Conference Paper

Structural and Functional Organization of Photosynthetic Apparatus in Wild Halophites

O. A. Rozentsvet, V. N. Nesterov, and E. S. Bogdanova

Institute of the Ecology of the Volga River Basin, Russian Academy of Science, 445003 Togliatti, Russia

Abstract

The structural and molecular parameters of photosynthetic apparatus in plants with different strategies for the accumulation of salts were investigated. The objects of the study were euhalophytes (*Salicornia perennans*, *Suaeda salsa*, *Halocnemum strobilaceum*), a crynohalophyte (*Limonium gmelinii*) and a glycohalophyte (*Artemisia santonica*). The euhalophytes *S. perennans* and *S. salsa* belong to plants of the halosucculent type, while the other three species represent the xerophilic type. Larger cells with a great number of chloroplasts, a high content of membrane glycerolipids and unsaturated C18:3 fatty acid and smaller pigment and light-harvesting complexes characterize the features of euhalophytes with a succulent leaf type. Therefore, the features of the halophyte photosynthetic apparatus structure are closely related to its functional indicators and are defined by a strategy in both the accumulation of salts and the method of water regime regulation.

Keywords: chlorophyll, lipids and fatty acids, photosynthetic apparatus, ultrastructure of chloroplasts

Corresponding Author:
O. A. Rozentsvet
rozentsvet@mail.ru

Received: 12 September 2018
Accepted: 15 October 2018
Published: 29 October 2018

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the Ecology and Geography of Plants and Plant Communities Conference Committee.

1. Introduction

Plant photosynthetic apparatus (PA) is a complexly organized multi-level system that ensures the light absorption and transformation of its energy into the energy of chemical bonds. Leaf architectonics is determined by the number of cells per leaf unit area, their size and shape; this forms the optimal structure for light passage and carbon dioxide diffusion from the leaf space to the chloroplasts [1–3].

Light photosynthetic reactions are carried out by pigment-peptide complexes embedded in the internal membranes of chloroplasts and are divided into two morphological domains: granal thylakoids and stromal thylakoids [4, 5]. The lipid composition

of the thylakoid membrane is unique and extremely conservative: uncharged galactolipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) constitute 60–80% of the total number of thylakoid lipids [6, 7]. The rest is made up of anionic lipids: sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). The dynamic and functional properties of membranes depend on the composition of fatty acids (FA) [8].

Photosynthetic pigments, chlorophyll (Chl) and carotenoids, are responsible for light absorption and photochemical reactions in chloroplasts [9]. Control of the Chl concentration and Chl *a/b* ratio are the photosynthetic function adapts to various environmental conditions, primarily light intensity and water supply [10].

Soil salinization is one of the factors that adversely affects many of physiological functions [11, 12]. At the same time, halophytes possess high adaptability to the action of salts. They have developed several ways to protect PA from salinity realized at the plant, plant tissue, cellular and molecular levels [13, 14]. The aim of this study was to investigate the PA structure features at the level of leaf anatomy and mesostructure, the chloroplast ultrastructure and the biochemical composition of membrane structural components in plants with different strategies for the accumulation of salts.

2. Methods

The work was conducted on the coastal strip of Lake Elton (49°07′ N latitude, 46°50′ E longitude) over several years in conditions of high insolation (1000–2000 μmol m^2/s) and a temperature at day/night of 30–35/25–30°C. Soil salinization in this region is caused by highly mineralized (15–30 g/L) groundwater lying at a depth of 0–3 m. The objects of the study were *Salicornia perennans* Willd., *Suaeda salsa* L., *Halocnemum strobilaceum* (Pall.) M. Bieb. (*Chenopodiaceae*), *Limonium gmelinii* (Willd.) O. Kuntze (*Plumbaginaceae*) and *Artemisia santonica* L. (*Asteraceae*). The middle part of leaves of 15–20 plants collected within the same phytocenosis were used for biochemical analyses. Three independent biological samples for each analysis type (2–4 g of fresh mass (FM)) were made from overall leaf biomass.

