

Influences of Soda Soil on the Ultrastructure and Storage Inclusions of Chloroplasts of *Syringa oblata* Lindl.

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How to cite this paper: Zhao, Z.G. and Zhao, F.J. (2019) Influences of Soda Soil on the Ultrastructure and Storage Inclusions of Chloroplasts of *Syringa oblata* Lindl. *Natural Resources*, 10, 337-345.

<https://doi.org/10.4236/nr.2019.109022>

Received: July 30, 2019

Accepted: September 13, 2019

Published: September 16, 2019

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Abstract

With a high pH value, soda soil restricts the growth of plants. It had previously been assumed that the inhibition of plant growth by neutral salt stress was partly due to the changes of the ultrastructure and storage inclusions of chloroplasts. The aim of this paper is to investigate the effects of alkali-salt mixed stress of soda soil on chloroplasts of higher plant *Syringa oblata* Lindl. Testing *S. oblata* plants had grown for more than five years in soda soil, and transmission electron microscope was used for determining the ultrastructure and storage inclusions of chloroplasts. The results showed that some chloroplasts were destroyed, the normal chloroplasts became smaller, and the envelopes of the functional chloroplasts were slightly expanded generally in soda-stressed plants. The most noteworthy fact was that much more starch grains accumulated in the chloroplasts of *S. oblata* growing in soda soil than in neutral soil. The total contents of plastoglobules in chloroplasts did not change considerably, and plastoglobules with different electron densities appeared in chloroplasts in the plants growing in soda soil. We presume that the reduction of the volume and number of functional chloroplasts and the occupation of carbon resources by starch grains accumulated in chloroplasts were the possible reasons for the inhibition of *S. oblata* growth by soda soil. The accumulation of starch grains was one of the adaptive traits of *S. oblata*, which promoted the survival of plants in soda soil for that the starch grains could serve as a steady source of soluble sugars, which were known as protectors of plant cells under stressed conditions. Soda soil did not significantly change the total content of plastoglobules in chloroplasts, but changed their compositions.

Keywords

Lilac, Saline-Alkali Soil, Structure, Inclusion, *Syringa oblata*

1. Introduction

Soil salinization is a severe environmental problem. According to the data from the United Nations Environment Program, approximately 20% of total agricultural land in the world is salt-stressed [1] [2]. Salinity kills varieties of kinds of plants, and limits the vegetative and reproductive growth of the survivals even at low salt concentration [3] [4]. In spite of the fact that soil salinization and alkalinization frequently co-occur, studies of soil salinization were mostly focused on neutral salt stress, whereas the alkali stress was generally neglected. In fact, the alkali stress caused by NaHCO_3 and Na_2CO_3 is more severe than neutral salt stress caused by NaCl and Na_2SO_4 [5] [6] [7].

Songnen Plain in the northeast of China is one of the three most serious soda-stressed areas in the world [8]. Soda soil is a type of alkalized soil caused by NaHCO_3 and Na_2CO_3 . Salinity, alkalinity and drought restricted the development of the regional economy. To exploit saline soil and protect the environment, it is a feasible way to grow salt tolerant plants in salt stressed regions [9] [10]. In order to study the adaptability of woody plants to soda soil and the influences of the environmental stress on the growth of plants, and to screen plant varieties adapt to growing in soda soil, we founded a trial base on soda soil in the suburb of Baicheng in the west of Songnen Plain in 2016, and planted dozens of species of salt tolerant woody plants, including *Syringa oblata*. The focus of the present study on chloroplasts of *S. oblata* was determined by two considerations: 1) though with a relatively low growth rate compared with *S. oblata* growing in neutral soil, *S. oblata* could grow well in soda soil on the trial base, and 2) as the primary site of photosynthesis, chloroplasts play an important role in plant growth. In addition, soil samples were taken from the habitats of testing and control plants, and the pH and electric conductivity of the soil solutions were also determined.

2. Materials and Methods

2.1. Plant Materials

Testing *S. oblata* plants had grown for more than five years in soda soil at the trial base in the suburb of Baicheng, China. Control *S. oblata* plant materials were taken from Zoological and Botanical Garden of Changchun City, China.

2.2. Determination of pH and Electric Conductivity of Soil

The soil samples of the trial base were taken from five evenly distributed sites on the land where testing *S. oblata* plants were grown at depths of 0 - 10 cm and 20 - 40 cm. The soil of the same depth were fully mixed, air-dried at a shady place,

pulverized with a clean glass bottle, and bottled for examine. Control soil samples were taken from the nearby of control plants roots at depths of 0 - 10 cm and 20 - 40 cm, and were pretreated with the above-mentioned methods. Both soil samples were taken on a fine day at the beginning of March, July and November. Soil samples were sent to the Research Center of Analysis and Measurement of Northeast Normal University to measure the pH and electric conductivity of soil solutions. The electric conductivity of soil solutions, in which the ratio of soil to distilled water is 1:5 in weight, was detected with a conductivity meter (SevenGo SG3, METTLER TOLEDO, Switzerland).

