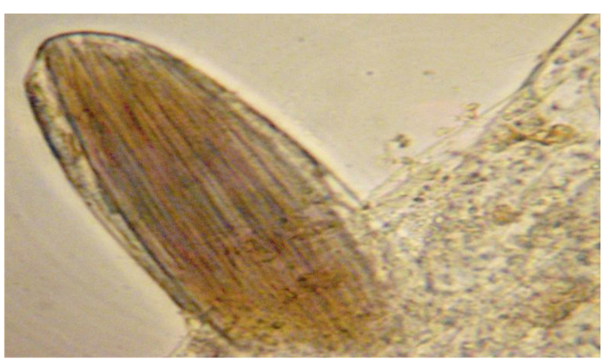

Fig.9 – Different types of idioblast of *Alocasia indica* from petiole

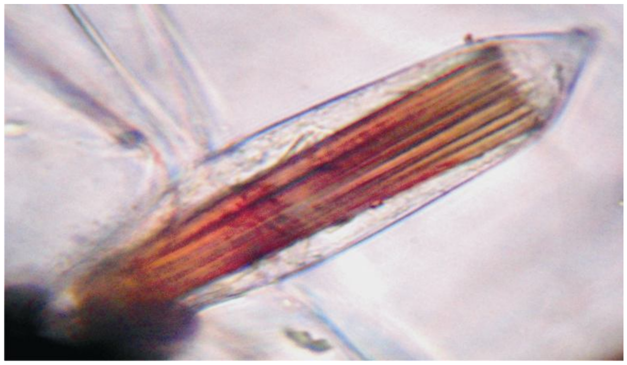

Fig.10- Different types of idioblast of *Alocasia indica* from petiole

Fig.11- Idioblast of *Colocasia* (black) from petiole.

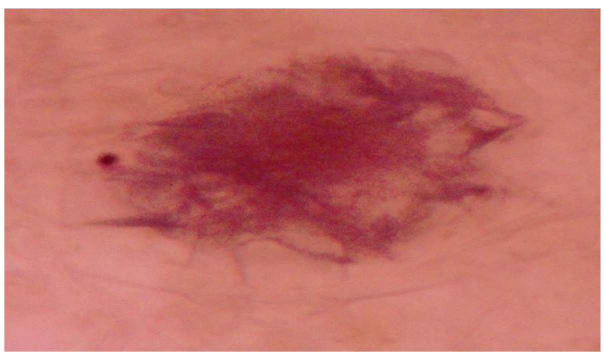

Fig.12- Druses of *Amorphophallus sylvaticus* from leaf.

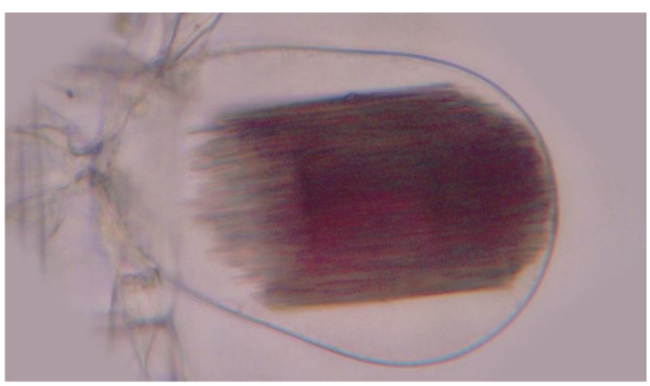

Fig.13- Idioblast of *Colocasia gigantia* from leaf.

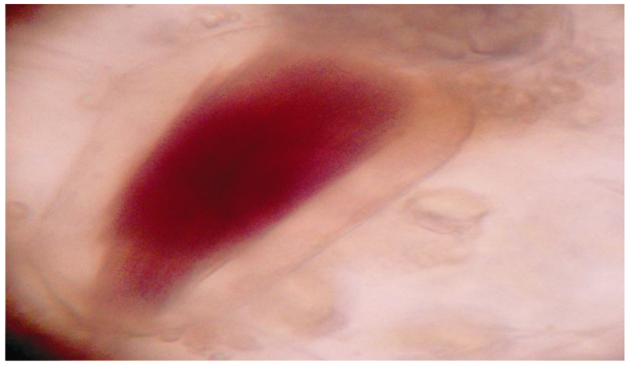

Fig.14- Idioblast of *Colocasia* (black) from leaf.