Water content in the plant leaves was calculated as a percentage of FM after determining dry mass (DM). DM was measured after drying plants at 80°C. The content of product lipid peroxidation (LPO) was determined by the method described in [15]. The numbers of cells and chloroplasts were counted using disks from the middle part of the leaf fixed in 70% ethanol according to Ivanova and Pyankov [10]. Photosynthetic

pigment content was measured in 3–4 biological replicates by means of a spectrophotometer (Shimadzu, Japan, UV-1700) in acetone extract at wavelengths of 662 and 644 nm Chl and 470 nm Car with corrections in absorbance maximums. The lipids were extracted three times by chloroform/methanol mixture (1:2, v/v) with simultaneous mechanical destruction of tissues [16].

In the figures, means ± standard error (SE) are presented. All statistical analyses were carried out in STATISTICA 6.0 (Tulsa, OK, USA, StatSoft Inc.). To test for differences between species following one-way ANOVAs, we used Tukey post hoc tests. Differences were considered to be significant at $P \leq 0.05$.

3. Results

The plants represent three ecological groups: salt-accumulating halophytes (euhalophytes); halophytes excreting excess salt on the surface of their leaves (crynohalophytes); and halophytes restricting the uptake of salt by the root system (glycohalophytes) [17]. They differed in the size and form of leaves: large (*L. gmelinii*), small (*S. salsa*, *H. strobilaceum*, *A. santonica*) and aphyllous succulent stems (with scale-like objects fused to the stem leaves) (*S. perennans*). The leaves of cryno- and glycohalophytes were characterized by a significant number of cells of smaller volume and surface area (Figure 1(a) & (b)). In general, the number of chlorenchyma cells per leaf area varied several times depending on the plant species – from 41.000 per cm^2 in *S. salsa* to 1060 per cm^2 in *H. strobilaceum*.

The highest number of chloroplasts in the palisade cells of the investigated halophytes was found in *S. perennans* – 125, which is two times higher than the average values of this parameter for most plants (60–80 chloroplasts per cell) [1]. In cryno- and glycohalophytes, the number of chloroplasts in the palisade tissue was more than 5 times lower compared to *S. perennans*, and 1.5 times lower than that in *H. strobilaceum* and *S. salsa* (Figure 1(c)). The number of chloroplasts is usually less in spongy parenchyma – 15–40 chloroplasts per cell. In the studied species, this index varied from 24 to 28 chloroplasts per cell, and the differences between the species were insignificant.

In halophytes, the structure of chloroplasts was typical for plants: the plastids of a proper lenticular shape, a well-developed thylakoid system and a fine-grained stroma. However, both the chloroplast volume and its surface area were 1.5–3 times higher in succulent halophytes compared to xerophytes (Figure 1(d)). Chloroplasts of euhalophyte *S. perennans* are more elongated and flattened. They contain 42% of the grana