2.3. Transmission Electron Microscopy

Fully expanded leaves of both *S. oblata* plants were cut into small pieces with a razor blade. These pieces were first fixed in 2.5% glaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2, under vacuum, for more than 24 h at room temperature, and then post-fixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature. After dehydration in a graded series of ethanol solutions and in acetone, leaf pieces were embedded in Epon 812 resin. Ultra-thin sections were cut with glass knives on a Reichert-Jung Ultracut-E ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a HITACHI H-600 transmission electron microscope. The Motic Image Advanced software was used for determining the areas of chloroplasts and storage inclusions and the diameter of the plastoglobules.

3. Results

3.1. Soil Property

According to determination, the soda soil of the trial base in the suburb of Bai-cheng is a kind alkalinized soil with high pH value, and the soil from Zoological and Botanical Garden in Changchun belongs to neutral soil (**Table 1**).

3.2. Ultrastructure and Storage Inclusions of Chloroplasts of *S. oblata* Growing in Neutral Soil

The longitudinal section of the chloroplast is oval in shape, with the major axis parallel to that of the cell (**Figure 1(A)**). The Chloroplast matrix is dense, with an electron density similar to that of thylakoids. Both the granal and stromal membranes are sometimes slightly expanded (**Figure 1(B)** and **Figure 1(C)**). Mitochondria of different sizes were observed near chloroplasts (**Figure 1(C)**).

In contrast with the chloroplasts of general mesophyll cells, which almost contained no starch grains, the chloroplasts of the mesophyll cells near the vascular bundle had big starch grains (**Figure 1(D)** and **Figure 1(E)**). The accumulation of starch grains near vascular bundle supports the transport of the products of photosynthesis from leaves to other organs via the vascular tissue.

Large amounts of plastoglobules accumulated in chloroplasts (**Figure 1(A)** and **Figure 1(C)**). Although Plastoglobules are primarily round in shape, oval

ones were also observed (**Figure 1(C)**). Inconsiderable difference in electron density between plastoglobules may depend on the thickness of ultrathin sections. There were numerous contacts between plastoglobules, plastoglobules and thylakoids (**Figure 1(B)** and **Figure 1(C)**). Cytoplasmic globules with the similar electron densities to plastoglobules were observed near chloroplasts (**Figure 1(C)**).

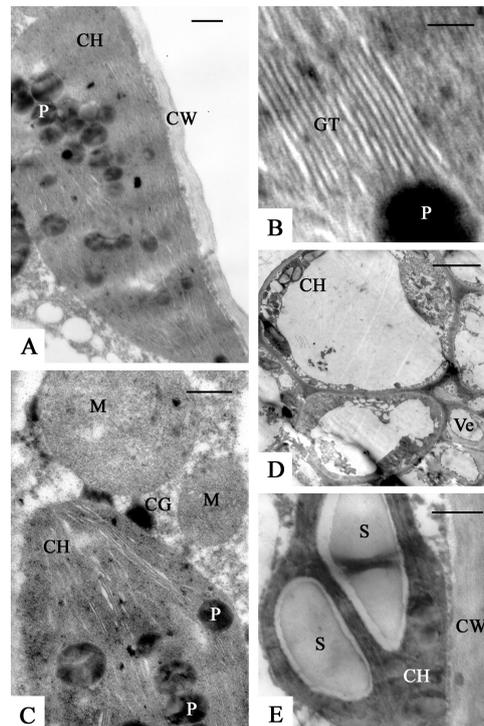


Figure 1. Ultrastructures and storage inclusions of chloroplasts of *Syringa Oblata* growing in neutral soil: A, Oval chloroplasts with vast of plastoglobules and contacts between plastoglobules. Scale bar = 0.5 μm . B, Magnification of the bottom part of A, showing the contacts between plastoglobules and slightly expanded granal thylakoids. Scale bar = 0.1 μm . C, Mitochondria of different sizes near chloroplast, cytoplasmic globules with similar electron density to plastoglobules, and the slight expansion of thylakoids. Scale bar = 0.4 μm . D, Starch grains accumulated in mesophyll cells near vascular bundle. Scale bar = 4 μm . E, magnification of the chloroplast indicated in D, showing big starch grains. Scale bar = 0.5 μm . Key (all figures): CH = chloroplast; CG = cytoplasmic globule; CW = cell wall; GT = granal thylakoid; M = mitochondria; P = plastoglobule; S = starch; V = vacuole; Ve = vessel.

