THE ROLE OF ER-BODIES IN BRASSICACEAE RESISTANCE UNDER CLINOROTATION

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ABSTRACT

Results of the electron-microscopic investigation of root apices of *Arabidopsis thaliana* 3– and 7–day old seedlings grown in the stationary conditions and under clinorotation are presented. It was shown the similarity in the root apex cell ultrastructure in control and under clinorotation. In the same time there were some differences in the ultrustructure of statocytes and the distal elongation zone under clinorotation. For the first time, the sensitivity of ER-bodies, which are derivative of granular endoplasmic reticulum and contain a β-glucosidase enzyme, to the influence of simulated microgravity that was demonstrated by increasing quantity and area of ER-bodies per cell section, as well as by higher variability of their shape under clinorotation. A degree of these changes correlated with the duration of clinorotation. On the basis of obtained data, a protective role of ER-bodies in adaptation of plants to microgravity is discussed.

1. INTRODUCTION

Important features of plants for space exploration missions are the stress tolerance to changes in the physical environment. There are relatively much data on the changes in the ultrastructure of *Arabidopsis thaliana* root and leaf cell organelles – plastids and mitochondria, as well as cell wall under the influence of real and simulated microgravity [1, 2]. In the same time, the information on the structure of ER-bodies in microgravity, which are derivatives of granular endoplasmic reticulum (GER) and characteristic for species of family *Brassicaceae* [3, 4], are absent. Recently, an enzyme β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21) with an ER retention signal has been shown to accumulate selectively in such bodies in response to different unfavorable factors [5–8]. β-glucosidase is appeared to perform the protective function. Therefore, the aim of our work was to study the ultrastructure of *A. thaliana* root apex cells under simulated microgravity, in particular root cap statocytes and cells of the distal elongation zone (DEZ) of a root proper, paying the main attention to the structure and topography of GER and ER-bodies.

2. MATERIAL AND METHOD

Seeds of *A. thaliana* (line Columbia) were sterilized and then sown on the MS' mineral medium in containers placed on the slow horizontal clinostat (2 rpm). Seedlings grew for 5 and 7 days at 23 ± 1°C in the dark in the stationary conditions and under clinorotation. Root apices were fixed in 2.5 % glutaraldehyde and 1 % OsO_4 on 0.1 M cacodylate buffer, pH 7.3. Samples were dehydrated in a graded ethanol series and propylene oxide and embedded in epon-araldite resin by a standard technique [9]. Sections of 50-70 nm of thickness were obtained on an ultramicrotome RMC MT-XL (USA), stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM 1200EX (Jeol, Japan) operated at 60 kV. Photonegatives were used for morphometry. Quantitative data have been statistically processed.by Statistica 6.0.

3. RESULTS AND DISCUSSION

It has been shown that ultrastructure of both statocytes and DEZ cells is typical for cells of these types described earlier [1]. Under clinorotation, the most prominent distinction in the statocyte structure was the localization of amyloplasts-statoliths, which did not sediment in the distal part of a cell but they revealed a tendency to group in its center, as that have been reported for other plants [2]. A nucleus was in the proximal part of a statocyte. Cells of the DEZ differed mainly on a mitochondrion size and number from those in the control GER in DEZ cells is presented with long cisterns, which have an ability to branch. Under clinorotation, a number of GER profiles on the cell section increased in comparison with control. On the statocytes and DEZ cells sections, it was clear seen that ER-bodies are the local enlargements of GER cisterns. Naturally, ER-bodies are surrounded of a single membrane with ribosomes and contain the thin fibrillar contents. On the cell sections, they have usually a rounded or oval shape (Fig. 1).

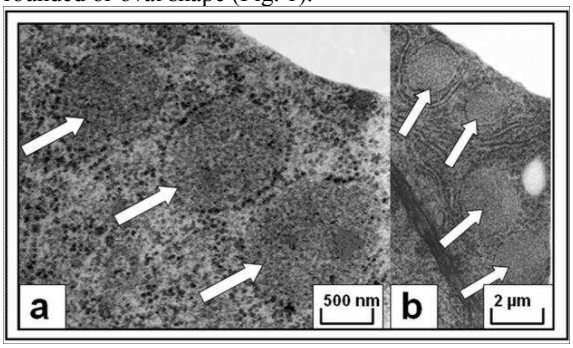

Fig. 1. Fragments of root DEZ cells of the 7-day old seedlings of A. thaliana: a – control, b – clinorotation. Arrows show ER-bodies.

