

# The Study of *Hydrilla verticillata*'s Cyclosis in Microgravity

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## Abstract

Cyclosis is the vital process in plant cell. In this work, the effect of microgravity on cyclosis of *Hydrilla verticillata* was investigated by cultured the plant under 12 hr light period of  $145 \pm 10 \mu\text{mol/s/m}^2$ , observed the rotation movements of chloroplasts from the leaves of the first to the fifth nodes which are next to the cluster of compressed leaves on the top of stem and measured the velocity of chloroplast movement under light microscope (4000x magnification) at  $27 \pm 2 \text{ }^\circ\text{C}$  (plane temperature) in the plane which provides 20 parabolic flights that operates microgravity for 20 seconds per flight. After measuring the average velocity of chloroplasts by ImageJ program, the average velocity of chloroplast in midrib cell is  $4.81 \pm 0.28 \mu\text{m/s}$  and  $4.99 \pm 0.30 \mu\text{m/s}$ ; in mesophyll cell is  $2.97 \pm 0.19 \mu\text{m/s}$  and  $2.51 \pm 0.17 \mu\text{m/s}$  in normal and microgravity condition, respectively. The average velocities of chloroplasts of two types of cell in the same gravity condition have significantly different. Furthermore, the average velocities of chloroplasts of the same type of cell in different gravity condition also have the significantly different ( $p < 0.05$ ). This work may suppose the basic knowledge about plant cell physiology and be the database for plant research in space.

**Keyword(s):** cyclosis, *Hydrilla verticillata*, microgravity

## 1. Introduction

Space experiments such as alter the gravity as none or micro level particularly in life science experiments are interested because the human explore other planet as new house and want to know can organisms survive in the space, and how they can. Plant is the most important part of our earth because it is the producer of every life. Plant preserves the equilibrium by using sunlight and carbon dioxide to produce glucose and oxygen gas which is very important for life. Long-duration human exploration missions in space or others planet also need plant for life support system. Thus, the study of plant in microgravity condition is very necessary.

Cyclosis or cytoplasmic streaming is the vital process in plant cell. It is driven by organells-associated myosin motors moving along actin filament bundles. Streaming may play a significant role in promoting the exchange of molecules and proteins across organelle membranes. This process is also responsible for mobility of ER, Golgi, peroxisomes, mitochondria and chloroplasts<sup>1</sup>. In addition, the entire vacuolar fluid is set in motion by forces generated in the cytoplasm<sup>2</sup>. The stirring of vacuolar fluid by streaming may also enhance mixing within a given cell, enabling the mobilization of metabolites and contributing to physiological homeostasis<sup>3</sup>. Furthermore, there are many researches on factors influencing the cyclosis. For instance, cytoplasmic streaming is inhibited by calcium ion and cytochalasin B<sup>4</sup>. Light also has an effect on cyclosis. However, there is no study about the effect of microgravity on cytoplasmic streaming.

Chloroplast movement is the obvious process of cytoplasmic streaming which is important for plant surviving to locate the most appropriate position to absorb efficient light for photosynthesis. Chloroplasts have alternative ways to response to different light intensity. They move toward weak light irradiated area (accumulation response) to absorb more light allowing efficient photosynthesis but move away from strong light when irradiated directly (avoidance response), avoiding absorb excess light lead to damage. Blue light receptor phototropin 2 (phot2) play role in avoidance from the strong light localized on the chloroplast envelop. Weak light-irradiated accumulation response is arranged by phot1 and phot2 localized on the plasma membrane. Movement is driven by chloroplast actin (cp-actin) filaments that must be polymerized by Chloroplast Unusual Positioning1 (CHUP1) at the front side of moving chloroplast<sup>5</sup>.

In this work, we are interested in the effect of zero-gravity condition on cytoplasmic streaming and focused our attention on *Hydrilla verticillata* because it is easy to see the movement of chloroplasts due to this specie has clearly cells and abundant in fresh water habitat. The aim of this work is to determine rate of *H. verticillata*'s chloroplast movement in zero-gravity condition. The results of this study may be an important data of plants in zero-gravity and also useful for space life in future.

