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Live Imaging Technique for Studies of Growth and Development of Chinese Cabbage Under Microgravity in a Recoverable Satellite (SJ-8)

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Abstract The live imaging techniques have been developed and applied to investigate for the first time the growth and development of Chinese cabbage for 18 days under microgravity conditions on board the Chinese SJ-8 recoverable satellite. These experiments offer insight into plant behaviors operating during plant development in space. Two automatic, preprogrammed CCD cameras were installed in the plant experimental chamber. The experimental objectives were: (1) seed germination; (2) seedling growth; (3) flower opening and pollination. The growth of seedlings and flowers were followed by time lapse photography at 2 h intervals. Serial real-time images of the Chinese cabbage plant growth under microgravity were successfully obtained through the remote operating system. The image data obtained from space experiment, in comparison with the results from ground control (1 g) and 3D clinostat stimulate experiments, showed that the height of plant and the number of leaves were significantly reduced under the microgravity conditions, but characters of leaf arrangement and leaf shape were not altered obviously. Flower opening and expansion were inhibited by exposed to space flight condition. The petals of flowers from both SJ-8 grown plants and clinostat rotated plants couldn't fully expand before wilted.

Keywords Chinese cabbage · SJ-8 · Live imaging technique

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Introduction

The study of plant growth and development under space flight conditions is of great significance to better understand the role of gravity in developmental biology and to explore the potential methods of using plant in controlled ecological life-support system under microgravity conditions. A series of space experiments with Arabidopsis, *Brassica rapa* and wheat have been performed to elucidate the effect of gravity on higher plant growth and development (Merkys and Laurinavicius 1983; Halstead and Dutcher 1987; Musgrave et al. 1997; Kiss et al. 1998; Kuang et al. 2000; Bubenheim et al. 2003). Kuang et al. (1996) reported that development of Arabidopsis flower was apparently affected by exposure to microgravity (Kuang et al. 1996). Ultrastructure and storage reserves in seed of *Brassica rapa* were changed in microgravity environment (Kuang et al. 2000). Abnormal development of the reproductive structures of "super dwarf" wheat grown on the Mir space station was also observed (Bubenheim et al. 2003). Most of these results, which were obtained by analysis of chemically fixed samples in space or living plant samples collected after returned to Earth (Brown et al. 1990; Kuang et al. 2000; Bubenheim et al. 2003), only could be reflected the state of samples at several time points and get results after flight. Since the opportunity to study the role of gravity in space is limited, application of living image in flight is important to enhanced scientific productivity by rapid

feedback of results during mission and increase on-board analysis capability.

Optical imaging combined with lineage analysis have been extensively used in studying animal and plant growth patterns at both organ and cellular levels, but the direct experimental determination of plant behavior, in real time, is generally lacking in space experiment. Detailed knowledge of dynamic patterns of plant growth and development, including phase transition, flower opening and seedling development, is important to understanding morphogenesis in space environment. In spite of many impressive engineering accomplishments in the space environment, and most of manipulations involved in space experiments in manned space lab or unmanned satellite were carried out automatically or by remote control, developing flight hardware and operational procedures to finish much sophisticate current biological research in a reasonable time in space is still a challenge to current space age technology. Thus, recording a dynamic spatiotemporal pattern of plant growth and development in space by in flight photography is needed to evaluate the immediate reasons for plant response to space environmental factors and to provide a framework for physiological and molecular analyses of the underlying mechanisms under microgravitation. In present study, we report the results of eighteen-day duration space experiments on plant phase transition and flower opening in real time by in flight photography carried out on the Chinese SJ-8 recoverable satellite using Chinese cabbage. More extensive studies on flower development under simulated weightlessness have also been performed using a 3D clinostat.

Materials and Methods

Plant Materials

Chinese cabbage (*B. parachinensis* L. H. Bailey) was used as plant material in Chinese SJ-8 recoverable satellite space experiments (September 9–27, 2006) to investigate the effect of microgravity on seed germination, developmental phase transition and flower development. For the seeds germination experiment, eight dry seeds were set in 1% agar containing MS basal medium (Murashige and Skoog 1962). For the plant development experiments, one plant with six leaves, respectively, without visible flower buds and elongated stem; the other plant with floral buds and two flowers were set in the root modules containing a commercially available vermiculite immersed by a medium containing macronutrients as described by Haughn and Sommerville (1986) in the plant growth chamber to investigate the effect of microgravity on the course from vegetative state to reproductive state and the flowering on the orbit (Haughn and Sommerville 1986). The previously mentioned items were sterilized by autoclaved and the plant samples used in space flight were also sterilized with a solution of 10% liquid bleach for 2 min, followed by extensive washing with sterilized water before mounted on the plant growth chamber.