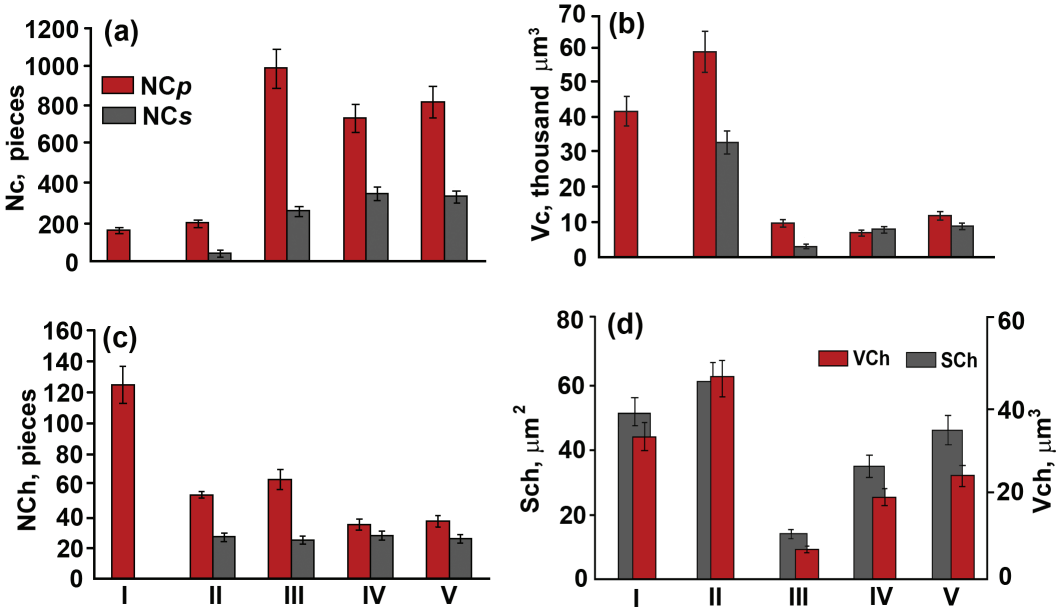

Figure 1: Quantitative characteristics of assimilating tissues of the studied halophytes: a – the number of palisade and sponge tissue cells; b – the volume of palisade and sponge tissue cells; c – the number of chloroplasts in the palisade tissue; d – surface area and volume of chloroplast. I – *S. perennans*; II – *S. salsa*; III – *H. strobilaceum*; IV – *L. gmelinii*; V – *A. santonica*. Different letters indicate statistically significant differences between treatments at $P \leq 0.05$. Source: Authors' own work.

with a small number of thylakoids (2–5 pieces) and the same number of grana with a large number of thylakoids in the granum (9–15 pieces). In the chloroplasts of cryno- and glycohalophytes, the main part (53–57%) consists of grana with 2–5 thylakoids in the granum.

The content of lipids containing one (MGDG) and two (DGDG) monosaccharide residues was more than 70% and reached 85% in both euhalophyte species. For the majority of species, MGDG are predominant in the general pool of glycerolipids, followed by DGDG, SQDG and PG. The lipids of *H. strobilaceum* membranes with observed equal amounts of MGDG and DGDG were an exception.

Lipid molecules form a matrix of thylakoid membranes, with the dynamic and functional properties depending on the FA composition. As expected, the share of unsaturated FAs was more than 60%. The tendency of a decrease in linolenic acid (C18:3) relative content has been revealed, together with a decrease in plant halophilicity. At the same time, an increase in the content of linoleic (C18:2) and oleic acids (C18:1) was found, which indicates differences in the activity of the desaturases responsible for the formation of double bonds in the FA molecule of the studied halophytes [8].

The physiological condition of photosynthetic cells was estimated by the number of green pigments, content of water and the intensity of LPO processes.

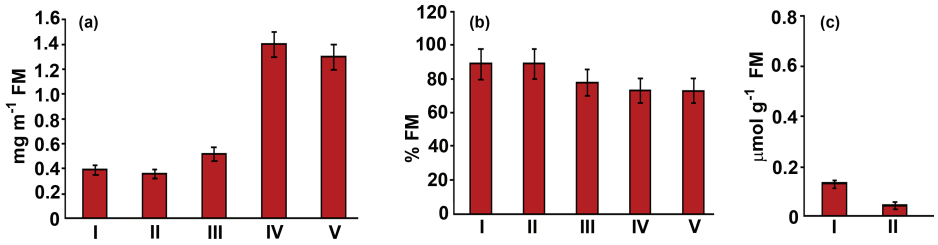

Figure 2: Physiological conditions of halophytes: a – content of pigments; b – content of water; c – LPO. I – *S. perennans*; II – *S. salsa*; III – *H. strobilaceum*; IV – *L. gmelinii*; V – *A. santonica*. Different letters indicate statistically significant differences between treatments at $P \leq 0.05$. Source: Authors' own work.

For the studied species, in the leaves of cryno- and glycohalophytes the content of both green pigments was two or more times higher than in euhalophytes, despite the fact that the latter were characterized by larger chloroplast size. Chl amount was not associated with the size of the chloroplasts, but Chl *a/b* ratio was inversely proportional to the chloroplast volume and surface area ($r = -0.97$ at $P \leq 0.05$ in both cases). The size of the chloroplast and the content of photosynthetic pigments positively correlated with the content of glycerolipids. Their total amount decreased in the same sequence as the number of chloroplasts in the cells of the palisade and spongy parenchyma. Three species of halophytes represent the xerophilic type (the water content in the leaves is 75–78%). Euhalophytes *S. perennans* and *S. salsa* belong to plants of the halosucculent type; the water content in the aboveground tissues is more than 90% of the wet weight. The content of LPO products was higher in plants of the xerophyte type compared with succulent eugalophytes.

