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Research article

Micromorphology and development of the epicuticular structure on the epidermal cell of ginseng leaves

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ABSTRACT

Background: A leaf cuticle has different structures and functions as a barrier to water loss and as protection from various environmental stressors.
Methods: Leaves of *Panax ginseng* were examined by scanning electron microscopy and transmission electron microscopy to investigate the characteristics and development of the epicuticular structure.
Results: Along the epidermal wall surface, the uniformly protuberant fine structure was on the adaxial surface of the cuticle. This epicuticular structure was highly wrinkled and radially extended to the marginal region of epidermal cells. The cuticle at the protuberant positions maintained the same thickness. The density of the wall matrix under the structures was also similar to that of the other wall region. By contrast, none of this structure was distributed on the abaxial surface, except in the region of the stoma. During the early developmental phase of the epicuticular structure, small vesicles appeared on wall–cuticle interface in the peripheral wall of epidermal cells. Some electron-opaque vesicles adjacent to the cuticle were fused and formed the cuticle layer, whereas electron-translucent vesicles contacted each other and progressively increased in size within the epidermal wall.
Conclusion: The outwardly projected cuticle and epidermal cell wall (i.e., an epicuticular wrinkle) acts as a major barrier to block out sunlight in ginseng leaves. The small vesicles in the peripheral region of epidermal cells may suppress the cuticle and parts of epidermal wall, push it upward, and consequently contribute to the formation of the epicuticular structure.

1. Introduction

The cuticle is an extracellular hydrophobic layer consisting of cutin and wax. It is deposited on the outer surface of epidermal cells of all land plants and has a primary role in water regulation, self-cleaning behavior, light reflection at the cuticle interface, and protection from biotic and abiotic stressors such as herbivores, pathogens, UV-B radiation, and high temperature [1–6]. A possible role for the cuticle as a suppressor of organ fusion early in organogenesis, and a connection between the cuticle and fertility have been described [7]. Various studies of the cuticle with diverse morphological structures and chemical compositions have been reported in different species [8–14]. Onoda et al [15] revealed that the leaf cuticle varies for > 100 times across species, and that a thicker cuticle is more resistant to tearing. They concluded that the thickness of the cuticle probably affects increasing mechanical resistance, and subsequently may confer a longer leaf lifespan among evergreen species. The cutin composition and fine structure of the cuticles were substantially changed during the growth and development of plant organs [16].

Epicuticular wax, which is the outermost layer of the cuticle, may be amorphous or may possess a crystalline structure or platelets. Modification or partial removal of epicuticular waxes by γ-irradiation reduced the barrier properties of the cuticle. Therefore, epicuticular waxes may be a major determinant of cuticle permeability [17,18]. Through anatomical and physiological

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changes, leaves generally acclimate to sun and shade conditions for photosynthesis [19]. Because most ginseng species are shade plants, their leaves may easily experience serious leaf burn under direct sunlight because of the simple structure of the epidermis. However, a few cultivars of ginseng have leaves that are healthier under sunlight [20]. The relationship between the epidermal structure of the leaf and light sensibility is largely unknown. The objectives of this study were to determine the characteristics and development of epicuticular structure and to correlate fine structure with leaf burning in ginseng leaves.

2. Materials and methods

2.1. Scanning electron microscopy

The five cultivars of *Panax ginseng*—Gopoong, Geumpoong, Sunpoong, Yunpoong, and Chunpoong—were provided by KT & G Central Research Institute (Daejeon, Korea). For scanning electron microscopy (SEM), 4-year-old ginseng leaves were fixed in 2% glutaraldehyde in 25mM phosphate buffer (pH 7.1) for 2 hours and dehydrated in a graded ethanol series, and placed in isoamylacetate. Critical point drying (Bioradical E3000 dryer; Nakahara, Tokyo, Japan) was performed using liquid carbon dioxide at 1000 psi. The dried specimens were mounted on the stubs, and a 180-nm coating of gold was applied with a sputter coater (JFC 1110E; JEOL Ltd., Tokyo, Japan). The results were observed with a scanning electron microscope (Hitachi S 3500N; Instruments, Hitachi, Ltd., Tokyo, Japan) operated at 20 kV.

