THREE-DIMENSIONAL OBSERVATION OF LEAF EPIDERMIS OF CREEPING BENTGRASS BY CONFOCAL LASER SCANNING MICROSCOPY

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ABSTRACT

The leaf epidermis of creeping bentgrass *Agrostis stolonifera* (L.) was observed with autofluorescence by confocal laser scanning microscopy (CLSM). The three-dimensional observation revealed that leaf epidermal cells in a layer were morphologically complicated, different in shape and thickness, adhered to each other like a jigsaw puzzle, and some stomata were slightly covered with adjacent cells. Leaf strings, fixed probably over veins, were remarkably observed to be fistulous, and “prickle hairs” or trichomes were hollow. These observations suggest that the complex structures of creeping bentgrass leaf epidermis may relate to growth efficiency or possibly a survival strategy. To our knowledge, this is the first and preliminary report on three-dimensional observations of turfgrass leaf epidermis by CLSM.

INTRODUCTION

Turfgrass on the land can be compared to phytoplankton in the ocean, as both have very high ratios of production to biomass (Agata, 2008; Whittaker, 1970). Turfgrass is possibly one of the keys to prevent global warming because of its high potential to reduce carbon dioxide and save energy (Agata, 2008). Gas exchanges of carbon dioxide and oxygen, transpiration, foliar fertilization, pathogen infection and pesticide application physically occur through the leaf epidermis (Couch, 2000; Eichert and Burkhardt, 2001; Shönherr, 2006; Stern et al., 2008), and leaf rigidity is related to sports turf playability (Turgeon, 2008). The current understanding of the foliar epidermis of turfgrass, especially from a three-dimensional anatomical aspect, is not sufficient despite two-dimensional views (Beard, 1973; Turgeon, 2008). In this study, we attempted to observe the leaf blade epidermis three-dimensionally by confocal laser scanning microscopy (CLSM) with image processing of optical sections.

MATERIALS AND METHODS

Creeping bentgrass *Agrostis stolonifera* L. ‘Penn A-1’ was transferred to the laboratory in Tokyo, Japan from a sand-based golf course green in Osaka, Japan and cultured in the room with foliar fertilization. Plant height for the experiment was ≥ 10 cm, which was grown from a 4 mm of original height. For three-dimensional observation, CLSM (Leica TCS SP5, Wetzlar, Germany) was used with image processing software (Leica Application Suite, Wetzlar, Germany). A leaf blade sample was removed by tweezers and transferred onto a glass slide with low autofluorescence immersion oil, mounted with a cover slip and sealed with clear nail polish, containing nitrocellulose and butyl or ethyl acetate. Only the adaxial (upper) side of the foliar specimen was observed with an excitation beam of 405 nm by AOTF (acousto-optic tunable filter), and emission band widths of 426-500 nm and 650-750 nm, or only 409-483 nm using a 40 x 1.25 or 63 x 1.4 objective lens and 10 ocular. Paradarmal (xy) and transversal (xz, yz) views were produced from optical sections by step sizes of 0.29, 0.50, or 0.59 μm using the image processing software.

RESULTS AND DISCUSSION

Leaf epidermal cell walls were clearly observed as emitted green autofluorescence, however, sub-epidermal mesophyll cells containing chlorophyll were partially detected as a reddish color, because epidermal cells largely attenuated excitation light from CLSM (Wuyts et al., 2010). Three-dimensional observation revealed that leaf epidermal cells were morphologically complicated, different in shape and thickness and adhered to each other like a jigsaw puzzle (Fig. 1a-d and Fig. 2 a-d). Leaf epidermal cells are generally acknowledged to be arrayed in a layer with almost similar thickness and shape (Beard, 1973; Turgeon, 2008). Such complexity of the leaf epidermis of *A. stolonifera* had been unexpected when compared to a previous leaf surface observation showing simple and smooth cell sides (Ushilo, 2010). These findings suggest that the leaf epidermis complexity may increase physical strength needed to protect the leaf from mechanical injury or even external pathogens infection. Moreover, a variety of epidermal cell shapes and thickness may intensify light use efficiency (Wuyts et al., 2010) in creeping bentgrass.