## 2. Materials and methods

### 2.1 Plant material

*Hydrilla verticillata* or hydrilla obtained from natural habitat

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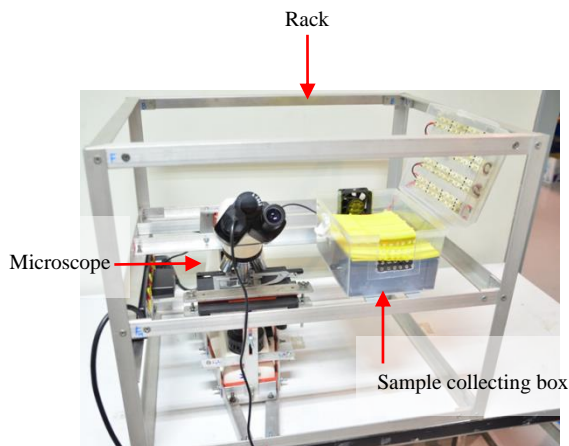
at Mahidol University and were grown submergible in a soil at ambient temperature with a 12 h. light /12 dark cycles in bottle containing drinking water (pH 7). Light intensity was  $145 \pm 10 \text{ umol/s/m}^2$ . Hydrilla was grown in laboratory around 1 month before experiment day. Leaves of hydrilla were used to observe chloroplast movement by light microscope.

## 2.2 Sample preparation

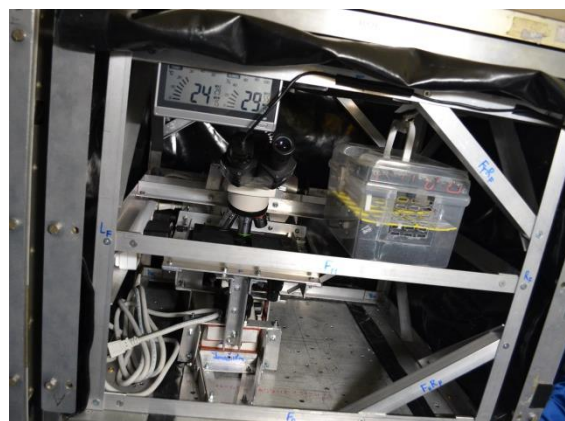
2 leaves of hydrilla were collected from the first to the fifth nodes, which are next to the cluster of compressed leaves, on the top of stem. Leaves were placed on plastic slide and dropped water around  $20 \text{ }\mu\text{l}$ . Then cover slip was covered on sample and sealed with scotch tape for preventing leakage of water. Samples were kept in collecting box with spongy for reducing the vibration in the flight.

## 2.3 Parabolic flights

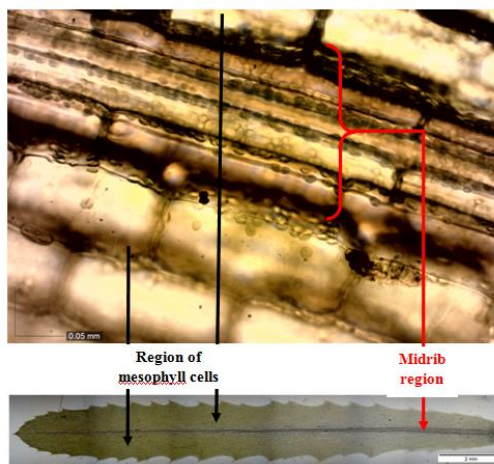
Movement of chloroplasts of hydrilla in response to normal- and microgravity was monitored during the parabolic flight operated by the Japan cooperation Diamond Air Service (DAS). A modified airplane G-II (gulfstream II) was applied to fly 20 times parabolas, which were carried out on two flight days. Each parabola consists of five phases: (1) 40 s at  $0.5\text{-}1.2 \times G$  (before pull-up phase), (2) 20 s at  $1.8 \times G$  (pull-up phase; maximal inclination angle =  $45^\circ$ ), (3) 20 s in microgravity ( $1 \times 10^{-3} \times G$ ; actual parabola), and (4) 20 s at  $1.5 \times G$  (pull-out phase, maximal inclination angle of  $-35^\circ$ , i.e. downward flight), (5) 40 s at  $0.5\text{-}1.2 \times G$  (after pull-out phase). The pattern of gravity changes are shown in Fig. 1. For observation the experiment, we used the light microscope (4000x magnification) connected with microscope eye-piece camera (Fig. 2). The microscope eye-piece camera was connected to computer and collecting the data with Dino-capture 2.0 programs in video capturing mode. The cells of hydrilla leaves were separated to 2 areas for observation including region of mesophyll cells and mid-vein region (Fig. 3). After each sample was placed on the stage of light microscope in experiment rack, we recorded video of chloroplasts from 1<sup>st</sup> phase to 3<sup>rd</sup> phase of each parabola. Temperature on board experiment was controlled at  $27 \pm 2 \text{ }^\circ\text{C}$ . Videos were further applied to analyze.



