Atomic Force Microscopic Observation of Early Maturing Barley Aleurone Cell Surface

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Objectives

Here we demonstrate that non-contact mode AFM leads high resolution topographical images of membrane proteins and channel proteins in barley aleurone cells. Also, we examined the aleurone thickness during the seed development stages.

Materials and Methods

○ Scanning electron microscopy

Isolated seed samples were washed 3 times with 0.05 M cacodylate buffer for 10 min after fixed in Karnovsky's solution at room temperature. These samples postfixed in 1% osmic acid for 2 hr at 4℃ and then followed by a wash for 10 min in 0.05 M cacodylate buffer. The samples were dehydrated in a series of ethanol solution(50-100%) and critical point dried(HCP-2, Hitachi) had used with liquid CO_2. Isoamyl acetate was used as the intermediate fluid. After the dried samples were longitudinally sectioned with blade, it were directly mounted on circular aluminum stubs with double-sided sticky tape, coated for 2 min with Au-Pd ion coater (E-1010, Hitachi), then examined and photographed in a Hitachi scanning electron microscope (S-3500N, Hitachi) at an accelerating voltage of 15 kv.

○ Atomic force microscopy

For AFM observation, the barley grain outer and inner glume were removed with razor blade and forceps (Fig.1.).

Dehulled seed was placed on a G scanner (XE 150 mode PSIA, Korea)(Fig. 2.), and then scanned in the non-contact mode and recorded as the topographical images. The scanning area was limited to 1x1 μm^2 to 10×10 μm^2 and scan speed was on 0.5 Hz. Images were composed of 256×256 pixels. The operating voltage was set as low as possible to avoid damaging the samples.

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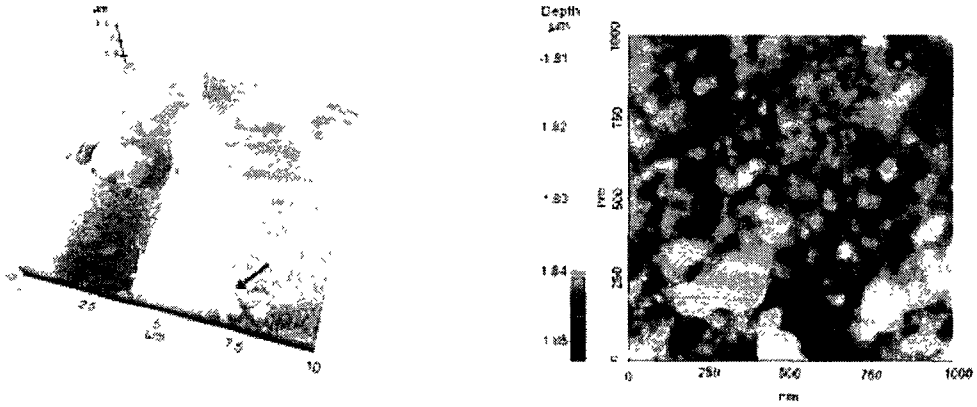
Results and Discussion

To observe and analysis ultra-microscopically barley aleurone cell surface, atomic force microscope(AFM) was used. Seed coat of early maturing germplasm, eam9, was dehulled and scanned by non-contact mode. We have obtained the high resolution topographic 3-dimensional image of barley aleurone layer with high resolution. These images showed the membrane proteins in barley aleurone cell. One channel protein and numerous peripheral or integral proteins were detected in a area of 100 μm^2. Furthermore, we found that their widths were ranged from 50 nm to 750 nm and lengths from 0 to 66 μm. The thickness of aleurone layer could not be measured by scanning electro-microscope. The thickness at early developmental stage was about 16 μm and then the aleurone cell enlarged upto 57 μm at least until 42 days after anthesis. In this study, we firstly reported on the ultrastructural AFM analysis of living aleurone cell as a biological specimen.

It was clearly suggested that AFM will become an powerful tool for probing both the structural properties of biological samples.



(A) (B)

Fig. 1. Three-dimensional (A) and 2-dimensional (B) images of barley aleurone layer using non-contact mode AFM (A, 10×10 μm^2) and topographic image(B, 1×1 μm^2).

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