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Keywords: autofluorescence, CLSM, leaf anatomy, leaf string, prickle hair, stomata, trichome, turfgrass

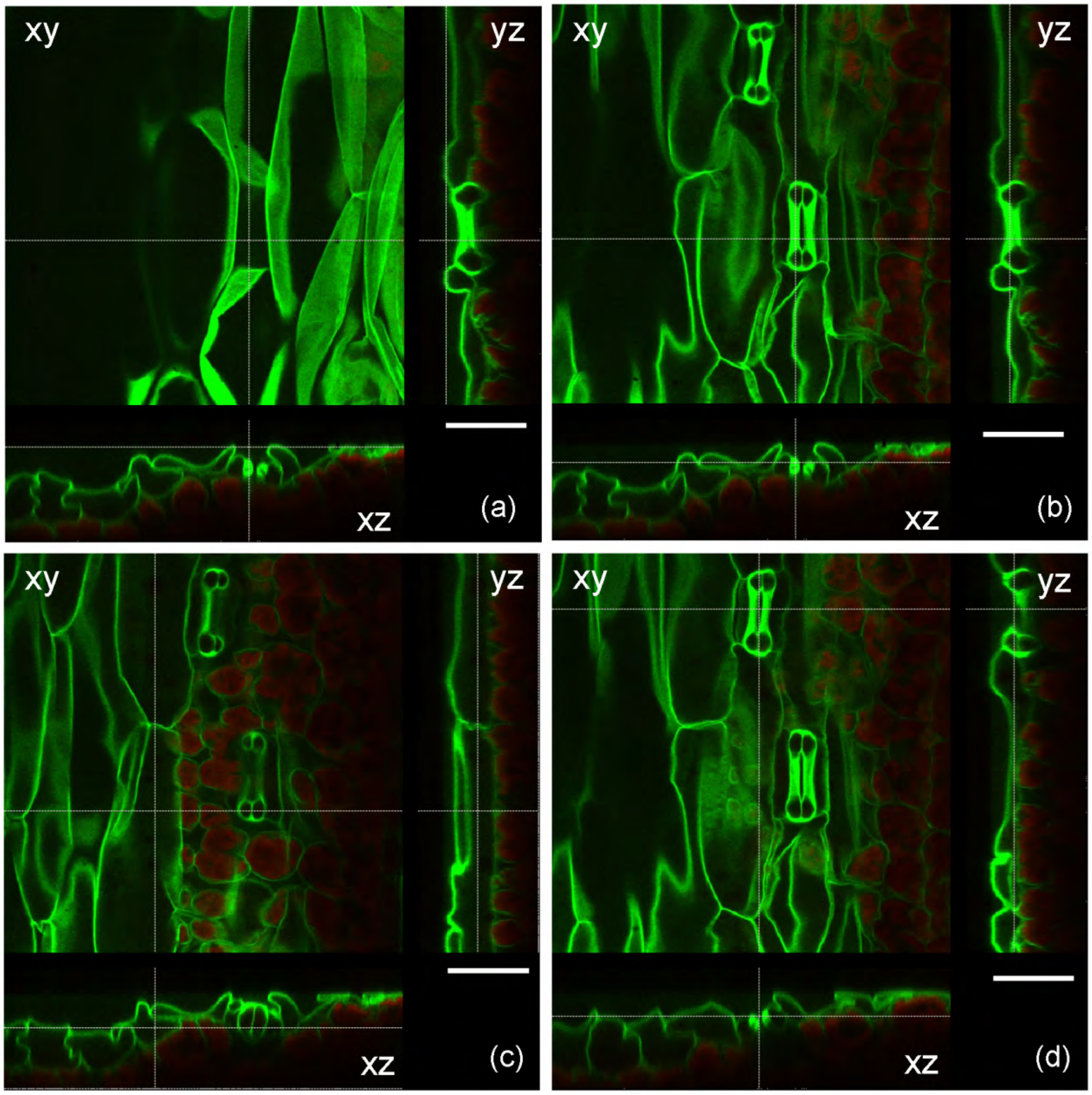

Fig. 1. Three-dimensional images of stomata and leaf epidermal cells of creeping bentgrass. (a) Adjacent cells to guard cells slightly covering a stoma by paradermal (xy) and transversal (xz) views. (b) Stomatal apparatus by the xy-view, and central regions of guard cells emitting strongest green autofluorescence from inside also by xz- and yz-views, indicating these parts consist of mostly cell walls. (c) Polar ends of guard cells with comparatively thin cell walls shown by the xy- and xz-views. (d) Adjacent cells to another guard cells not covering a stoma but rising nearby by the xy- and xz-views. White scale bar of 30 μm for all images.