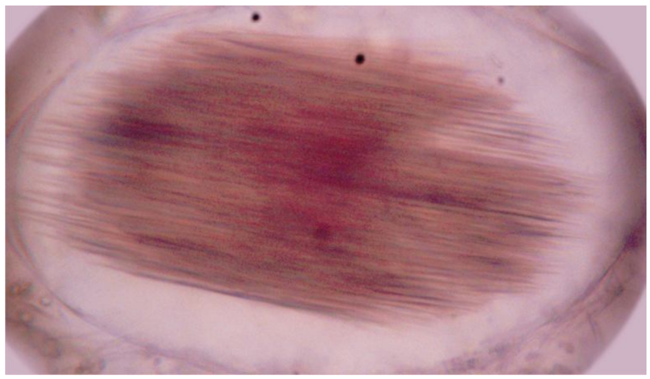

Fig.15- Idioblast of *Colocasia* (black spotted) from petiole.

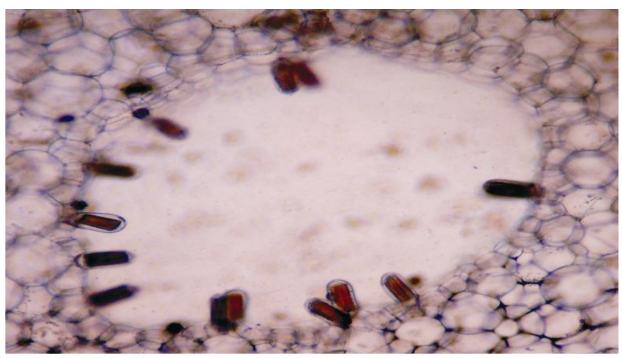

Fig.16- *Idioblast* of *Alocasia indica* arrangement in a ring from petiole

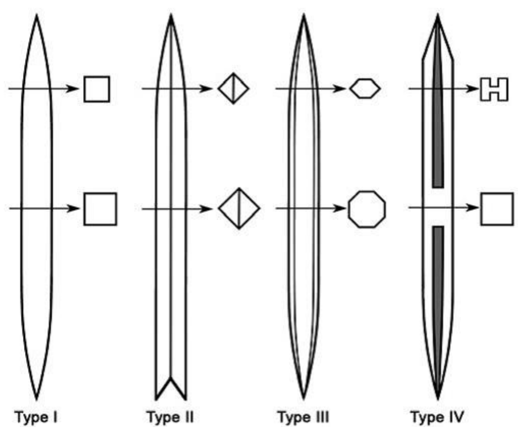

Fig.17-Diagram showing the four basic types of raphide cross sections.End forms are described in text (redrawn from Horner and Wagner 1995:58)

DISCUSSION

In case of *Colocasia esculenta* storage organ shows oblong shaped, petiole of *Colocasia gigantia* [Fig-13] *Colocasia esculenta*(black) [Fig-11], *Amorphophallus sylyaticus* shows slightly rectangular to oval shaped; petiole of *Colocasia esculenta* (black spotted) [Fig-15] shows oval shaped; petiole of *Alocasia odora* (Fig.9 – 10) leaf of *Colocasia esculenta* (black) [Fig-14] shows elliptical shaped; leaf of *Colocasia esculenta* (black spotted) [Fig-8]shows lanceolate idioblast. Our study shows, that some also possess barbs near their tips and along their edges, which occur in some other Araceae.

Type I is the most common raphides form and consist of four sided single crystals that have two symmetrical pointed ends. In our work we observed mainly that *Lasia heterophylla*[Fig-1,6], *Epipremnum pinnatum* [Fig-7] possess Type I raphides. And also petiole, root of *Diffenbachia picta;* leaf, petiole, root of *Dracontium nivosum*, storage of *Colocasia gigantia* possess Type I raphides.

Leaf, length of raphides is longer in *Lasia heterophylla* (154.332 µm), shorter in *Dracontium nivosum* (13.799 µm). Breadth of raphides is greater in *Lasia heterophylla* (4.499 µm), smaller in *Dracontium nivosum* (0.617 µm).