2.2. Transmission electron microscopy

The ginseng leaves were also fixed in 4% (v/v) glutaraldehyde in 25mM phosphate buffer (pH 7.1) for 2 hours, and postfixed in 2% (w/v) osmium tetroxide solution in same buffer for 1 hour. Dehydration was accomplished in a graded ethanol series. The samples were placed in propylene oxide prior to further treatment, and then embedded in a Spurr mixture. Semithin sections were cut with a glass knife and ultramicrotome (Reichert Ultracut S, Vienna, Austria). They were then stained with toluidine blue and basic fuchin for preliminary screening with a light microscope. Ultrathin sections approximately 70 nm thick were cut with a diamond knife, and then stained on copper grids with 1% (w/v) uranyl acetate and

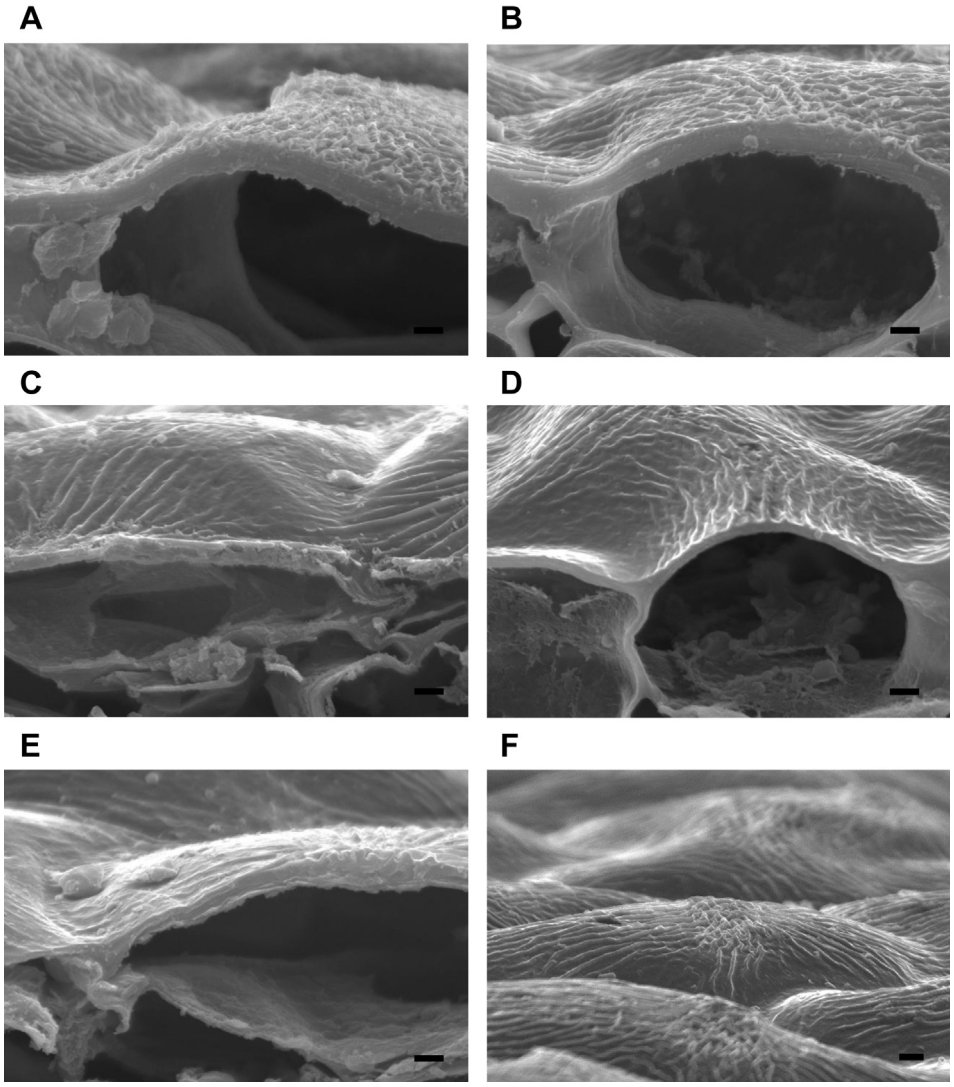

Fig. 1. Scanning electron microscopy (SEM) micrographs of epidermal cells on the adaxial leaf surfaces of ginseng cultivars. A longitudinally sectioned epidermal cells show the wrinkled epicuticular structure on the adaxial surface and epidermal wall of (A) Gopoong, (B) Geumpoong, (C) Sunpoong, (D) Yunpoong, and (E) Chunpoong. (F) The epidermal cell surface shows a radial arrangement of the epicuticular structures of Sunpoong. Bars, 2 μm (A–E) and 1 μm (F).