Hardware Design and Conditions for Plant Material Growth Under Microgravitation

The plant growth chamber used for this experiment had a growing area of 0.09 m^2 and a height of 40 cm. It consisted of three growth compartments, illumination system and photograph system (Fig. 1). Illumination was provided by light banks made up of 200 solid state light emitting diode (LED) lamps (400–700 nm white light) on a 12-h photoperiod. Inside the chambers, temperatures were 25°C light/17°C dark, relative humidity was between 90% and 100%, and the photosynthetically active photon flux density produced by LED lamps was 120 μmol m^{-2} s^{-1} at surface of the first leaf of the experimental plant. Temperature and humidity were recorded every 1 min during flight. Light levels were also recorded with the same frequency. These data were used to set the ground control in a control growth chamber

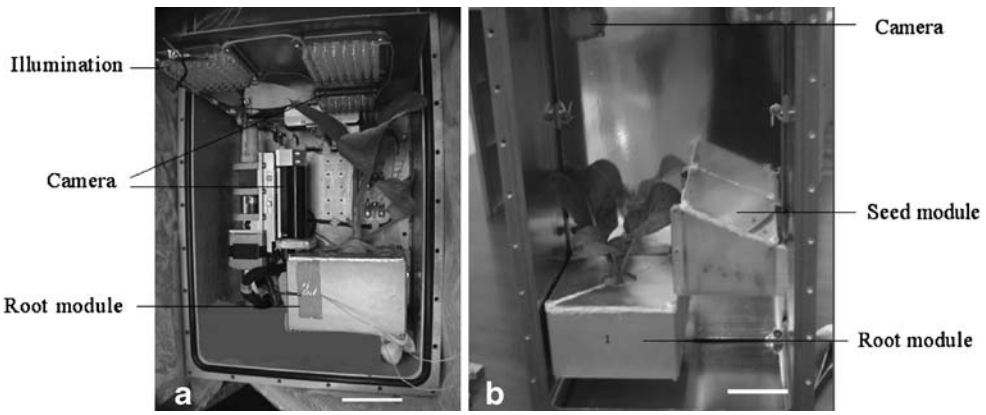

Fig. 1 The plant growth chamber was used in Chinese SJ-8 recoverable satellite. The chamber consisted of three growth modules included two root modules and one seed module; two cameras, one camera used for monitoring plant flowering (**a**); the other used for photographed seedling growth and seed germination (**b**). *Bar* 5 cm

or in the clinostat rotation experiment. Ethylene concentration in the growth chamber was regulated by placing potassium permanganate (10 g) packed in a box made from waterproof microporous film (30 μm; Wills et al. 1995) for removal of virtually all released ethylene.

Photographic equipment developed by SITP (Shanghai Institute of Technical Physics, Chinese Academy of Sciences, Shanghai) was used to record images of the seedlings and flowers. Images were recorded by two automatic, preprogrammed cameras (image size 720× 480 pixels). The photographs were taken at 2-h intervals. One camera was used for photographed seedling growth and seed germination; the other camera connected with an small automatic microscope (2× objective) was used to follow the detailed growth pattern of flower opening. Time-lapse imaging requires that the plant remain healthy and develop normally while it is being imaged, but it is difficult to continually and automatically image the flowers, which position changed with the rapid elongation of inflorescence axes. In order to obtain long-term time-lapse pattern of flower opening under microgravity in space, a meshwork was placed between photograph system and the plant growth compartment (Fig. 2), two of the flower buds caught in the meshes were selected to image over time (time-lapse image). All manipulations involved in the experiment were automated or carried out by remote control.

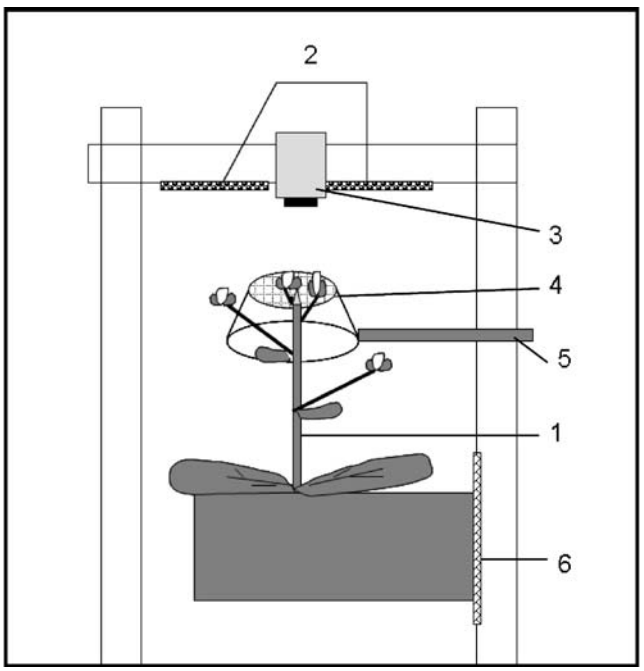

Fig. 2 The detail mechanical set up for the optical measurement of flower in the plant growth chamber (as showed in Fig. 1a) in Chinese SJ-8 recoverable satellite. *1* A Chinese cabbage plant in the growth chamber; *2* panel of LED for illumination; *3* CCD camera; *4* a meshwork for the fixation of the flower base; *5* a rod for supporting the frame of meshwork; *6* an adjustable stabilizer

Clinostat Experiment

A 3D clinostat (SM-31 tow-axis driving clinostat) was designed and constructed for the simulated weightlessness (made by Center for Space Science and Applied Research, Chinese Academy of Sciences, 2005). The main power of rotation was provided by two geared stepping motors and the sample stage was three dimensionally rotated by changing the rate and direction of rotation at random from 1 to −1 (reverse direction) rpm every 1 min. The module was illuminated by light banks made up of fluorescent lamps with a photoperiod and temperature conditions as the SJ-8 space experiment described above.