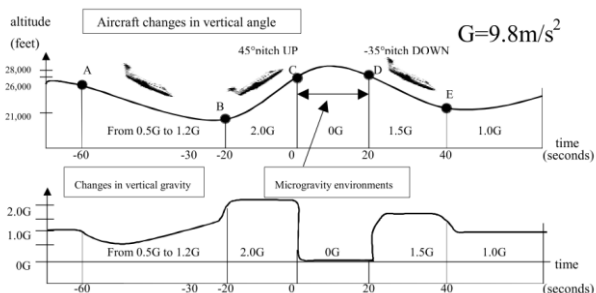
**Fig.2** The experiment rack



**Fig.3** The experiment rack for observation on parabolic flight experiment



**Fig.4** The observed regions of *Hydrilla* leaf



**Fig.1** The pattern of gravity changes

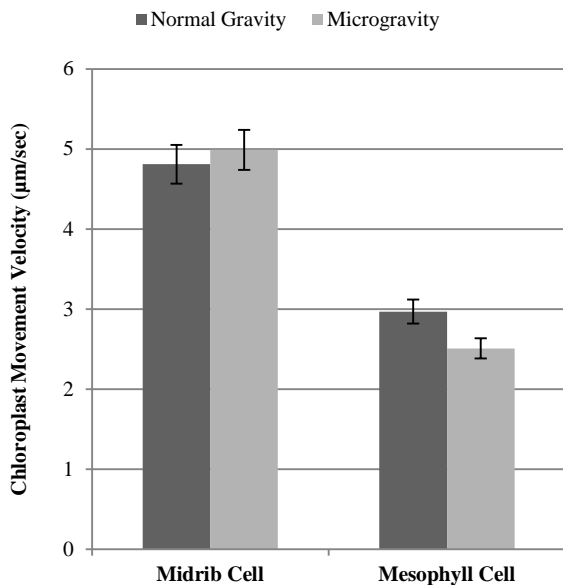
## 2.4 Analysis

The videos of chloroplasts movement were analyzed by manual tracking method in ImageJ. The velocity of chloroplasts at each gravity condition was calculated by Manual Tracking. Comparison of velocity in normal gravity and microgravity was analyzed by t-test at ( $p < 0.05$ )

### 3. Results and discussions

Conditions	Chloroplast Movement Velocity ( $\mu\text{m}/\text{sec}$ )	
	Midrib Cell	Mesophyll Cell
Normal Gravity	4.81 $\pm$ 0.28	2.97 $\pm$ 0.19
Microgravity	4.99 $\pm$ 0.30	2.51 $\pm$ 0.17

**Table 1** Chloroplast movement velocity in normal gravity and microgravity of different cell.



**Fig.5** Bar chart of Chloroplast movement velocity in normal gravity and microgravity of different cell.

Cyclosis or cytoplasmic streaming is the term of fluid circulation and organelles within cell. This process aids in transport products of the cell including metabolite, gene, protein, ion, and others to all area. The molecular mechanism of cyclosis is generated by myosin XI sliding associated with organelles along actin filaments, using the hydrolysis energy of ATP. Chloroplast movement is the obvious result from cyclosis. The movement occurs to adjust the most appropriate position to absorb sufficient light for photosynthesis.

*Hydrilla verticillata* is an aquatic monocot plant found commonly in freshwater habitat. Due to this species being aquatic, which has high water content in cells, it has a very clear cell that can be seen through and is easy to observe chloroplasts. This plant is the best documented case of an inducible C4 photosynthetic system that concentrates  $\text{CO}_2$  in the chloroplasts without enzymatic compartmentation in mesophyll and bundle sheath cells, i.e. it lacks Kranz anatomy. *H. verticillata* exhibits C3 when  $\text{CO}_2$  concentration is abundant, but when an environment is low  $\text{CO}_2$ , the photosynthetic system is induced into C4. A unique trait of this system is that the C4 and Calvin cycles exist

together within the same cell, and the site of  $\text{CO}_2$  concentration is the leaf mesophyll chloroplasts<sup>6</sup>.

