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Leaf Anatomy and Minimal Structure in Leaves of *Hydrocotyle umbellata* L., Obtained from Water Stress, were Examined under Electron Microscope and Light Microscope

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ABSTRACT

Leaf anatomy and ultrastructure in leaves of *Hydrocotyle umbellata* L. were examined under light microscope. The anatomical features measured included leaf thickness and number and area of bundle sheath and mesophyll cells. The results indicated differences in the cells. Transmission electron microscopy provided details of the internal structure of the cells. There were differences between the subcellular structure of mesophyll and bundle sheath cells from well-watered and water-stressed plants. Furthermore, quantitative measurements based on micrographs of ultrastructure of chloroplasts and starch grains were made. The knowledge from this research can be applied in other scientific fields such as botany, agriculture, and environmental science.

INTRODUCTION

Transmission Electron Microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick per grid. Preparation technique for transmission electron microscopy can be divided into eight major steps: primary fixation, washing, secondary fixation, dehydration, infiltration with transitional solvents, infiltration with transitional resin, embedding and cutting [1,3,9]. The aim of the present investigation was to study leaf anatomy and ultrastructure using TEM at high magnification power to enhance the identification pattern.

Hydrocotyle umbellata L. is a single leaf plant with a round, long, and slender stalk attached to the leaf at the center of the leaf. The leaf diameter is about 3-5 cm, and the leaves are green. *H. umbellata* is spread throughout the tropics. Originally, it was native to North America. [2] In Thailand, it is found in much of the north and central parts of the country. The stem is long, round, and slender. The stems are scattered to the ground with cracked roots and leaves. It can be consumed fresh and used as a folk remedy, as anti-inflammatory, and bladder-stressing agent, as well as for wound healing and arthritis. Furthermore, it is popular as a potted plant. It is used as an ornamental plant in fish bowls, aquariums and it is also easy to maintain [4,5].

METHODOLOGY

Plant material

H. umbellata grown naturally were collected at Kasetsart University, Bangkean campus, Thailand during January 2017 (Figure 1). The leaves were studied using light and transmission electron microscopes

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Specimens were cut into small pieces (1x2 mm^2) and then were fixed in primary fixative containing 2.5 % glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 for 12 hours at 4°C. After that the specimens were washed 3 times (10 min per time) in the same buffer. After washing the specimens, they were post-fixed in 2 % osmium tetroxide in distilled water for 2 hours, and then washed with distilled water 3 times (10 min per time). The specimens were dehydrated in

Figure 1 ***Hydrocotyle umbellata*** **L.**

a graded series of acetone (10 min per series) [3]. The specimens were then embedded in Spurr's low viscosity epoxy resin. After polymerization at 70°C for 8 hours using a formulation of Spurr's resin (1969) [1], the specimens were cut into pieces 1µm thick using an ultra microtome EM UC7 (Leica, Austria) with a glass knife, and mounted onto a glass slide. The specimens were stained in Toluidine blue 1% in borax at 85°C for 1 minute and stained in Basic Fuchsin 1% in distilled water at room temperature for 2 minutes, then dried at 85 °C for 5 minutes, then closed with a cover slide.= Thirty areas per specimen were examined under light microscope (Zeiss; Axiostar plus), which was equipped with a photographic camera under normal bright-field imaging.

Transmission Electron Microscopy

For transmission electron microscopy, the analysis procedure was the same as that of light microscopy. Ultrathin section (100 nm) leaf specimens were prepared using a Leica ultra microtome EM UC7 (Leica, Austria), and the specimens were mounted on copper grids. The specimens were stained with 5% aqueous uranyl acetate for 15 minutes and Reynold's lead citrate for 15 minutes and examined under a HT7700 transmission electron microscope (Hitachi, Japan).