An average size of ER-bodies vary in the seedlings of different age: it was 0,24±0,08 µ² in 3-day old seedlings and 0,25±0,11 µ² in 7-day old ones, total area of ER-bodies per section was 0,54±0,18 µ² and 0,60±0,20 µ²,

respectively. Under clinorotation, the total area of ER-bodies per cell section increased more than twice and was 1,14±0,30 μ² in 3-day old seedlings and 1,32±0,25 μ² in 7-day old ones. Such increasing in the ER-body total area is caused by the augmentation of ER-body number and size (Table 1, Fig. 2).

Table 1. An average number of ER-bodies per cell section of statocytes and root DEZ cells of A. thaliana seedlings, (each, M ± m, n = 144).

Cells	Control	Clinorotation
3-day old seedlings		
Statocytes	1,40±0,12	2,2±0,61
DEZ	2,37±0,77	3,45±0,84
7-day old seedlings		
Statocytes	1,50±0,05	2,87±0,13
DEZ	2,62±0,80	3,66±0,87

The variety of ER-bodies also increased, especially in 7-old seedling: an average area of the smallest bodies was from 0,7 till 0,14 μ², an area of the largest bodies reached ~ 0,49μ², and bodies had frequently an elongated shape. So, the formation of ER-bodies both in

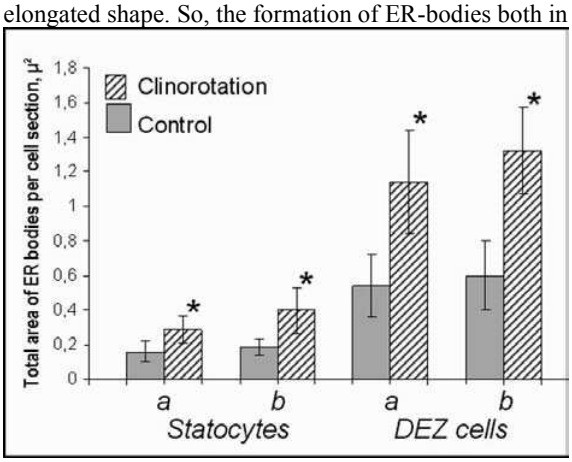

Fig. 2. Total area of ER-bodies per section of statocytes and root DEZ cells in A. thaliana in control and under clinorotation: a - 3-day old seedlings, 7-day old seedlings. Significant changes between control and experiment (<0.05).*

statocytes and DEZ cells turned out sensitive to the influence of clinorotation. As it is known, ER-bodies in cotyledon and hypocotyl cells of *A. thaliana* seedlings are characteristic with the high lability and sensitivity to wounding, mechanical pressure, toxic substances, diseases, and eating by insects [5]. A number and size of ER-bodies increased under the influence of such agents. Just detection of β-glucosidase in ER-bodies was conditioned much attention to the formation and role of these bodies in a cell. Whereas it is thought that an enzyme β-glucosidase is the main component of ER-bodies, they are assumed to be a depot of accumulation and keeping this protein for the defense of a cell from an action of stress agents. Existing appreciable differences in the total area of ER-bodies per cell in statocytes and in the DEZ cells may be explained with various functions of these cells in root development. Statocytes are highly specialized cells for gravity perception. DEZ cells distinguished themselves with special physiological properties and, therefore, respond to the action of exogenic, including gravity, and endogenic signals in a different way than other root cells [10–12]. DEZ cells provide the preparation to cell fast growth in the central elongation zone. As highly metabolizing cells, they are the most sensitive to altered gravity [13]. Thus, the influence of clinorotation on the formation dynamics of ER-bodies, which are derivative of GER and contain an enzyme, in statocytes and cells of the root distal elongation zone of *A. thaliana* seedlings was in the first time detected. The obtained data allow to assume that ER-body volume increasing under clinorotation is an adaptive cell reaction on the influence of simulated microgravity.

4. References

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