Transmission electron microscopy of extra ultrathin sections from *Nicotiana occidentalis*.

J. Nebesarova[1,2], M. Vancova[1,3], S. Svidenska[3]

1. Biology Centre of ASCR, v.v.i., Institute of Parasitology, Branisovska 31, 37005 Ceske Budejovice, Czech Republic
2. Faculty of Science, University of South Bohemia, Branisovska 31, 37005 Ceske Budejovice, Czech Republic

nebe@paru.cas.cz
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Transmission electron microscopes (TEM) working at accelerating voltage 100 kV require to cut the majority of biological specimens in ultrathin sections with the thickness in the range 50-80 nm. The resulting image from TEM represents a superimposition of all structures into the section occurring in an observed area. The low voltage transmission electron microscope (LVTEM) works at accelerating voltage 5 kV and that is why it needs much more thinner sections from specimens with the thickness only 15-20 nm [2]. The advantage of using so low accelerating voltage is the substantial reduction of the superimposition in addition to the contrast enhancement.

LVTEM represents completely new branch of microscopy. It was designed by Armin Delong especially for the observation of samples composed from elements with low atomic numbers [1]. It surprises by its unusual design. The microscope consists of two parts; the first one is a miniature 5 kV transmission electron microscope with the maximum magnification 500 times equipped by emitter source of Schottky type, permanent magnetic lenses for image formation, and electrostatic lenses to control magnification. The invisible electron images are converted to the light ones by means of a single crystal YAG fluorescent screen. The second part of the device is a conventional optical microscope with the maximum magnification 400 times equipped by CCD camera for image recording. The resolution power of LVTEM is 2 nm.

We used LVTEM for studying the chloroplast ultrastructure of *Nicotiana occidentalis*. A small pieces of fresh leaves were fixed in 2,5 % glutaraldehyde in 0.1 M phosphate buffer, only part of them were postfixed by 1 % osmium tetroxide. Then the samples were dehydrated through acetone series and embedded in the hardest variation of the epoxy resin Spurr (Polysciences). The specimens were carefully irradiated by microwaves in each preparation step and we used also changes of pressure and prolonged times during the infiltration and embedding [3, 4]. The using of the oscillating diamond knife (Diatome) [5] and the ultramicrotome UCT (Leica) facilitated considerably cutting of ultrathin sections with the thickness in the range from 20 to 50 nm. Sections were observed without any staining procedure at first in LVTEM 5 (Delong instruments) and then in TEM JEOL 1011 working at 80 kV.

As the Fig.1a shows LVTEM revealed a new detail in the ultrastructure of chloroplast – a network of starch channels. The detail was visible only on sections from the specimen which wasn't treated by the fixation of osmium tetroxide (Fig.1b). The presence of the network of starch channels was proved also by TEM JEOL 1011; in this case the visibility of the ultrastructure has depended on the thickness of sections in addition to the osmium tetroxide fixation. The channels weren't recognizable when the thickness of the section exceeded 40 nm.

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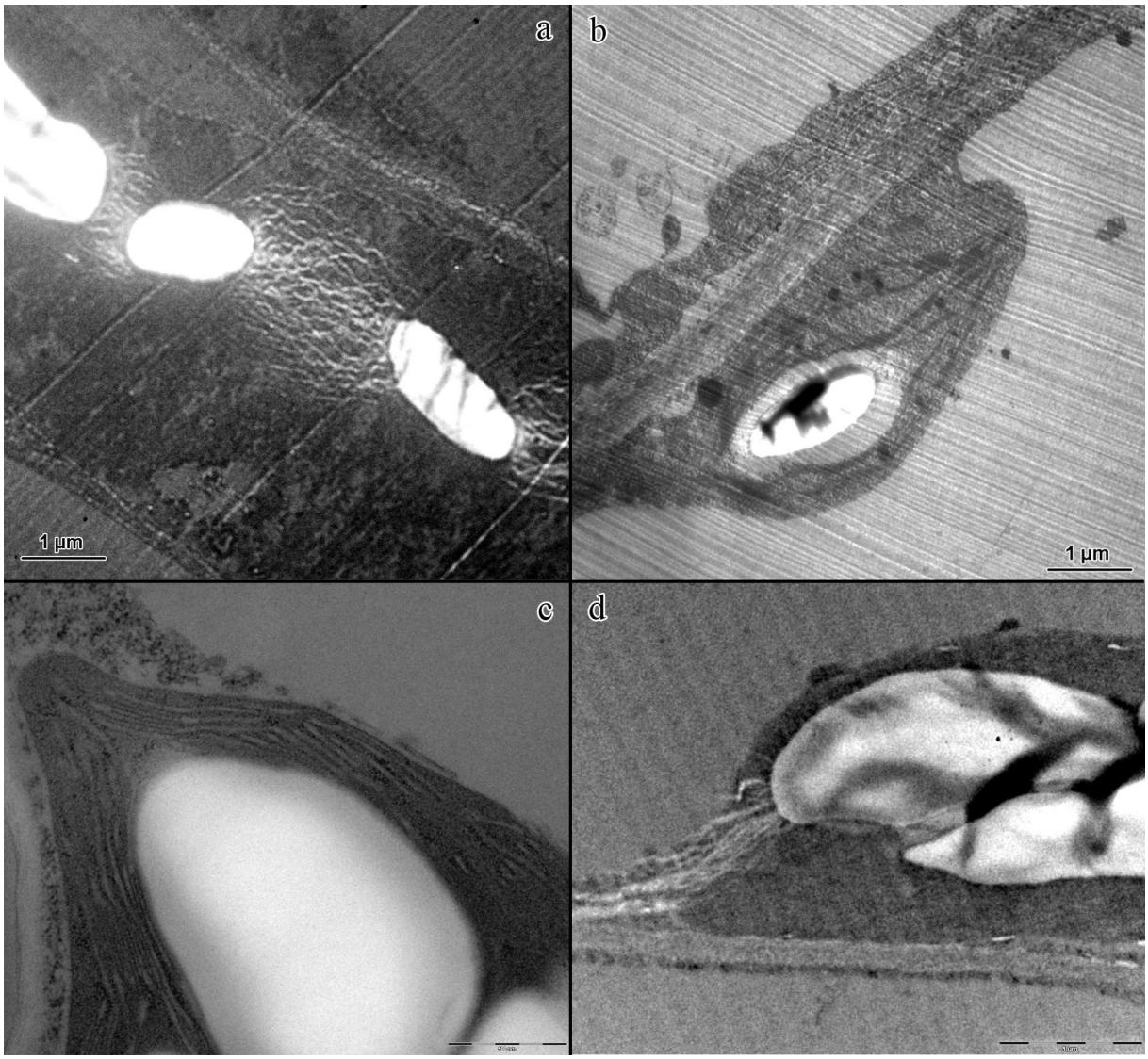

Figure 1. The appearance of the chloroplast ultrastructure of *Nicotiana occidentalis* in LVTEM working at 5 kV:
1a – 20 nm thin section without any staining including OsO_4 postfixation, **1b** – 20nm thin section with OsO_4 postfixation;
And in TEM JEOL 1011 working at 80 kV: **1c** – 50 nm thin section with OsO_4 postfixation, **1d** – 20 nm thin section without any staining including OsO_4 postfixation.

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