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The Study of Maize Epidermal Replica by Oblique Illumination Microscopy

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Surface replication is one of the commonly used methods in the study of plant surfaces; since surface replicating technique allows multiple observations from a living specimen at various developmental stages, the method thus frequently used in the study of the development of plant surfaces. Nitrocellulose based nail enamel, silicon rubber and methyl methacrylate (PolySciences**,** 23679-1) resin are among the most commonly used materials for making the surface replica. All these materials produce flexible, clear, transparent films, therefore, it can be studied by phase contrast or DIC microscopy. Because of the present of surface topography replicated from the specimen, it is also possible to obtain excellent images by using oblique illuminating method [1].

In the study of cell type, shape and size along a developing maize stem (*Zea mays* L., Ohio43/KYS, field grown material) [2], epidermal replicas were obtained by applying a thin layer of clear nail enamel (New York Color^{TM} 138B)[5] coating on the surface of maize stem and leaf sheath. After allowing the nail enamel to dry, the coating was carefully peeled off from the surface of the specimen and floated on a drop of water on a glass slide. The slide was then heated slightly to flatten the replica and then the water was carefully withdrawn. It is important to float the replica with its cell-replicating surface facing away from the microscope slide. Optical microscopy was performed on an Olympus BX51 upright microscope equipped with an Edge dynamic oblique illumination condenser [1, 3] and an Olympus DP11 digital camera. Figures 1, 2, 3 and 4 show replica images obtained from maize stem surface (c-d region) by oblique illumination at 0^o, 90^o, 180^o and 270^o respectively. In comparing with conventional trans-illuminated wild-field image (Fig 5), the oblique illumination provides significantly better contrast. As a reference, Fig. 7 shows the surface and longitudinal section of a maize stem; the nodal region is subdivided into a-b, b-c, c-d regions. Region above “a” bears a surface typical of leaf sheath while region below “d” consists of a surface typical of internodes (Fig. 8). Our microscopy results show complex variations in the arrangement and distribution of cell types from internodes to node to leaf sheath (Fig. 8). The epidermal cells on the surface of internodes are aligned in files with rows of stomata. When approaching to the nodal region (Fig. 8, d-b region), the stomata (S) become less organized and intermixed with other epidermal cells (c-d region, Fig. 4), then at the b-c region, stomata is completely absent. The a-b region develops significant number of hair cells, otherwise, the arrangement of epidermal cells remain similar to the b-c region. Above the “a” level, the arrangement of epidermal cells is basically the type found on leaf sheath, with long files of silica cells (Si) demarcating the position of the vascular bundles (Fig 6) [4]. Stomata are arranged in between the files of silica cells. In summary, our result has demonstrate the use of oblique illumination for the observation of large area plastic replica obtained from plant surface.

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[5] New York Color™ 138B nail enamel consists of isopropyl alcohol, butyl acetate, ethyl acetate, nitrocellulose, tosylamide/formaldehyde resin, n-butyl alcohol, dibutyl phphalate, camphor, acrylate copolymer, and benzophenone-1.