Concerning stomata, some were slightly covered with adjacent cells next to subsidiary ones (Fig. 1a,b), thus appearing to protect stomata mechanically or related to transpiration, although the true meaning is unknown. Central regions of guard cells, directly forming stoma, emitted strongest green autofluorescence from inside (Fig. 1b,d and 2c,d), which suggests these parts consisted of mostly cell walls of possibly lignin (Ziegler, 1987) and/or callose (Albersheim et al., 2011) without lumen. Meanwhile, both polar ends of guard cells were bulbous and having lumen radiating no fluorescence (Fig. 1c). Such stomatal apparatus of *A. stolonifera* is similar to the two-dimensional view of grasses by Ziegler (1987), which implies that the mechanism of stomatal opening and closing of monocotyledon *A. stolonifera* differs from a dicotyledon's based on the asymmetrically thickened cell walls (Albersheim et al., 2011).

Leaf strings, fixed probably over vascular veins which were not absolutely recognized, were observed with strong autofluorescence (Fig. 2a-d). The string consisted of two small, fistulous fibers with torsional, caved structure over it as shown by paradermal (xy) and transversal (xz, yz) views (Fig. 2). These structures may be important for rigidity, thus providing "uprightness" and wear stress tolerance.

The "prickle hairs", produced mainly from silica and widely categorized as trichomes (Cutler et al., 2008), were also seen as hollow with strong autofluorescence (Fig. 3a-c), which is not usually observed by routine optical microscopy. Our three-dimensional images indicated that trichomes were apparently unfilled with silica, suggesting the plant likely saves or utilizes the silica for other purposes. The functions of nonglandular trichomes are to slow the rate of transpiration, prevent insect attack, and protect against excessive sunlight (Bowes and Mauseth, 2008), and hypothetically to collect and