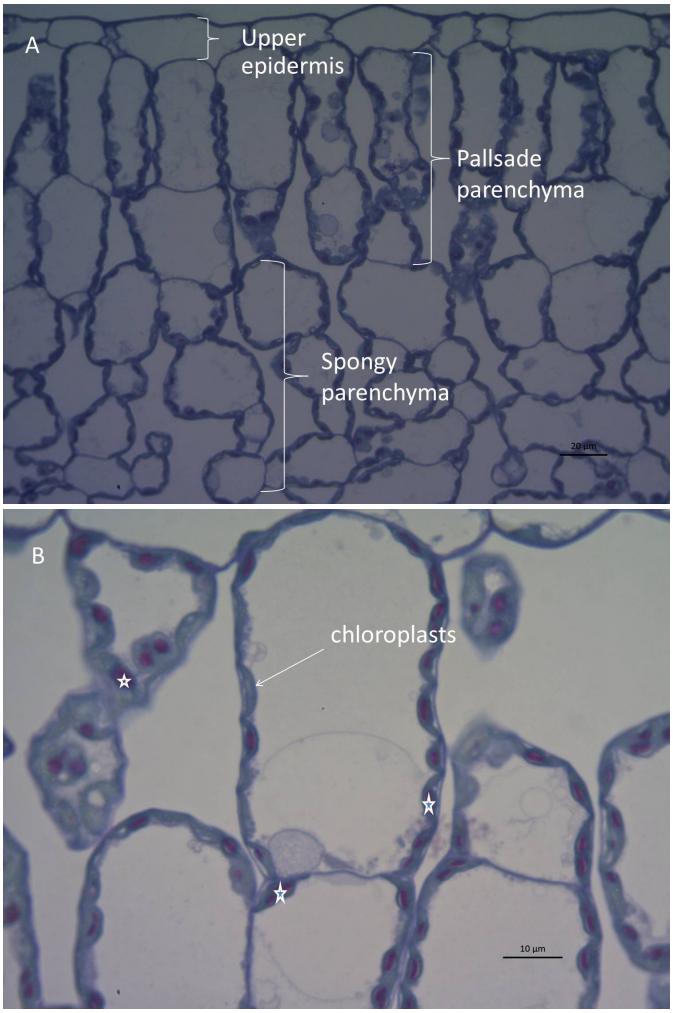

Figure 2 Bright field micrographs of cross section of a well-watered leaf of *Hydrocotyle umbellata* L. under light microscopy.
A: shows well-watered leaf surface upper epidermis, palisade parenchyma, spongy parenchyma (40x)
B: shows well-watered leaf chloroplasts in palisade parenchyma (arrow), starch granules (star) (100x)

Table 1 Differences in cell characteristics of well-watered and water-stressed leaves of *Hydrocotyle umbellata* L.

Type of cells	Well-atered leaf		Water-stressed leaf	
	Range	Average	Range	Average
Thickness cross section of leaf (µm)	271.71 -306.81	285.69 ± 8.12	284.42-307.61	168.80±196.30
Thickness of upper epidermis cell (µm)	8.76-31	20.73±5.15	23.23-30	26.61±4.79
Thickness of lower epidermis cell (µm)	9.85 -27.90	17.48±4.09	15.14-30	22.57±10.51
Length of parenchyma cells (µm)	31.85-75.79	61.75±8.12	56.23-56.23	43.11±18.55
Width of parenchyma cells (µm)	18.60-35.89	25.45±4.54	16.81-30	23.40±9.33
Length of spongy cells (µm)	15.39-70.40	33.9±13.73	44.40-44.40	37.20±10.18
Width of spongy cells (µm)	12.16-33.47	22.01±5.10	25.51-30	27.75±3.18

