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Structural Features of Nuclei in Leaf Mesophyll Cells of Salt-Tolerant *Artemisia marschalliana*

Galina Semenova

Institute of Theoretical and Experimental Biophysics of Russian Academy of Sciences, Pushchino, Russia
Email: gals1041@rambler.ru

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Abstract

All nuclei in mesophyll cells of *Artemisia marschalliana* are located in vacuoles and occupy up to 90% of their volume. The ultrastructural organization of chromatin in nuclei shows different degrees of its decondensation, up to complete separation of DNA from histones. It is possible that the separation of DNA from histones enables Artemisia to grow in soils with high salinity.

Keywords

Meso- and Ultrastructure of Mesophyll, Decondensation of Nuclear Chromatin, *Artemisia marschalliana*

1. Introduction

In studies devoted to the effect of salinization on the ultrastructure of cell components, the main attention is paid to the structure of chloroplasts [1] [2]. In [3], meso- and ultrastructural patterns of salt stress on wheat seedlings. It can be seen that nuclear chromatin undergoes significant changes under salt treatment (50, 100, and 200 mL NaCl). Through a stage of strong condensation, heterochromatin proceeds to fusion into a single mass followed by disintegration into a finely divided substance.

A structural study on a wild *Artemisia marschalliana* plant in soil salinity conditions has shown an unusual state of the components of leaf mesophyll cells. All cell nuclei are located not in the cytoplasm but in the vacuolar space and occupy up to 90% of their volume. Nuclear chromatin has varying degrees of decondensation up to complete separation of DNA from histones. Nuclear envelopes and other cell membranes are not visible.

Such an unusual state of cell nucleus chromatin in the mesophyll cells of an actively growing plant requires interpretation.

2. Materials and Methods

The material was collected in Biologicheskaya balka of Eltonsky Nature Park, Volgograd region, Russia. Salt content in the soil in Biologicheskaya balka is 4 - 16 mg/g dry soil.

Leaves from a young non-flowering plant were collected on June 9, 2019, at 1 p.m. The pieces of leaves (2 × 2 mm) were fixed in 3% glutaraldehyde dissolved in 30 mM phosphate buffer (pH 7.2) with postfixation in 1% OsO_4 solution.

The fixed material was dehydrated in alcohols and acetone and then embedded in the Epon 812 epoxy resin (Fluka, Germany).

Ultrathin sections were contrasted in saturated aqueous solution of uranyl acetate (Sewa, Czech Republic) and 0.25% solution of lead citrate (British Drug Houses, England) and were examined with an electron microscope (Jeol, Japan).

Sections for the optical microscopy (2 - 3 μm) were not stained; they were examined under a transmitted-light microscope (Axiostar Plus, Carl Zeiss, Göttingen, Germany) and photographed by a digital camera.

3. Results and Discussion

Figure 1 shows a young non-flowering plant *Artemisia marschalliana* of the Asteraceae family, the leaves of which were taken from the middle of the stem. The leaves of the plant are small, 1 mm wide, and have no signs of degradation even in the lowest leaves.

Figure 1. *Artemisia marschalliana* plant, the leaves from which were taken for fixation.

Figure 2 demonstrates a cross section of a leaf photographed with a light microscope.

Epidermal cells are large with a thick lower membrane. At the very top, there is a structure of a possible salt gland (Figure 2).

Chloroplasts in mesophyll cells are located in the parietal layer of the cytoplasm, and nuclei in all cells are located in the center and occupy up to 90% of the vacuole volume (Figure 2).

The data obtained at the ultrastructural level show that all cell membranes (plasmalemma, tonoplast, nuclear and chloroplast envelopes) are hardly noticeable in mesophyll cells (Figure 3(a) and Figure 3(b)).

The ultrastructure of chloroplasts shows that thylakoids are assembled into grana located in the electron-dense matrix. The presence of starch grains indicates their functioning (Figure 3(a) and Figure 3(b)).

Cell nuclei located in vacuoles show varying degrees of chromatin decondensation. The low level of decondensation is shown in Figure 4(a); the high level of decondensation is shown in Figure 4(b). The extreme level of chromatin decondensation is accompanied by the separation of DNA strands from histones (Figure 5(a) and Figure 5(b)). Figure 5(b) shows a pattern of DNA strands that can be compared with a structure of proplastid DNA [4].

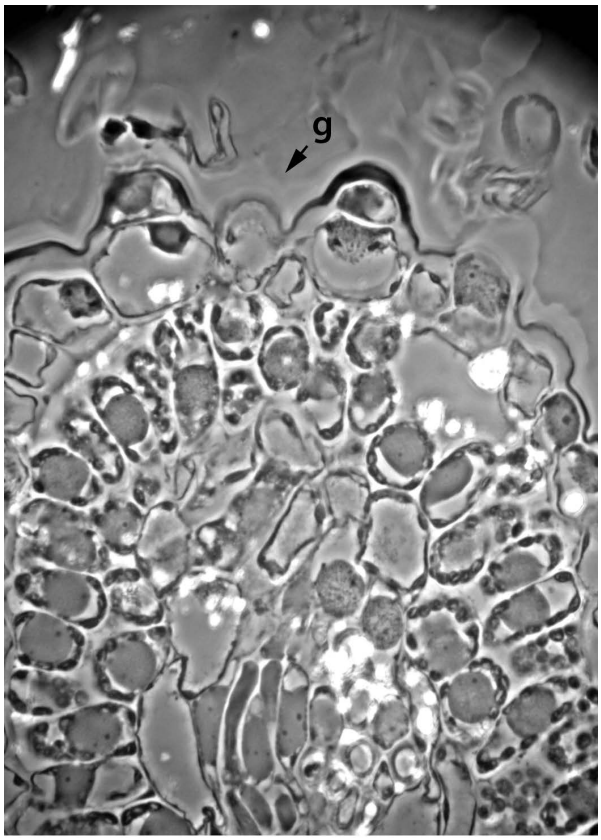

Figure 2. *Artemisia marschalliana* leaf section. In all mesophyll cells, nuclei occupy vacuolar space. A gland-like structure can be seen (g). Lens × 100.

