DIFFERENTIATION OF PLANT GRAVIPERCEIVING AND GRAVIRESPONDING CELLS IN ALTERED GRAVITY

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ABSTRACT

A main goal of our work was to compare the anatomy and ultrastructure of a root cap, including statocytes (graviperceiving cells), and a root proper meristem and elongation zone (graviresponding cells) of *Beta vulgaris* seedlings grown in the control and under clinorotation as a root apex is a very convenient model for the study of plant cell gravisensitivity. The comparison of the ultrastructure and topography of cell organelles clearly showed the differences in growth by elongation and differentiation in time and space between statocytes and cortex cells of the distal elongation zone (DEZ), in dependence on their main functions. A root graviperceptive apparatus develops under clinorotation but it does not function. DEZ cells reveal the highest metabolism activity in both variants that can underlie their specific physiological properties and provide cell rapid growth in the central elongation zone.

1. INTRODUCTION

A discovery of plant cell gravisensitivity is based on the changes in the cell metabolism and ultrastructure in microgravity. In order to better understanding of the mechanisms of these phenomena it was supposed to distinguish between the terms "graviperception" and "gravisensing". The first is related to actively using by cells a gravity stimulus for realization of plant normal space orientation, growth and vital activity. The second is related to the stability of cell metabolism and ultrastructure in the gravitational field and their changes in microgravity [1]. Despite of intensive studies dealing plant gravisensitivity, including cell graviperception and gravisensing, many steps are controversies and many questions remain unsolved.

One of the experimental approaches for understanding of cell gravisensitivity is the performance of investigations in the conditions of low microgravity – real microgravity in space flight and partially simulated microgravity by using clinorotation. The proposed object, namely root apices, is a very convenient model for such the complex comparative research. Therefore, a main goal of our work was the analysis of the anatomy and ultrastructure of a root cap columella (graviperceiving cells) and the root proper meristem and elongation zone (graviresponding cells) of *B. vulgaris* seedlings grown in the stationary conditions and under slow horizontal clinorotation**.**

2. MATERIAL AND METHODS

The calibrated dry fruits of *B. vulgaris* (cv Bordo 237) were wrapped in the tubes from a moist filter paper and placed into glass vessels allocated in special containers. One part of the containers was placed on the slow (2 rpm) horizontal clinostat, the second one was in the stationary conditions. Fruits germinated and seedlings grew at the temperature 23 ± 1 °C and 67 % humidity in darkness.

For the investigation, we used root apices of 3-day old seedlings, in which there are meristematic initials of the epidermis, cortex and central cylinder and initials of root cap columella and peripheral zone. The differentiation of meristematic cells proceeds in two opposite directions: to the basal part of a root proper – elongation and differentiation zones, and to the apical part – zones of elongating and differentiating cells, namely statocytes and secretory cells. Root apices were fixed in 3% glutaraldehyde (0.1M phosphate buffer, pH 7.2) and 1% OsO_4 (the same buffer). Fixed samples were dehydrated in ethanol series followed by acetone and embedded in the mixture of epoxy resins. Semithin and thin longitudinal sections were obtained on the ultramicrotome LKB III, stained with 1% methylene blue or lead citrate and analyzed with a light microscope Axioscope (Zeiss, Germany) and an electron microscope JEM 1200EX (Jeol, Japan).

3. RESULTS AND DISCUSSION

We revealed that the columella cell height decreased under clinorotation that leads to decreasing of a root cap size. At the same time, a columella diameter increased. Under clinorotation, a mitochondrion size in cap meristematic cells decreased. In general, a root graviperceptive apparatus develops under clinorotation, but does not function (amyloplasts-statoliths do not sediment in the distal part of a statocyte) due to permanent rotation of seedlings in the gravitational field.

In the main root of *B. vulgaris*, a length of the growth region in the elongation zone, where cells elongate slowly, is 380 μm in average. This region located between the apical meristem and the distal portion of the zone of rapid cell elongation is now defined as the transition zone. Cells of this zone accomplish a developmental transition recently from cytoplasmically driven expansion to vacuome driven elongation. Cortex

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and epidermis cells show a prominent transition zone composed of around 25 cells in each longitudinal cell file "postmitotic isodiametric growth zone" [2]. We use terms of [3] “distal elongation zone” (DEZ). This terminology indicates that these cells are near the distal end of the elongation zone but does not categorize them in terms of mitotic activity, shape, or allometric coefficient of expansion. The main distinguishing feature of these cells is their special physiological properties. The cells in DEZ differ from cells of the main or central elongation zone (CEZ) with respect to auxin sensitivity and exogenous factors, such as ethylene, extracellular calcium, water and salt stress, gravity, aluminium and microorganisms [4-6]. The differences in gene expression in the DEZ and the CEZ indicate that these zones are distinguished by more than their differing rates of elongation. The DEZ seems to hold the “key” for plant development.

According to our data, a cortex of the *B. vulgaris* DEZ consists of 4 layers, the innermost is differentiated to an endoderm. The epidermis and cortex as a rhizoderm provide apoplastic ion transport and take part in ion selective adsorption from apoplast and their including in symplast [7]. Cortex parenchyma cells connected between themselves in the radial and longitudinal directions that provides upwards and downwards symplastic transport inside this tissue. The characteristic feature of DEZ cells is the numerous functional contacts of organelles. Close attachment of mitochondria and plastids with a nuclear envelope and lipid droplets is usual. Analogous contacts of dictyosomes and plastids with ER are also observed. A connection between mitochondria and plastids can be established through a mitochondrium narrow elongated part. Lipid droplets are often closely surrounded with GER and also contact with Golgi vesicles. In addition to the described direct contacts of organelles and organelles with lipid droplets, in cells there are local associations of plastids, mitochondria, dictyosomes, GER and AER cisterns. We believed that the ultrastructure of cells in the DEZ visualizes clearly a sharp activation of metabolism connected with the formation of specific enzyme systems [8] as well as the accumulation of the cell wall material and low-molecular substances, that provides a rapid growth of cells in the central elongation zone (CEZ) and their special functions on uptake of water with mineral substances and its transport. The gradual vacuolization of cells in the DEZ occurs due to an activity of the Golgi apparatus and endoplasmic reticulum. The DEZ is ended with forming of a central vacuole. The highest metabolism state of cells in the DEZ is assumed to underlie their specific physiological properties which have been described early [3-6]. As actively metabolizing cells, they are the most sensitive to environmental signals.

Under clinorotation, more complex cytoplasmic membrane folds in DEZ cells in comparison with the control were observed. In addition, there were more of longer GER cisterns situated parallel both one to another and the nuclear envelope. As in the stationary conditions, amyloplasts surrounded a nucleus and the numerous contacts between organelles were also observed. Thus, root cell growth and differentiation processes occurred under clinorotation similarly with the stationary control. Some changes in the DEZ cell ultrastructure under clinorotation can indicate possible metabolism alterations in altered gravity.

The comparison of the ultrastructure and topography of organelles in cells originated from the meristems of a root cap and a root proper and transferred in statocytes and DEZ cells accordingly clearly shows the peculiarities of the elongation and differentiation in time and space of graviperceiving and gravireacting cells, in dependence on their main functions that is demonstrated below:

Root cap	**Root proper (epidermis and cortex)**
Short-term differentiation	***Long-term differentiation***
Meristem	Meristem
↓	↓
Transition zone, one-cell layer	Transition zone, DEZ, ~25-cell layers
↓	↓
Secretory cells	CEZ
↓	↓
Abscission	Mature zone
	↓
	Aging and Death

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