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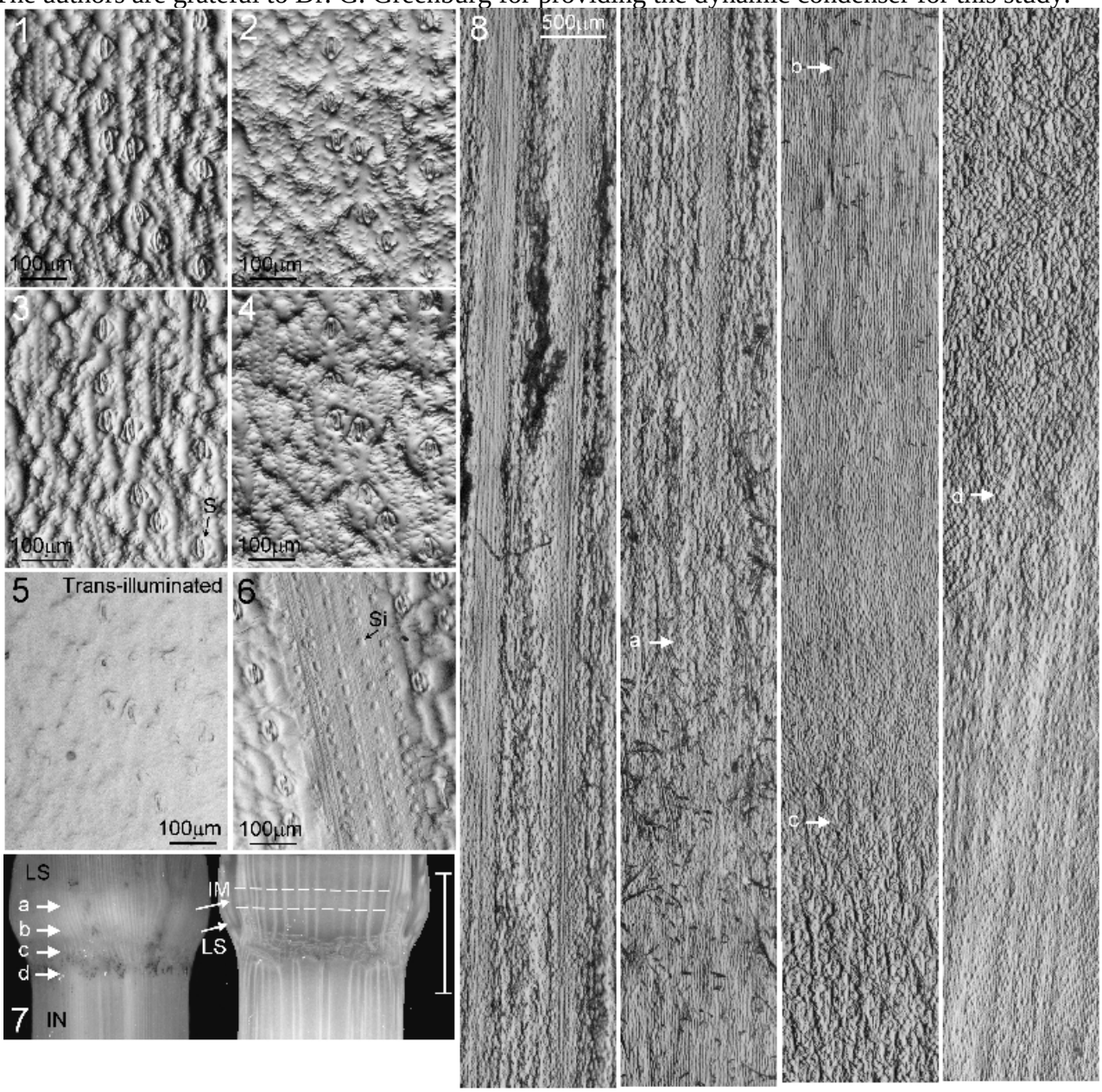

FIG 1, 2, 3 and 4. Four epidermal replica as viewed by oblique illumination from 0^o, 90^o, 180^o and 270^o respectively.

FIG 5: Conventional trans-illuminated image of the same specimen as shown in Fig 1-4.

FIG 6. Replica of the abaxial surface of leaf sheath showing files ofsilica cells (Si).

FIG 7.Surface view and longitudinal section of a maize (Oh43/KYS) stem showing the nodal region. White arrows indicate the approximate positions corresponding to the labels in Fig 8. Two white dotted lines indicate the position of intercalary meristem (IM) of stem. LS: leaf sheath; IN: internodes. The vertical white bar indicates the approximate range of Fig 8.

FIG 8. Four image panels were obtained from a continues strip of epidermal replica showing the variation of cell types and arrangement from leaf sheath (top of left panel) to internodes (bottom of right panel). Labeled white arrows corresponding to the positions shown in Fig. 7.

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