Petiole, length of raphides is longer in *Lasia heterophylla* (284.975 µm), shorter in *Diffenbachia picta* (21.927 µm). Breadth of raphides is greater in *Lasia heterophylla* (8.727 µm), smaller in *Dracontium nivosum* (0.981 µm)

Storage organ or corm, length of raphides is longer in *Lasia heterophylla* (89.959 µm), shorter in *Colocasia gigantia* (43.474 µm). Breadth of raphides is greater in *Lasia heterophylla* (3.464 µm), smaller in *Colocasia gigantia* (1.509µm).

Root, length of raphides is longer in *Diffenbachia picta* (84.629 µm), shorter in *Lasia heterophylla* (16.350 µm). Breadth of raphides is greater in *Lasia heterophylla* (4.493 µm), smaller in *Diffenbachia picta* (1.573 µm).

Our observation revealed that leaf, petiole, storage organ or corm, root of *Colocasia esculenta*, *Colocasia nymphaeifolia*, *Caladium schomburgkii*[Fig-3,5], *Alocasia indica, Alocasia odora, Amorphophallus campanulatus, Amorphophallus sylvaticus, Colocasia esculenta* (black spotted) [Fig-4], *Colocasia esculenta* (black) [Fig-2,], shows H- shaped raphides. And also the leaf, petiole, root of, leaf of *Diffenbachia picta,* storage organ of *Dracontium nivosum* shows H- shaped raphides

Leaf, length of raphides is longer in *Amorphophallus campanulatus* (250.957µm), shorter in *Diffenbachia picta* (17.992 µm). Breadth of raphides is greater in *Amorphophallus campanulatus* (6.116 µm), smaller in *Colocasia esculenta* (black spotted) (0.401 µm).

Petiole, length of raphides is longer in *Amorphophallus campanulatus* (327.892 µm), shorter in *Caladium schomburgkii* (21.08 µm). Breadth of raphides is greater in *Amorphophallus sylvaticus* (8.596 µm), smaller in *Colocasia esculenta* (black spotted) (0.309 µm).

Storage organ or corm, length of raphides is longer in *Amorphophallus campanulatus* (210.554 µm), shorter in *Colocasia nymphaeifolia* (38.445µm). Breadth of raphides is greater in *Amorphophallus campanulatus* (5.533 µm), smaller in *Colocasia esculenta* black spotted (0.436 µm).

Root, length of raphides is longer in *Colocasia esculenta* (black spotted) (209.677 µm), shorter in *Colocasia esculenta* (14.466 µm). Breadth of raphides is greater in *Alocasia indica* (3.491 µm), smaller *in Colocasia* (black spotted) (0.310 µm).

From the above discussion we can draw the following inference that Type I and Type IV (H -shaped), both types of raphides present in the family Araceae and therefore it offer great potential for taxonomic marker. Shape, size of idioblast will be taken in consideration for taxonomic marker.

CONCLUSION

The formation of crystals idioblast is a complex process involving changes in development of cells and the formation of specific raphides structure. The presence or absence of crystals is an important character of understanding the evolutionary relationships of plant species. The specific distribution and shape of the raphides of this angiosperms are consistent the character of calcium crystals may be genus and species specific. The observation of Calcium oxalate crystals occur in 14 species of Araceae, revealed that the occurrence and shape of crystals can be useful in the intraspecific level of phylogenetic relationship.

Table: 1 Measurement of Different Types of Calcium Oxalate Crystals of Some Selected Plant Taxa (14) Including Different Plant Parts

