Structure and functioning of statocytes in *Lepidium* seedlings grown in weightlessness and under 1 g conditions

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To compare the structure and sensitivity of gravisensing cells in *Lepidium* axial organs formed under the action and in the absence of gravity (1 g), experiments on board the Bion 10 satellite as well as on a ground centrifuge-clinostat were carried out. Significant changes were observed in the structural polarity of statocytes formed in real weightlessness. If the mean position of amyloplasts comprised about 25% of the whole cell length under 1 g (reference centrifuge and ground control), in microgravity their distance from the morphological lower cell wall increased up to 48% in statocytes of both positive and negative gravitropic organs. A study of root responses to gravitropic ($0.004 \leq g \leq 0.1$) stimulation revealed that the bending of roots grown under simulated weightlessness was stronger than under 1 g, however, the evaluated g-threshold values were almost the same – 0.0016 and 0.0015 g, respectively.

Key words: statocyte, amyloplast, gravisensing, threshold, *Lepidium*, root, hypocotyl, gravitropism

INTRODUCTION

To date, it is clear that the structural organization of specialized gravisensing cells – statocytes plays an essential role in gravity sensing [1, 2]. The structural changes of root statocytes developed in microgravity [3–7] address the question concerning the competence of such cells to fulfil their main function – gravisensing. However, only a limited experimental support of such a viewpoint exists at present [8, 9]. Attention should be given to the fact that most of the studies on gravitropic sensing were performed either on root or on aerial axial organs, but not on different organs of the same plant. Taking into consideration the both above-mentioned problems, we focused on a comparison of the structure and sensitivity of the statocytes in *Lepidium* axial organs formed under the action and in the absence of gravity (1 g). The objectives of the present investigation were: 1) to compare the structural properties of statocytes in hypocotyls and roots of seedlings grown in authentic weightlessness (microgravity) and on a 1 g reference centrifuge during the Bion 10 mission; 2) to determine the minimal mass acceleration (g-threshold) of gravitropic stimulation for roots of seedlings grown under simulated weightlessness (horizontal clinostat) and normal (1 g) conditions.

MATERIALS AND METHODS

For all experiments the test material was *Lepidium sativum* L., Crysant, Bonn, Germany. The experiment carried out during the Bion 10 satellite flight was performed with a Neris-5 automatically operating system consisting of a stationary setup under real weightlessness (MG) and in a 1 g reference centrifuge (RC). A detailed description of experiment methods is presented elsewhere [5]. After 27 h of growth the space and ground control seedlings were fixed in 4% glutaraldehyde, postfixed in 1% OsO_4, and embedded in Epon. Morphometrical measurements were performed by light microscopy on median longitudinal sections of root apices and hypocotyls.

To determine the g-threshold for *Lepidium* roots, experiments were carried out on a centrifuge-clinostat with the equipment and by the methods described earlier [10, 11]. Differently from the earlier methodology, the roots were selected before the g-treatment. It was shown that the root populations of cress grown for 25 h vertically under 1 g and on a horizontal clinostat differed significantly in their deviation from straight growth direction [11]. Roots of both variants in which the deviation exceeded ±2.5° from straight growth were excluded from the sample. Gravitropic responses to lateral g-treatment on the centrifuge-clinostat were measured as described previously [10].

RESULTS AND DISCUSSION

Morphometry of the statocytes in *Lepidium* seedlings grown in space experiment. It has been suggested that along with gravity, the elastic forces of the cytoskeleton determine the polar structure of statocyte and take part in the positioning of amyloplasts acting as statoliths [1, 7]. This is supported by data on the spatial location of amyloplasts in root statocytes of different plant species subjected to changed gravity. In spaceflight or on clinostat, where the constant amplitude and/or direction of gravity vector are removed, the polar structure of the statocytes was altered on account of centrally-located statoliths [3, 4, 7, 6]. Our data concerning amyloplast localization in the statocytes of roots and hypocotyls of *Lepidium* seedlings developed in space and on Earth are presented in Fig. 1. Statolith position was measured as a distance of plastid center from the morphological lower wall of the statocyte and expressed in % of the whole cell length. The mean position of statoliths comprised about 25% in the statocytes of seedlings germinated and grown under the action of 1g (ground control and reference centrifuge). Interestingly, there was no considerable difference in the localization of these plastids between the gravisensing cells of roots and hypocotyls. As expected, significant changes took place in the structural polarity of statocytes formed in microgravity. The statoliths in MG were found to be concentrated around the cell center and their mean position was increased by up to 48% in the statocytes of roots as well as hypocotyls. Thus, our results showed that the positioning of amyloplasts and its changes under MG in the gravisensing cells was similar in positively and negatively gravitropic organs of *Lepidium* seedlings. These data coincide with the results of recent space experiments with cress and lentil roots [4–7]. The obtained amyloplast location in MG statocytes is consistent with that determined for *Lepidium* roots grown on clinostat in the previous study [6]. Taken together, these findings demonstrate the importance of gravity in keeping the statocyte polarity. If the structural modifications occur in gravisensing cells formed in microgravity or on clinostat, a shift of their gravisensitivity might be expected, too.