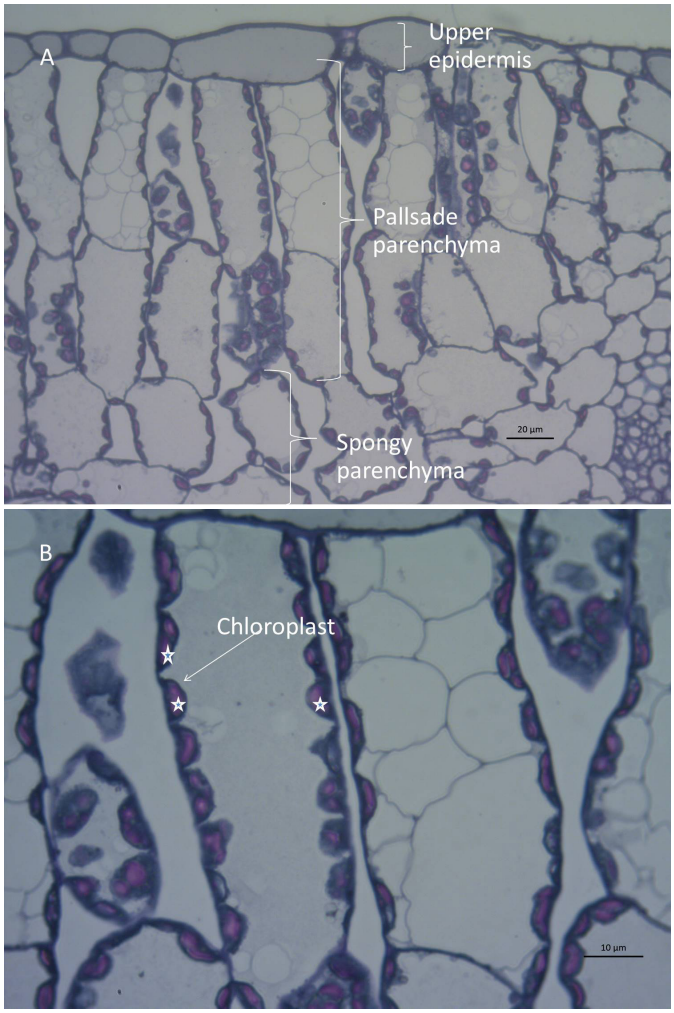

Figure 3 Bright field micrographs leaf cross section water- stressed leaf of *Hydrocotyle umbellata* L. under light microscopy. A: shows water- stressed leaf surface upper epidermis, palisade parenchyma, spongy parenchyma (40x).
B: shows water- stressed leaf chloroplasts in palisade parenchyma (arrow), starch granules (star (100x)

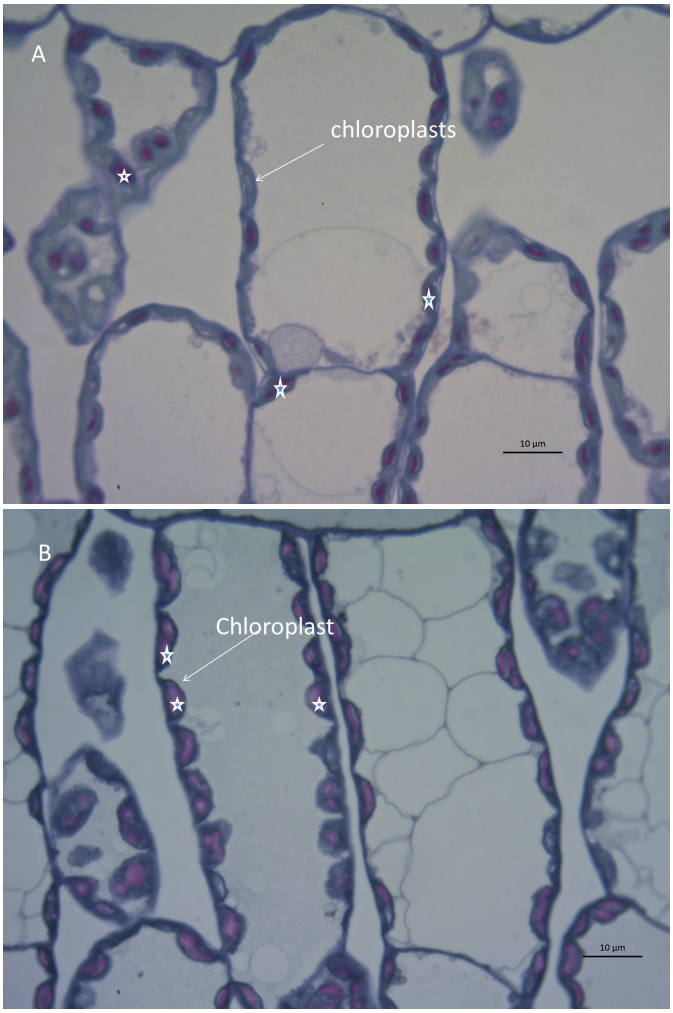

Figure 4 Bright field micrographs of cross section of a *Hydrocotyle umbellata* L leaf. under light microscopy. A: shows palisade parenchyma (arrow), starch granules (star) of a well-watered leaf (100x). B: shows chloroplasts in palisade parenchyma (arrow), starch granules (star) of a water-stressed leaf (100x)