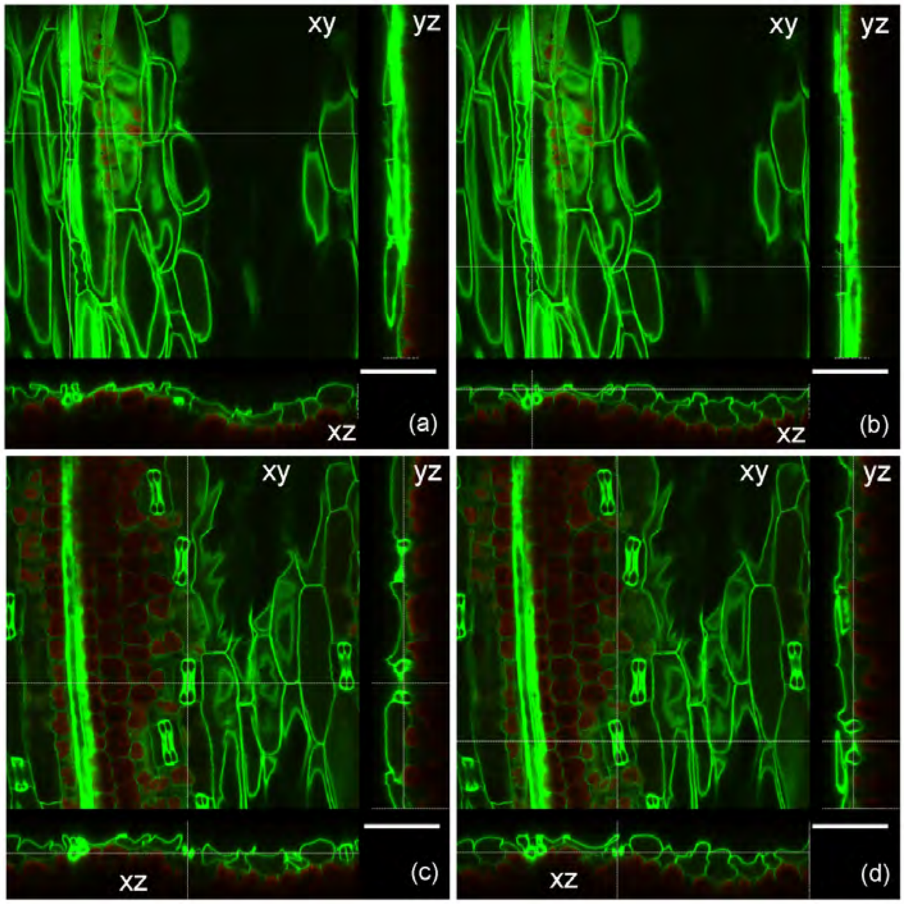

Fig. 2. Three-dimensional images of a leaf string, stomata, and epidermal cells of creeping bentgrass. (a) and (b) A leaf string probably consisted of two small, fistulous fibers with torsional, caved structure on a string by paradermal (xy) and transversal (xz, yz) views. (c) and (d) Stomatal apparatus by xy-, xz- and yz-views, and central regions of guard cells from inside similar to Fig. 1a and 1b. Epidermal cells connected to each other like a jigsaw puzzle. White scale bar of 50 μm for all images.

control electromagnetic output of the sun as a dielectric waveguide (Callahan, 2001). Structures and functions of leaf string and trichomes of turfgrass should be investigated further, to clarify the true meaning of their existence and consider the index possibility of turf quality or vitality.

In conclusion, our results demonstrate that the leaf epidermal cells of turfgrass are different-shaped, diversely constructed, and arranged like a jigsaw puzzle, which have not been disclosed clearly in previous work, and warrants further study (Wuyts et al., 2010). Such complexity of leaf epidermis is presumably important to be considered from the view point of contribution to growth efficiency or survival strategy. These findings, however, are limited under the conditions of comparatively indoor low light intensity (i.e. 2,000 lux), one species of *A. stolonifera*, tall plant height, and leaf adaxial side only observation. Further three-dimensional observation of turfgrass under different factors by CLSM could help clarify the mechanisms of high production/biomass ratio, the CO_2 reducing potential, and foliar fertilizer absorption.

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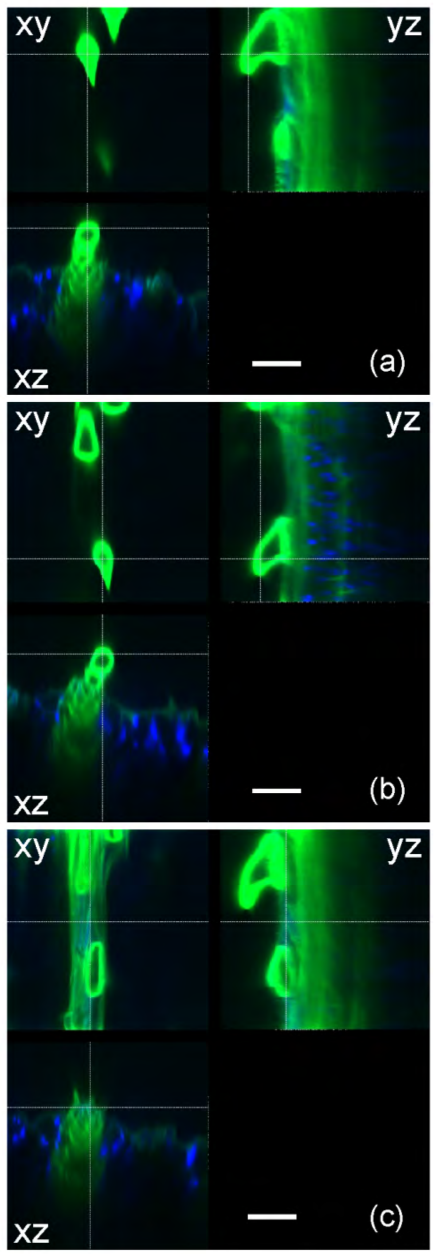

Fig. 3. Three-dimensional images of “prickle hairs” or trichomes of leaf epidermis of creeping bentgrass. (a), (b), and (c) Trichomes considerably hollow from the base to the point by paradermal (xy) and transversal (xz, yz) views. White scale bar of 30 μm for all images.