Seed Surface Micro Morphological Features of the Holoparasitic Angiosperm Aeginetia spp. (Orobanchaceae) in South India

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The seed surface features of three Indian species of the genus Aeginetia (Orobanchaceae) was studied with the help of light microscopy and Scanning Electron Microscopy to understand the comparative morphology of the seed coat surface, and its utilization as a source of taxonomic evidence at the species level. The seeds of the three taxa show marked differences in their size, shape and surface ornamentation. Although Aeginetia pedunculata and A. sessilis show resemblances in their morphological and floral characters but differ in their seed coat sculpture. In A. indica the cells of the seed coat are alveolar and look like empty bunch of baskets with ribbed to scalariform thickenings on their lateral walls. In A. pedunculata outer tangential walls are retained but for a central ovoid depression or pore. In A. sessilis, however, the cells of the seed coat surface have a shallow depression. The micropylar end of the seed is smooth surfaced without any ornamentation. The comparative morphology of the seed coat surface in these three species of Aeginetia is discussed and its use as a source of taxonomic evidence is presented.

Keywords: Aeginetia spp.; Orobanchaceae; Root Parasite; SEM Studies; Seed Coat Morphology

Introduction

Scanning Electron Microscopic studies on the seed coat morphology of Angiosperms have contributed significantly to the systematics of flowering plants. The SEM studies on seed coat surface of parasitic flowering plants are relatively scanty. Chuang and Heckard (1972) described four types of seed coat ornamentation in Cordylanthus. Musselman and Mann (1976) studied the seed surface characteristics of 23 species of Scrophulariaceae and two species of Orobanchaceae. Olsen and Olsen (1980) while studying the seed coat morphology of Boschniakia hookeri concluded that the seed surface structure probably helps in the maintenance of early phase of host root/parasite connection. Based on seed coat morphology, Chuang and Heckard (1983) distinguished different species and infrageneric groups in Orthocarpus. The Scanning Electron Microscopic study of seed coat morphology in parasitic plants like Agalinis by Canne (1979), surface features of Striga seeds by Muselman and Parker (1981), seed coat ornamentation in Striga hermonthica by Jones and Safa (1982) and on Orobanche by Plaza et al. (2004) are said to be of taxonomic significance. Heywood (1969) highlighted the importance of seed characters in detailed taxonomic studies.

The genus Aeginetia L. (Orobanchaceae) comprises 10 Indomalayan species (Airy Shaw 1973). Three species viz., A. indica L., A. pedunculata Wall. and A. sessilis Shiva & Raja are reported from India (Vijay 2007). All are leafless annual herbaceous holoparasitic Angiosperms. In recent years A. sessilis was reported as a new taxon from Kerekatte, Kuduremukha ranges of Western Ghats (Shivamurthy & Rajanna 1994). Morphologically A. indica is clearly distinct from the other two taxa while there are some morphological resemblances between A. pedunculata and A. sessilis. There was no information on the seed

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coat morphology of these three species at the Scanning Electron Microscope level and hence the present study.

**Materials and Methods**

Seeds of the three species of *Aeginetia* were collected from the grass lands and forests near Kerekatte of Kuduremukha ranges of Western Ghats during September-October 2005-2007. Ripe fruits were separated from the plants and shade dried. Seeds were separated manually. For Scanning Electron Microscopic observation, mature seeds were dried to a critical point in a biorad CPD 750 apparatus. Seeds were then mounted on specimen stubs with a double adhesive tape and coated with gold in an Edwards S150B sputter coater. Examination and photomicrography of the seeds were done by using a LEO 435VP Scanning Electron Microscope at 20kV.

**Results**

The seeds of *A. indica* are ovoid (Fig. 1B), yellowish and measure 232-279µm in length and 155-187µm in diameter. The integument transforms into the seed coat and their cells are polygonal in outline. The radial and inner tangential walls of these cells gradually undergo thickening due to the deposition of additional cell wall materials to produce rib like anastomosing bands (Fig. 1B). During maturity the outer peripheral (surface) walls and the protoplasmic contents of these cells gradually disintegrate completely and a ‘deep well’ like depression can be seen. The rim of each of these cells is highly thickened due to additional cell wall materials. Under the electron microscope the mature seed coat surface appears reticulate and the whole seed looks like a bunch of polygonal baskets held firmly by the ridges of the polygonal cell walls (Fig. 1B).

In *A. pedunculata* (Fig. 1C) the seeds are brown and somewhat top shaped with the micropylar end more pointed. The seed size ranges from 274-356µm in length and 198-232µm in diameter. Seed coat surface cells at the micropylar end are smaller and become somewhat narrower at the lower end (Fig. 1D). The surface of the seed coat shows reticulate pattern with the raised ridges. Unlike in *A. indica*, the outer tangential walls of each of the cells of the seed coat are found to be somewhat hard, as a result of which they remain intact in a mature seed except at the centre where it disintegrates to create an ovoid deep perforation. The outer rim of each of the cells is raised and forms distinct hexagonal to pentagonal ridge. All along the rim of the tangential walls minute punctae are noticed (Fig. 1D).