SL. NO.	SCIENTIFIC NAME OF THE PLANT	LEAF		PETIOLE		STORAGE ORGAN OR CORM		ROOT	
		L (µm).	B(µm)	L(µm)	B(µm)	L(µm)	B(µm)	L(µm)	B(µm)
1	*Colocasia esculanta* (Linn.) Schott.	32.785 31.732 30.809	2.182 1.753 2.350	40.683 44.965 43.200	3.147 2.182 2.618	89.459 89.900 82.130	2.182 2.794 2.544	14.466 24.877 29.418	0.614 0.617 1.851
2	Alocasia *indica* (Roxb.) Schott.	80.419 90.704 91.092	1.301 1.109 1.002	60.093 103.82 56.778	1.799 1.573 2.760	103.264 101.290 101.050	4.023 3.728 4.556	26.874 31.178 27.259	1.745 3.491 2.380
3	*Alocasia odora* (Roxb.) K.Koch	88.470 90.744 121.090	1.380 1.309 1.809	57.938 57.045 55.544	1.573 1.254 0.967	86.880 46.089 55.081	2.618 2.544 2.045	37.725 32.913 41.234	1.234 2.618 1.799
4	*Caladium schomburgkii* Schott.	106.130 34.580 33.747	2.225 3.491 3.480	37.661 26.036 21.081	0.873 0.783 0.801	72.861 72.625 65.169	1.951 1.234 1.951	28.162 29.279 16.198	0.783 0.873 0.436
5	*Colocasia gigantia* Hook.f.	32.220 39.032 40.009	2.102 1.703 2.596	38.630 36.878 33.450	1.799 1.851 1.309	54.504 60.618 43.474	1.509 2.225 1.573	23.921 21.927 22.463	0.463 0.436 0.489
6	*Colocasia nymphaeifolia* Kunth.	81.470 77.744 89.090	1.307 0.989 1.333	72.226 57.602 74.179	0.436 0.436 0.617	128.398 68.881 38.445	2.205 1.005 0.873	39.333 48.444 21.818	1.309 1.309 0.873
7	*Epipremnum pinnatum* (L.)Engl.	65.571 68.746 83.609	2.350 2.760 4.140	58.456 65.549 34.602	1.380 1.799 1.309	N.S.O.F.	N.S.O.F.	24.534 30.483 64.800	2.182 1.309 1.851

<table>
<tr><td>8</td><td>Diffenbachia picta
Schott.</td><td>17.992
28.919
20.676</td><td>1.796
1.380
1.436</td><td>21.927
76.978
72.646</td><td>2.182
3.491
2.760</td><td>N.S.O.F.</td><td>N.S.O.F.</td><td>73.637
59.193
84.629</td><td>1.573
1.573
1.851</td></tr>
<tr><td>9</td><td>Lasia heterophylla
(Roxb.) Schott</td><td>150.393
154.332
132.369</td><td>4.499
3.995
3.111</td><td>269.909
284.975
250.957</td><td>8.727
6.293
4.329</td><td>89.654
89.959
83.919</td><td>2.654
2.670
3.464</td><td>56.460
26.650
16.350</td><td>4.493
3.001
3.007</td></tr>
<tr><td>10</td><td>Dracontium nivosum
(Lem.) GHZhu.</td><td>21.422
17.068
13.799</td><td>0.617
1.234
0.617</td><td>35.492
42.954
28.299</td><td>1.056
1.987
0.981</td><td>53.260
41.397
42.549</td><td>2.618
2.350
1.380</td><td>21.059
29.551
21.659</td><td>2.225
1.745
1.989</td></tr>
<tr><td>11</td><td>Colocasia esculenta (black spotted)
(Linn.) Schott</td><td>208.191
212.311
146.811</td><td>0.436
0.401
2.621</td><td>170.812
156.941
173.811</td><td>0.309
0.399
1.451</td><td>173.733
183.331
135.855</td><td>0.436
0.556
0.656</td><td>209.677
147.081
94.277</td><td>0.309
0.331
0.301</td></tr>
<tr><td>12</td><td>Amorphophallus sylvaticus
(Roxb.) Kunth</td><td>216.466
215.654
213.877</td><td>5.433
5.443
5.212</td><td>326.764
322.111
326.909</td><td>6.502
7.702
8.596</td><td>91.415
87.012
91.977</td><td>0.617
0.412
0.654</td><td>41.717
43.252
41.601</td><td>1.245
0.481
0.412</td></tr>
<tr><td>13</td><td>Amorphophallus campanulatus
Blume.ex.Decne</td><td>226.171
216.466
250.957</td><td>5.656
5.343
6.116</td><td>327.892
327.515
326.223</td><td>8.391
7.108
7.002</td><td>208.621
210.554
197.616</td><td>5.533
5.022
4.652</td><td>51.671
57.301
54.112</td><td>1.231
1.212
1.454</td></tr>
<tr><td>14</td><td>Colocasia esculenta (black)
(Linn.) Schott</td><td>112.811
109.292
135.294</td><td>4.171
3.833
4.022</td><td>141.599
142.499
139.884</td><td>1.201
0.651
1.841</td><td>136.599
148.701
153.632</td><td>4.982
4.696
4.401</td><td>43.584
44.277
39.011</td><td>0.921
1.051
0.720</td></tr>
</table>

[L= Length; B=Breath]; [N.S.O.F.- No Storage Organ Found]

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