4. Conclusion

Thus, in halophytes growing under natural conditions, the differences were revealed at the level of leaf anatomy and mesostructure, the chloroplast ultrastructure and the biochemical composition of the membrane structural components. Larger cells with a great number of chloroplasts, a high content of membrane glycerolipids and unsaturated C18:3 FA and smaller pigment are the characteristic features of euhalophytes with a succulent leaf type. Plants of the xerophyte type, including both *H. strobilaceum* euhalophyte and cryno- and glycohalophytes, are described by lower values of these characteristics. Therefore, the features of the halophyte PA structure are closely related to its functional indicators and are defined by a strategy for both the accumulation of salts and the method of water regime regulation.

Funding

The authors would like to acknowledge the financial support of the Russian Foundation for Basic Research Grants № 04-12-01110-a, 14-04-1018914-k toward the present work.

References

[1] Mokronosov, A. T. and Gavrilenko, V. F. (1992). *Photosynthesis. Physiological, Ecological and Biochemical Aspects*. Moscow: Publishing House of Moscow University.

[2] Reich, P. B., Wright, I. J., Cavender-Bares, J., et al. (2003). The evolution of plant functional variation: Traits, spectra, and strategies. *International Journal Plant Science*, vol. 164, no. S3, pp. 143–S164.

[3] Li, L., Ma, Z., Niinemets, Ü., et al. (2017). Three key sub-leaf modules and the diversity of leaf designs. *Frontiers in Plant Science*, vol. 8, no. 1545, pp. 1–8.

[4] Rochaix, J. D. (2011). Assembly of the photosynthetic apparatus. *Plant Physiology*, vol. 155, no. 4, pp. 1493–1500.

[5] Nevo, R., Charuvi, D., Tsabari, O., et al. (2012). Composition, architecture and dynamics of the photosynthetic apparatus in higher plants. *Plant Journal*, vol. 70, no. 1, pp. 157–176.

[6] Deme, B., Cataye, C., Block, M. A., et al. (2014). Contribution of galactoglycerolipids to the 3-dimensional architecture of thylakoids. *The FASEB Journal*, vol. 28, no. 8, pp. 3373–3383.

[7] Kobayashi, K., Endo, K., and Wada, H. (2016). Roles of lipids in photosynthesis, in *Lipids in Plant and Algae Development*, pp. 21–49. Switzerland: Springer International Publishing AG.

[8] Los, D. A. (2014). *Fatty Acid Desaturases*. Moscow: Scientific World.

[9] Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids pigments of photosynthetic biomembranes, in *Methods in Enzymology*, pp. 350–382. New York, NY: Academic Press, Inc.

[10] Ivanova, L. A. and Pyankov, V. I. (2002). Influence of environmental factors on the structural parameters of the leaf mesophyll. *Botanicheskii Zhurnal*, vol. 87, no. 12, pp. 17–28.

[11] Flowers, T. J. and Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, vol. 179, no. 4, pp. 945–963.

[12] Rozentsvet, O. A., Nesterov, V. N., Bogdanova, E. S., et al. (2016). Biochemical conditionality of differentiation of halophytes by the type of regulation of salt metabolism in prieltonye. *Contemporary Problems Ecology*, vol. 9, no. 1, pp. 98–106.

[13] Dajic, Z. (2006). Salt stress, in *Physiology and Molecular Biology of Salt Tolerance in Plant*, pp. 41–99. Netherlands: Springer.

[14] Rozentsvet, O. A., Kosobryukhov, A. A., Zakhozhiy, I. G., et al. (2017). Photosynthetic parameters and redox homeostasis of *Artemisia santonica* L. under conditions of Elton Region. *Plant Physiology Biochemistry*, vol. 118, pp. 385–393.

[15] Uchiyama, M. and Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, vol. 86, pp. 287–297.

[16] Kates, M. (1972). *Techniques of Lipidology: Analysis, Isolation and Identification of Lipids, 2rd*. Amsterdam: Elsevier.

[17] Rozentsvet, O. A., Nesterov, V. N., and Bogdanova, E. S. (2014). Membrane-forming lipids of wild halophytes growing under the conditions of prieltonie of South Russia. *Phytochemistry*, no. 105, pp. 37–42.