Table 1. The pH values and electric conductivities of soil samples from different habitats.

	Soil sample	pH	*EC ($\text{s}\cdot\text{m}^{-1}$)
A	0 - 10 cm	8.73 ± 0.03	85 ± 1
	20 - 40 cm	9.08 ± 0.01	148 ± 10
B	0 - 10 cm	6.45 ± 0.11	57 ± 1
	20 - 40 cm	6.79 ± 0.01	38 ± 1

Values are mean \pm s.d., n = 3. *EC: electric conductivity of soil solution. The ratio of soil to distilled water is 1:5 in weight. A: soil sample from the trial base in the suburb of Baicheng, China. B: soil sample from the Zoological and Botanical Garden in Changchun, China.

3.3. Ultrastructure and Storage Inclusions of Chloroplasts of *S. oblata* Growing in Soda Soil

Chloroplasts are in the cytoplasm, which was separated from the cell wall (Figure 2(A)). The chloroplasts were smaller than those of the control plants (Table 2). The chloroplast somewhat rounded up, especially of the side toward the vacuole (Figure 2(A)). Sometimes, the envelope of chloroplasts bulged out to form protrusions comprised plastoglobules (Figure 2(B)). Most chloroplasts had well-developed thylakoids system with regular granal stacking (Figure 2(C)); in contrast, some chloroplasts expanded destructively, and their thylakoids system could not be discerned (Figure 2(D) and Figure 2(E)).

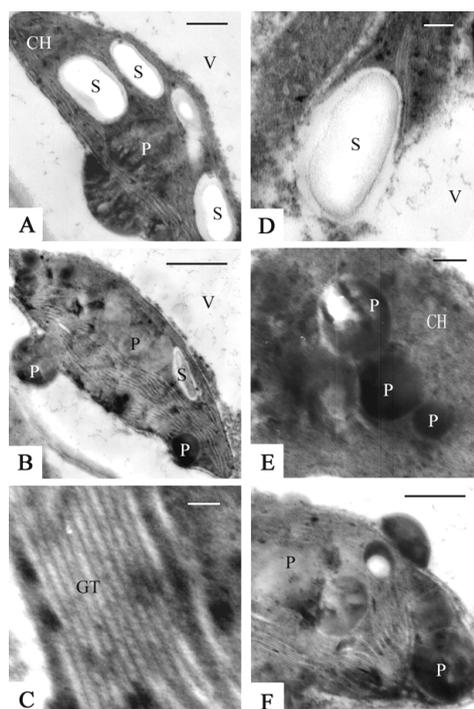


Figure 2. Ultrastructures and storage inclusions of chloroplasts of *S. Oblata* growing in soda soil: A, Large amounts of starch grains contents and slight expansion of chloroplasts toward vacuole. Scale bar = 0.5 μm . B, Plastoglobules of different electron densities and chloroplast protrusions comprised plastoglobules. Scale bar = 0.1 μm . C, Well-preserved granal stackings. Scale bar = 0.5 μm . D, Disorganization of a chloroplast contained a big starch grain. Scale bar = 0.2 μm . E, The electron-transparent zone of irregular shape in dark plastoglobules and contacts between plastoglobules. Scale bar = 0.2 μm . F, Plastoglobules of different electron densities. Scale bar = 0.5 μm .

Table 2. Sections of chloroplasts and storage inclusions.

Habitats	*Chloroplast area (μm^2)	*Starch grain area/chloroplast area (%)	*No. plastoglobules per chloroplast section	*Diameter of plastoglobules (μm)	*Plastoglobules area per chloroplast area (%)	**Ratio of broken chloroplasts and normal chloroplasts (%)
Neutral soil	9.26 \pm 0.53	1.36 \pm 0.07	13.31 \pm 1.67	0.29 \pm 0.06	29.89 \pm 3.12	0
Soda soil	5.71 \pm 0.68	23.40 \pm 5.44	6.20 \pm 0.54	0.56 \pm 0.06	32.01 \pm 2.31	5

*Values are mean \pm s.d., n = 30. **For 100 chloroplasts samples.

As compared to *S. oblata* growing in neutral soil, the starch grains content of chloroplasts considerably increased in *S. oblata* plants growing in soda soil (**Figure 2(B)**) (**Table 2**). Starch grains tended to accumulate at the chloroplast side close to the vacuole; probably due to this fact, the chloroplasts slightly projected towards the tonoplast (**Figure 2(A)**). Starch grains of different electron densities were observed in different chloroplasts; some of them were almost electron transparent (**Figures 2(A)-(D)**).