The seeds of *A. sessilis* (Fig. 1F) are obovate, brown to black and measure from 247-298µm in length and 174-206µm in diameter. The cells of the seed coat are polygonal, highly thickened and the seed coat show reticulate pattern on the outer surface. The boundaries of the reticulum are slightly raised. Some of the outer tangential walls on the surface of the seed coat cells are intact where as in others they show a round to oval shaped shallow depression (Fig. 1F). Punctae are scattered on the ridge of the cell walls. At the micropylar end of the seed, there is a smooth surfaced collar without any ornamentation (Table 1).

**Discussion**

In *Aeginetia* the seed coat develops from the cells of the integument. Due to the continued deposition of additional cell wall materials, the cells of the seed coat acquire various patterns. In *A. indica*, the outer surface walls of the seed coat surface collapse

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Seed surface features</th>
<th><em>Aeginetia indica</em> L.(L x Dia)</th>
<th><em>Aeginetia pedunculata</em> Wall.</th>
<th><em>Aeginetia sessilis</em> Shiva &amp; Raja</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Size of the seed</td>
<td>232-279 µm x 155-187 µm</td>
<td>274-356 µm x 198-232 µm</td>
<td>247-298 µm x 174-206 µm</td>
</tr>
<tr>
<td>2</td>
<td>Shape and colour of</td>
<td>Ovoid &amp; yellowish</td>
<td>Top shaped &amp; brown</td>
<td>Obovate &amp; brown</td>
</tr>
<tr>
<td></td>
<td>the mature seed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Seed coat surface</td>
<td>Reticulate &amp; polygonal</td>
<td>Reticulate with ridges raised</td>
<td>Reticulate &amp; boundaries of reticulum are slightly raised</td>
</tr>
<tr>
<td>4</td>
<td>Seed coat cells</td>
<td>Alveolar &amp; look like empty bunch of baskets</td>
<td>Aereolate with reticulate network</td>
<td>Polygonal, edges with slightly raised ridges</td>
</tr>
</tbody>
</table>
SEM Studies of Aeginetia Seeds

Fig. 1: A. *Aeginetia indica*; B. SEM of seed showing spiral thickenings on radial wall and reticulate thickenings on inner tangential wall of cells of seed coat; C. *Aeginetia pedunculata*; D. SEM of seed showing reticulate (R) nature of seed coat. Each cell of the seed coat is hexagonal in outline with raised border and deeper central aperture (P); E. *Aeginetia sessilis*; F. SEM of seed showing the reticulation (R) on the seed coat, prominent collar (C) at the micropylar end and shallow well-like depression in the center of each polygonal cell.
completely and disappear at maturity of seeds. On the other hand in *A. pedunculata* the outer surface walls of seed coat are retained but for a central ovoid deep depression or pore, while in *A. sessilis* the cells of the seed coat surface are provided with a shallow depression.

The seeds of the three taxa of the present study show marked differences in their size, shape and sculpture of the seed coat and this could be used to distinguish one from the other. The study also showed that the seeds of *A. pedunculata* are larger than *A. sessilis*. In *A. pedunculata* and *A. sessilis*, the plants look morphologically similar except for lack of peduncle and just one (rarely up to 3) flower/s in the latter. In *A. sessilis* the cells of the seed coat on the surface especially the polygonal edges of the reticulum have slightly raised ridges. The depression in the centre of the outer tangential walls of the cells is shallow. In *A. indica* the cells of the seed coat are alveolar and look like empty bunch of baskets with ribbed to scalariform thickenings on their lateral walls. Such a seed coat has also been observed in *Boschniakia hookeri* (Olsen & Olsen 1980) and called it as alveolar. This alveolar seed coat helps in dispersal by water and also in anchorage of the host roots.

Based on the SEM studies of the seed coat of *Cordylanthus*, Chuang and Heckard (1972) described four types of ornamentations namely irregularly crested, deeply reticulate, shallowly reticulate and irregularly striate. However in *A. pedunculata* the surface of the seed coat was found to be aereolate with reticulate network. Study carried out by Musselman and Parker (1981) in nine species of *Striga* revealed that all species have aereolate surfaces with prominent ridges. They concluded that surface features of seeds are of some taxonomic value in certain species complexes. However, Jones and Safa (1982) contradicted this with their studies on *Striga hermonthica* and stated that ornamentation on seeds from one species were constant but varied within and between populations probably due to out breeding. Rauh et al. (1975) opined that the pitted or chambered seed surfaces are common features in small seeds, which are dispersed by wind, typical of many Scrophulariaceae, Orobanchaceae and a number of other families and this finding which is also noticed in *Aeginetia* species of the present study.

Micromorphological studies on the seeds of *A. indica* by Anuradha and Kumbhojkar (1996) also showed that seed coat ornamentation is of systematic importance. Since the seeds are relatively stable in external morphology, the Scanning Electron Microscopic study on seed coat morphology provides effective and simple data to distinguish taxa which are morphologically alike.

The present study helped in proving conclusively that *A. pedunculata* and *A. sessilis*, even though show many morphological resemblances, are distinct taxa based on their micro morphological features and they also differ from *A. indica*.

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