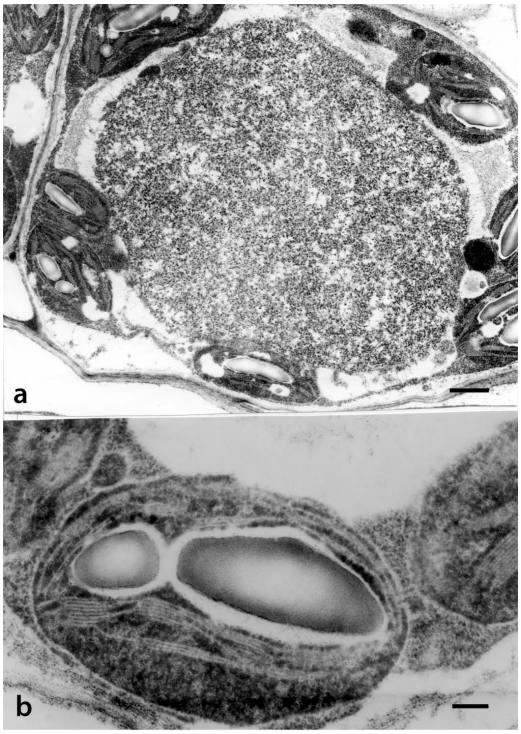

Figure 3. Leaf mesophyll cell. (a) The nucleus almost completely occupies the vacuolar volume; the nuclear envelope is absent; nuclear chromatin is sparse. Bar is 1 μm; (b) The chloroplast is at high magnification its structure is intact. Bar is 0.2 μm.

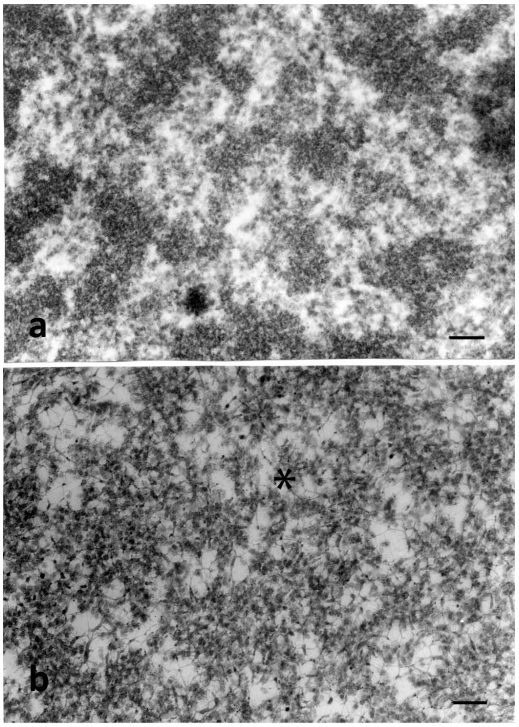

Figure 4. Nuclear chromatin. (a) Nuclear chromatin in normal state; (b) Slight decondensation of nuclear chromatin. Bars (a) and (b) are 0.2 μm.

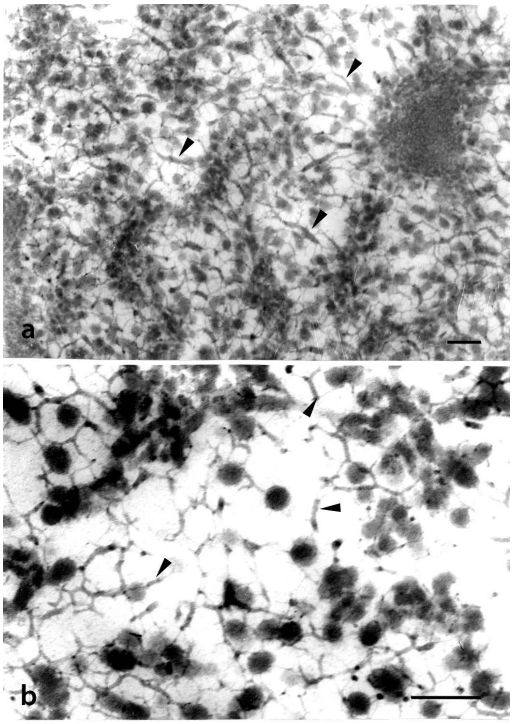

Figure 5. (a) Strong decondensation of nuclear chromatin; (b) Leading to complete separation of DNA strands (arrows) from histones. Bars (a) and (b) are 0.2 μm.

It has been shown that the treatment of isolated DNA with NaCl solutions leads to the complete separation of DNA from histones [5]. In tomatoes growing under NaCl salinity conditions, alkaline proteins of the plants including histones are most affected. It has been suggested that there is dissociation of histone complexes with the subsequent nuclear exclusion [6]. Cultivation of barley on NaCl solutions resulted in complete degradation of nuclei in leaves [2].

When growing wheat seedlings in soil containing 100 - 200 mM NaCl, very strong condensation of nuclear chromatin was observed in mesophyll cell nuclei at first, and then chromatin turned into a homogenous mass of reduced density [3].

In our case, neither condensation nor complete dissociation of chromatin was observed in *Artemisia marschalliana* nuclei. However, separation of DNA strands from histones was observed. It is possible that alkaline proteins and histones neutralize Na ions that accumulate in vacuoles.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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