Compared with control plants, the plastoglobules of soda-stressed plants increased in volume, but decreased in number; the total content of plastoglobules did not change notably (**Table 2**). Plastoglobules with different electron densities were observed in chloroplast matrix (**Figure 2(B)**, **Figure 2(E)** and **Figure 2(F)**). Within some dark plastoglobules, there were electron-transparent zones of irregular shape (**Figure 2(E)**). Contacts between plastoglobules were clearly shown (**Figure 2(E)** and **Figure 2(F)**).

4. Discussion

With low amounts of precipitation and soda soil, the trial base is an alkaline, saline and droughty habitat for the *S. oblata* plants growing in it. The analysis of the electron-microscopic pictures showed that both the chloroplast ultrastructure and their storage inclusions under soda stress differed from control plants.

4.1. Ultrastructure of Chloroplast

Smaller chloroplasts could decrease the photosynthesis rate of *S. oblata* growing in soda soil. The slight expansion of chloroplasts envelopes of soda-stressed *S. oblata* might be due to the osmotic stress induced by salinity and drought [11]. The maintenance of well-developed thylakoids system, which was necessary for the growth of plants, manifested the adaptability of *S. oblata* to soda soil after growing in it for a long period. The destructive expansion of some chloroplasts decreased the number of functional chloroplasts, and consequently blocked the plant growth to some extent.

4.2. Starch Grains

The differences between the soda-stressed and control *S. oblata* plants were primarily related to the storage inclusions, especially to the content of starch grains. Hydrolysis of starch could produce sugars, which raised the osmotic pressure in the vacuoles and promoted water influx. Under stress conditions, sugars always accumulate as cell protectors, and starch serves as a specific sugar depot, which is important for its osmoprotective function [12]. Therefore, the capacity of *S. oblata* plant to accumulate large amounts of starch grains in the chloroplasts might promote its survival under soda stress. The inclination of starch grains to accumulate at the chloroplast side facing the tonoplast probably facilitated the entry of sugars into the vacuole. Various electron densities of starch grains were probably due to the dissimilar degrees of starch hydrolysis; and the electron-

transparent areas of starch grains might be the cavities left after starch lysis.

As a storage form of the outcome of photosynthesis, starch is an important carbon bank in plant. Although the vast amounts of starch grains accumulated in chloroplasts provided a steady source of sugars for plants under soda stress, they also occupied lots of carbon resources that did not, at least temporarily not, participate in the carbon metabolism, and consequently slowed down the growth of plants to a degree [13].

4.3. Plastoglobules

Plastoglobules were observed in chloroplasts of both soda-stressed and control *S. oblata*. Plastoglobules consist mainly of lipids, probably due to which they are primarily round in shape; in addition, plastoglobules also contain proteins, pigments and wax [14]. The well-preserved membranes of thylakoids system in chloroplasts suggest that the accumulation of plastoglobules did not result from the destruction of membranes. Lipids could be synthesized in chloroplasts, and moved into the cytoplasm later [15]. The chloroplast protrusions that consist of plastoglobules probably represented the midway step of the plastoglobules exit from the chloroplast into the cytoplasm. The similar electron density of plastoglobules and cytoplasmic globules might be due to the same origin of them.

It was presumed that the initial plastoglobules occurred at the thylakoid cavities; and when they later moved into the chloroplast matrix, their contacts with the thylakoids were not broken [16]. The contacts between different plastoglobules probably indicated the future fusing of them.

It was reported that salinity and water stress could induce the changes of phospholipid composition [17] [18]. Therefore, we presumed that it was the qualitative changes in plastoglobules that caused the appearance of various plastoglobules with different electron densities under soda stress.

5. Conclusions

In sum, we presumed that the reduction of the volume and quantity of functional chloroplasts, and the accumulation of large amount of starch grains in chloroplasts are the possible reasons for the inhibition of *S. oblata* growth by arid soda soil, and that both the accumulation of starch grains and the slight expansion of the envelope of chloroplast are the adaptive traits of *S. oblata* to arid soda soil, and that soda soil do not change the total plastoglobules content of chloroplast notably, but change their composition.

Both the soda-stressed and control *S. oblata* plants in this study grew in the natural environments; therefore, the results could be used for reference during the control and utilizing of arid soda soil via screening and introduction of salt tolerant plants.

Funding

This work was financially supported by a grant from the Natural Science Foun-

dation of Shandong Province (No. ZR2014CL032; ZR2015CL013) and Open Fund Project of Binzhou Key Laboratory of Environmental Engineering Technology (No. bzhks201404), also supported by the fund of key discipline of environmental engineering in Binzhou University.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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