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OPTIMAL CLINOROTATION SETTINGS FOR MICROGRAVITY SIMULATION IN A. THALIANA SEEDLINGS

Abstract

Due to high cost and low availability of space (real microgravity) experiments, ground based facilities (GBF) for simulating microgravity are commonly used. The clinostat is one of the most widely GBF used in simulation experiments. Up to date, there is no clear set of rules on how to use 2D clinostats concerning the speed of rotation. With the objective of defining the optimal settings, we have used a mathematical model and compared the physiological response of *A. thaliana* 5-day-old seedlings to two speeds of the 2D clinostat: low speed (1 rpm) and high speed (60 rpm) for short (1, 2, 3 hours) and long (24 h) period of time. Seedlings were grown for five days in conditions of photoperiod, oriented according to the gravity vector. Then, they were subjected to clinorotation (1 or 60 rpm) in darkness to avoid the possible effect of light on the auxin gradients. Auxin is a phytohormone driving the physiological changes resulting in gravitropism. In a control experiment, we positioned seedlings perpendicular to the gravity vector in order to observe statoliths displacement producing changes in auxin gradient and to record the timeframe of the process, by collecting samples at defined time intervals. The gravitropic reaction of plants starts with the displacement of statoliths in columella cells. We investigated the displacement of statoliths by staining starch in these organelles with Lugol solution. Next, we observed the auxin gradient using two reporter lines: DR5::GUS and DII Venus. The changes in the position of statoliths were observed as soon as 1 h after experiment started. In the seedlings positioned perpendicular to the gravity vector, statoliths moved from the bottom of statocytes to the side of the cells, whereas in the seedlings exposed to clinorotation they appeared dispersed in the cells, which confirmed that plants were unable of sensing the direction of gravity vector. Accumulation of auxin was observed on the side of the root where gravity vector was acting after 1h in case of DII Venus and 2h in case of DR5::GUS. We observed qualitative and quantitative changes in auxin gradient in the meristems of seedlings exposed to two different speeds of clinorotation. We compared the results with previous experiments using RPM and in real microgravity. We conclude that the proposed mathematical model, as well as the physiological reaction of plants, confirms that the low speed (1 rpm) is more suitable for performing microgravity simulation experiments.