The sensitivity of roots grown vertically under 1 g and in simulated weightlessness. One of the basic parameters of gravitropic sensitivity is the minimum mass acceleration still allowing to overcome thermal noise and to evoke gravitropic response. To test this parameter, *Lepidium* roots grown vertically and on the clinostat were then placed in a centrifuge-clinostat and stimulated for 60 min by mass acceleration in the range of 0.004 g to 0.1g. The data presented in Fig. 2 indicate that the graviresponse of roots grown on clinostat was greater than that of roots grown vertically. Due to a logarithmic relationship between mean responses and the mass acceleration applied, the regression lines of the type $R = a + b\ ln(g)$ were calculated, where $a = 45.72$ and $b = 7.12$ (r = 0.965) for clinorotated and $a = 31.25$, and $b = 4.81$ (r =

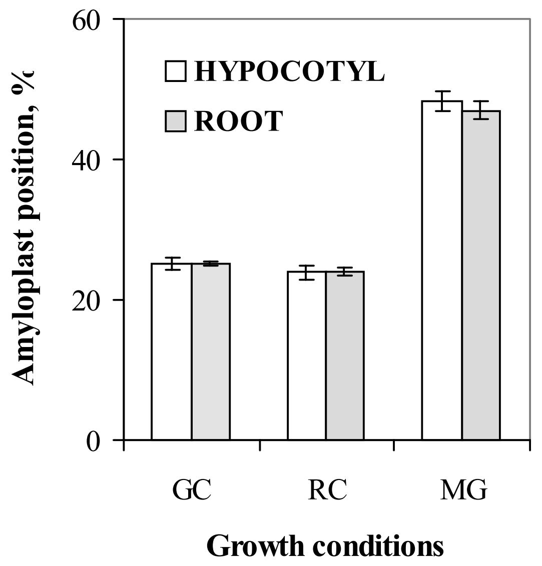

Fig. 1. The position (mean ± SE) of statoliths in hypocotyl and root statocytes of *Lepidium* seedlings grown in space experiment. GC – ground control, RC – reference centrifuge and MG – microgravity in spaceflight

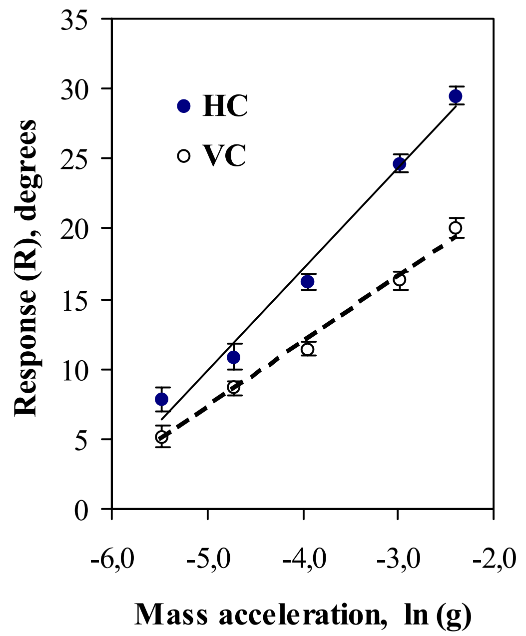

Fig. 2. Gravitropic response as a function of mass acceleration in *Lepidium* roots grown vertically (VC) and on horizontal clinostat (HC). Each point (mean ± SE) was obtained from N ≥ 50 of VC and N ≥ 103 of HC roots

= 0.995) for control roots. The g-threshold values of 0.0016 g for HC and 0.0015 g for control roots were evaluated by extrapolation of these regression lines to zero response. It is evident that these values are very close, however, lower than those determined in our previous studies of gravitropism in whole populations of *Lepidium* and *Lactuca* organs grown under 1 g [10, 11]. For comparison, the g-threshold values of about 0.0024 g and 0.0034 g have been evaluated for roots and hypocotyls, respectively.

In conclusion, despite differences in the structure of the statocytes of *Lepidium* roots grown in weightlessness and under 1 g, they possess a gravisensing system of similar sensitivity to mass acceleration.

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***Lepidium* DAIGŲ, AUGUSIŲ NESVARUMO IR 1 G SVARUMO SĄLYGOMIS, STATOCITŲ STRUKTŪRA IR FUNKCIONAVIMAS**

S a n t r a u k a

Siekiant įvertinti gravitacijos įtaką teigiamai ir neigiamai gravitropiškų organų gravisensorinių ląstelių struktūrai ir funkcionavimui, atlikti eksperimentai su *Lepidium* daigais biopalydove Bion 10 ir centrifugoje-klinostate Žemėje. Mikrogravitacijos sąlygomis augusiuose daiguose nustatyti patikimi ir panašūs hipokotilių ir šaknų statocitų struktūros pokyčiai. Veikiant 1 g (borto centrifugoje ir Žemės gravitacijai) amiloplastų vidutinis atstumas nuo morfologiškai apatinės statocitų sienelės lygus maždaug 25% viso ląstelės ilgio, o mikrogravitacijos sąlygomis šis atstumas siekia 48%. Tiriant šaknų reakciją į gravitropinį ($0{,}004 \leq g \leq 0{,}1$) dirginimą nustatyta, kad imituoto nesvarumo sąlygomis klinostate augę šaknys linko intensyviau už kontrolines. Tačiau abiem sąlygomis augusių šaknų gravisensorinės sistemos jautrumas, apibūdinamas ekstrapoliacijos metodu apskaičiuotomis g slenksčio reikšmėmis (0,0016 g ir 0,0015 g), beveik vienodas.