In this work, we investigated the rotational movement of chloroplast in *H. verticillata*'s leaf cell. The leaf cell can be divided into 2 types due to their different characteristics, including mesophyll cell – the photosynthetic parenchyma cell (50-100  $\mu\text{m}$  long, 30-42  $\mu\text{m}$  in length), and companion cell (130-150  $\mu\text{m}$  long, 11-17  $\mu\text{m}$  in length) which is in midrib region<sup>7</sup>.

In normal gravity, the mesophyll chloroplast has a significantly less velocity than chloroplast in midrib region. Mesophyll cell is the major photosynthetic area of *H. verticillata*<sup>8</sup>.

Companion cell is the part of phloem which transports nutrients to all regions of the plant. The cell transports nutrients to sieve tubes via branched plasmodesmata<sup>7</sup>. This difference of the cell may lead to different velocities of chloroplast movements.

In microgravity, the velocity of chloroplast movement in mesophyll decreased significantly, resulting from cyclosis, which is the process involved in photosynthesis<sup>9</sup>. Previous studies had found that microgravity can affect photosynthesis of dwarf wheat. There was a reduction in whole chain electron transport (13%), PSII (13%), and PSI (16%) activities observed under saturating light conditions, suggesting that microgravity-induced responses at the canopy level may occur at higher photosynthetic photon flux (PPF) intensity<sup>10</sup>. The electron transport chain of light reaction is the process of ATP synthesis. ATP plays an important role in cyclosis. If an electron transport chain was reduced, it would be the effect from a less light reaction, leading to a decrease in ATP of the cell. Thus, the velocity of cyclosis also declined in microgravity. In addition, the change of velocity was the result of actin-myosin association. Myosin XI involves in cyclosis by associating with organelles and sliding along actin filaments<sup>5</sup>. The structure of actin filaments is the component of globular protein subunits that can rapidly assemble and disassemble in the cell. The assembly and disassembly of such labile structures is governed by gravity<sup>11,12</sup>. Thus, microgravity causes the slow assembly and disassembly of actin, leading myosin to carry an organelle and move along actin filaments slowly, and the velocity of cyclosis was reduced.

Actin-myosin processes of cyclosis use intracellular  $\text{Ca}^{2+}$  as secondary messengers which can change the intracellular concentration by microgravity, leading to a change in the system of cyclosis. In addition, intracellular  $\text{Ca}^{2+}$  stores were discussed as an important role in the regulation of chloroplast movement<sup>13</sup>. Consequently, microgravity can affect the movement of chloroplasts as a result of  $\text{Ca}^{2+}$  concentration change.

Surprisingly, chloroplasts in companion cells in microgravity conditions moved significantly faster than in normal gravity conditions. The interesting chloroplast movement velocity in midrib is found in *Anacharis densa*, which is in the Hydrocharitaceae family like *H. verticillata*. The experiment

was treating herbicide: 2,4 dichlorophenoxyacetic acid to *A. densa* found that four days the leaves had bleached to a light yellow, but the midrib still was faintly green at that time. Cyclosis was present only in the midrib cells where it was very active. Apparently the midrib cells are the last to be affected<sup>14</sup>. It seems like the velocity of cyclosis in midrib was increased when exposure to a stress condition or not have ability of photosynthesis. As microgravity is the one of stress condition, our experiment found the chloroplast movement velocity of mesophyll which is the main cell of photosynthesis was declined, but faster in midrib like cyclosis velocity of midrib was faster in *A. densa* treated with herbicide. It is noteworthy that cyclosis in midrib is the region of homeostasis maintenance of plant.

#### 4. Conclusions

Cyclosis can indicate by measure chloroplast movement velocity. Microgravity affected cyclosis that chloroplast movement velocity in mesophyll cell was decreased, but increased in companion cell. The effect was the result from change of ATP synthesis, intracellular Ca<sup>2+</sup> concentration, and actin-myosin association from altered gravity condition. The decrease of the velocity in mesophyll cell elucidate that photosynthesis of plant in microgravity may also decrease. In the future, growing plant in space should to find how to maintain the cyclosis and the mechanism of cyclosis in companion cell although it is not the major region of photosynthesis, because both cell involve together in synthesis and transport nutrients to all part of the plant.

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