Application of atomic force microscopy in seed surface studies

Atomic force microscopy (AFM) is a valuable tool for studying physical and biological structures. Since the initial reports of Binnig et al., considerable progress has been made, but applications in the field of biology are scanty. Bustamente and Keller reviewed the possibilities of exploring biological structures under conditions in which living organisms exist. AFM is a kind of scanning probe microscope where imaging of the sample is realized by interaction of the probe with the sample surface and no imaging beam (light or electron) is involved in the process. The tip of the probe is mounted on the end of a flexible cantilever. As the sample is scanned beneath the tip, small forces of interaction with the sample cause the cantilever to deflect, revealing the sample’s topography. The most common approach, called an optical lever approach, is to reflect a laser beam off the backside of the cantilever into a segmented photodetector. The photodetector generates a measurable signal voltage to make the image.

Internet search reveals that no plant materials (including seeds) have so far been studied with the help of this new tool. We therefore attempted AFM imaging of mung bean seeds, a valuable commodity in the food market of India.

Seeds (mung bean) were collected from various local geographical sites with and without the seed coats in connection with a project by these authors.

We approached with the rationale that of the three modes of AFM operation, contact, non-contact and tapping, the first would be most suitable for the hard surface. As this mode is also likely to offer the best resolution, we tried to scan the surface of the seed coat and the cotyledon surface of different strains of mung bean. (In this microscopy no sample preparation is necessary, the image of the ‘native’ seed is captured.) The cantilever was of the 100 µm wide-legged, triangular type in which an optical lever reflects a laser beam off the backside of the cantilever. The forces selected in this mode of imaging are the capillary force and atomic repulsion force between the tip and the sample surface. The microscope (Digital Instruments) was a standard top view contact AFM, the NanoScope ESPM system. The image was simultaneously captured and displayed from the native seed surface at 512 × 512 resolution. The image acquired was stored in the system computer hard disk.

Figure 1a and b shows the cotyledon surface of two strains of mung bean (from Malda and Lalagola in West Bengal, respectively) at the same magnification (scan size). The differences in the strains reflect the different genomic imprint at this ultrastructural level. SEM micrographs even at the highest magnification do not reveal some of these features. Figure 2, the highest magnification that we obtained, shows 5–6 structural units (of the seed coat) within 20 Å (2 nm). Thus each unit size is ~3–4 Å.

A large number of mung bean varieties are found in India and a few of them are endowed with unusually good aroma. A project on the aroma molecules of mung bean by Brahmachary and Ghosh (unpub-

Figure 1. Cotyledon surface: a, Malda strain; b, Lalagola strain.

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Heneicosane: An oviposition-attractant pheromone of larval origin in *Aedes aegypti* mosquito

Oviposition aggregation pheromone can specifically influence many insect females to lay eggs in the same site resulting in more eggs deposition. The first unequivocal evidence for an oviposition pheromone occurrence in an insect vector mosquito was in *Culex*. However, studies on the influence of eggs of conspecific and heterospecific larval stages on the site selection by various *Aedes* species have given conflicting results. Surprisingly, in *Anopheles* mosquito the presence of conspecifics may actually be a deterrent.

*Aedes aegypti* prefers to oviposit on water containing the larvae of the same species. This larval conditioned water (LCW) is found to be effective after removing the larvae by filtration and the attractant activity is retained for several weeks. Many groups earlier tried to identify the oviposition-attractant factors present in the LCW, but the extremely small amount released by the larvae thwarted its characterization. Here we report the chemical primarily responsible for the oviposition activity of the LCW using gas chromatography coupled with mass spectrometry (GC/MS) followed by biological evaluation in the laboratory.

For these studies, water used for rearing *A. aegypti* larvae only for twenty days continuously was taken after filtration as the LCW. We extracted this LCW with hexane and ether (HPLC grade) sequentially, combined the extracts, concentrated and analysed by GC/MS. Similarly control water was extracted for comparison (blank). GC/MS analyses were performed and identified the compounds listed in Table 1.

**Table 1.** Fragmentation pattern of the additional components in LCW

<table>
<thead>
<tr>
<th>Peak retention (min)</th>
<th>Compounds identified</th>
<th>MW</th>
<th>Fragmentations</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.25</td>
<td>Octadecane</td>
<td>254</td>
<td>254 (M+), 57 (100), 71, 85, 99</td>
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<tr>
<td>17.63</td>
<td>Isopropyl myristate</td>
<td>270</td>
<td>270 (M+), 43 (100), 228, 102, 60</td>
</tr>
<tr>
<td>20.20</td>
<td>Heneicosane</td>
<td>296</td>
<td>296 (M+), 57 (100), 71, 85, 99</td>
</tr>
<tr>
<td>21.10</td>
<td>Docosane</td>
<td>310</td>
<td>310 (M+), 57 (100), 71, 85, 99</td>
</tr>
<tr>
<td>26.82</td>
<td>Nonacosane</td>
<td>408</td>
<td>408 (M+), 57 (100), 71, 85, 99</td>
</tr>
</tbody>
</table>

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