RESULTS AND DISCUSSION

Leaf anatomy and ultrastructure under light microscopy

Examination using light microscopy (Table 1) revealed that cross section thickness of well-watered leaves were 271.71-306.81 µm and thickness of water stressed leaves were 284.42-307.61 µm. The epidermis consisted of a single layer (Figure 2A and Figure 3A). [10]. The upper and lower epidermal cells of the well-watered leaves were thinner than the water-stressed leaves. The parenchyma cells of the well-watered leaves were longer and wider than those of the water-stressed leaves. The chloroplasts of the water- stressed leaves were very dense with starch granules (Figure 4A and Figure 4B). The sponge cells of the well-watered leaves were smaller than those of the water-stressed leaves.

Leaf anatomy and ultrastructure under Transmission Electron Microscopy

Examination using transmission electron microscopy , There were differences between chloroplasts well-watered and water-stressed plants (Figure 5 and Figure 6). Internal structure of water-stressed leaves chloroplasts were very dense with starch granules.

CONCLUSION

In this study, transmission electron microscopy and light microscopy technique were used to examine changes in cell structure and ultrastructure and can also be used in disease diagnosis of industrial crops, and environmental studies. There were differences between the subcellular structure of mesophyll and bundle sheath cells of well-watered and water-stressed plants. This finding provides a basis for further research on plants.

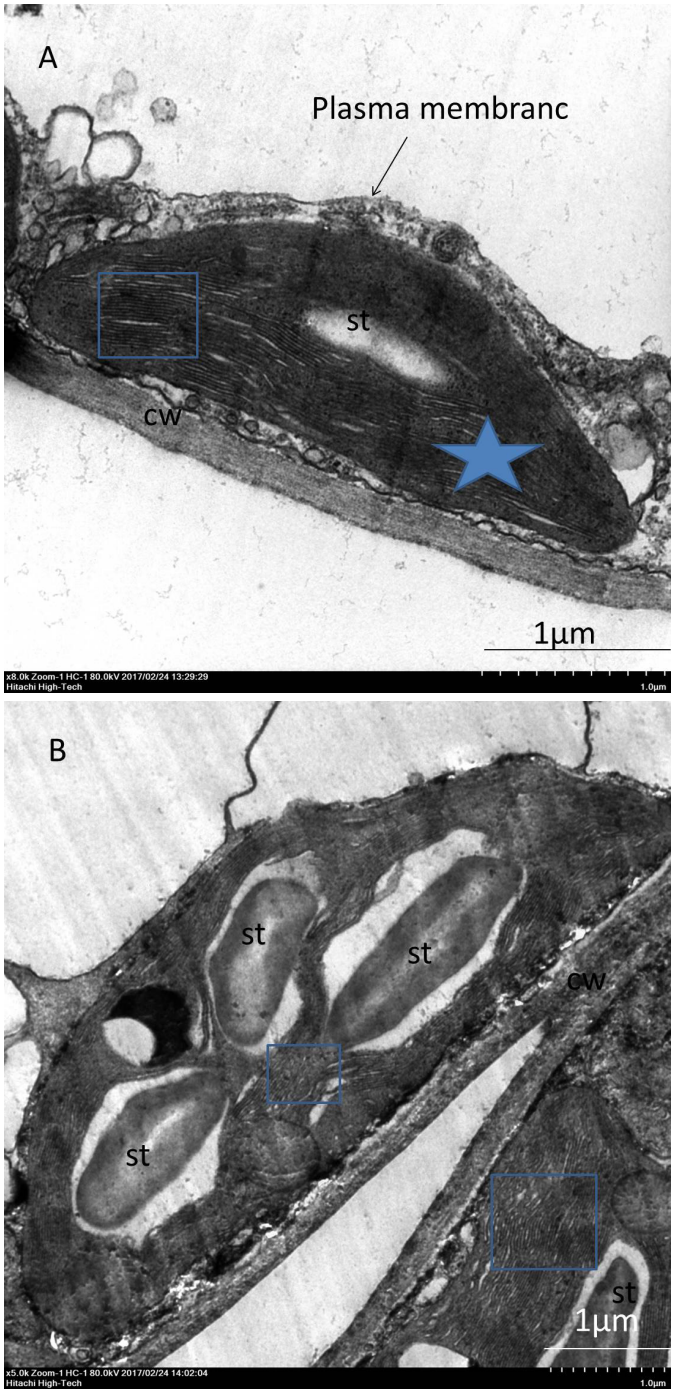

Figure 5 Transmission electron micrographs of *Hydrocotyle umbellata* L. well- watered leaf. A: shows cw = cell wall, ch = chloroplasts (star), st = starch, granum thylakoids (square), Plasma membrane (arrow). B: shows cw = cell wall, st = starch, thylakoids (square).

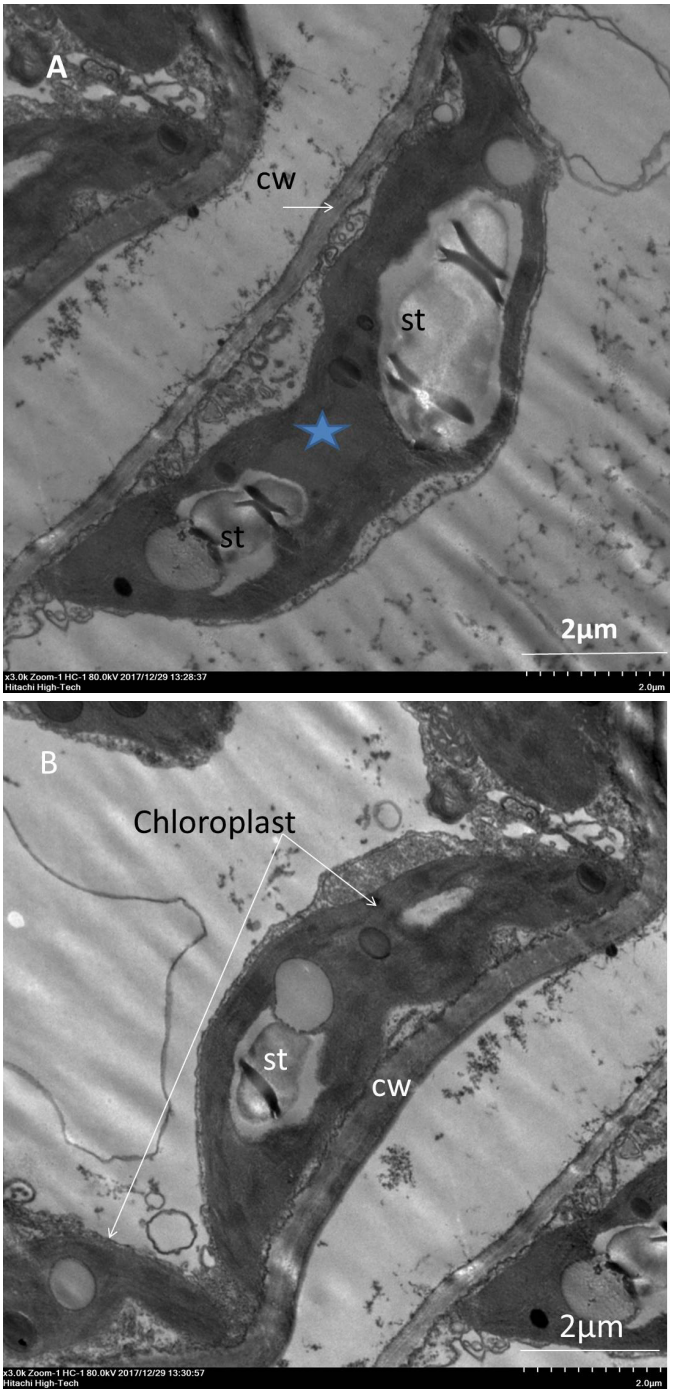

Figure 6 Transmission electron micrographs of *Hydrocotyle umbellata* L. Water stressed leaf. A: shows cw = cell wall (arrow), chloroplasts (star), st = starch. B: shows cw = cell wall, st = starch, chloroplasts (arrow).

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