Quantifying the Effect of Simulated Weightlessness on Flower Attributes

The flowers were collected from the clinostat rotated plants and control according to described by Cresswell et al. (2001). Two flowers were collected from each plant that was analyzed, one fully open (petal laminas perpendicular to style, pollen dehiscent) and one still in bud. After collection, each flower was preserved in 70% alcohol and, subsequently, the following measurements were made on fully opened flowers: petal length, petal width at widest point, length of long stamen, length of short stamen and pistil length. Where the measurements were obtained from multiple flower parts, the mean was calculated prior to further analysis. Pollen production was quantified in each collected bud, which had also been preserved in 70% alcohol. Anthers were removed by dissection, macerated, and the number of pollen grains in the flower was estimated from haemocytometer preparations. For statistical analysis, mean values were calculated from four replicates, four plants being used for each replication.

Experimental Results

Effects of Microgravity on Growth and Development of Chinese Cabbage

Chinese cabbage showed synchronized seedling emergence after 3 days of sowing under ground condition, while seeds germinated about 5 days after sowing in space. The first true leaves appeared 4 days after seedling emergence in space flight samples as well as those in the control samples. The habit of an indeterminate growth, continuing their

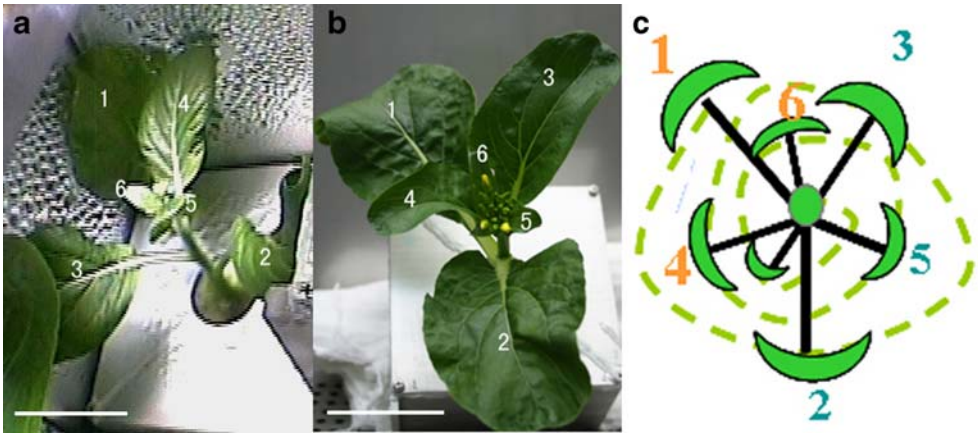

Fig. 3 A Chinese cabbage "SJ-8-flight" plant (**a**), a ground reference plant (**b**), and a model of the arrangement of leaves in the control plant (**c**). *Bar* 5 cm

vegetative growth during the reproductive stage, was observed in both space flight samples and ground control (Fig. 3).

Positioning of the leaves was not affected by microgravity. As shown in Fig. 3, the angle between the leaf petioles of two neighbor leaves in the space flight sample was about 150°, similar to those produced in the plants grown under 1 g conditions. The morphogenesis and direction of growth was also not affected during 18 days space flight in SJ-8 satellite (Fig. 3). These results suggested that the arrangement of the leaves might be independent on gravity during seedling development.

Effects on Elongation of Inflorescence Axes

The transition duration from the start of stem elongation to the appearance of the first flower was 6.5 days in SJ-8 satellite much longer than 3 days of those grown under ground condition. The growth rate of inflorescence axes in space was not significantly different from the samples grown on the ground during 50 h of flight, but the difference gradually became evident as the plant grown under microgravity after 50 h of flight (Fig. 4a). The height of inflorescence axes of spaceflight Chinese cabbage was reduced to approximately 56% of the control at 144 h after the satellite took off (Fig. 4a). Figure 4b shows the time course of successive rosette and flower leaves appeared under space flight. The rate of leaf formation decreased significantly under the microgravity condition. Four new leaves formed were observed by the end of 12 days space flight, while more than nine new leaves appeared in control samples during this time. (Fig 3).

Effects on Flower Opening

The plant with flower buds but without opening flower was set on the grown chamber for spaceflight. Two buds (2.5–3.5 mm in length, showing in Fig. 5a) at 2–3 days before the flower opening were selected and imaged repeatedly at 2 h intervals during the flight (Figs. 5 and 6). The flower buds in the same stage opened within 12 h on the ground condition (1 g), in which opening angle (the angle between the opposite petals) was more than 150°

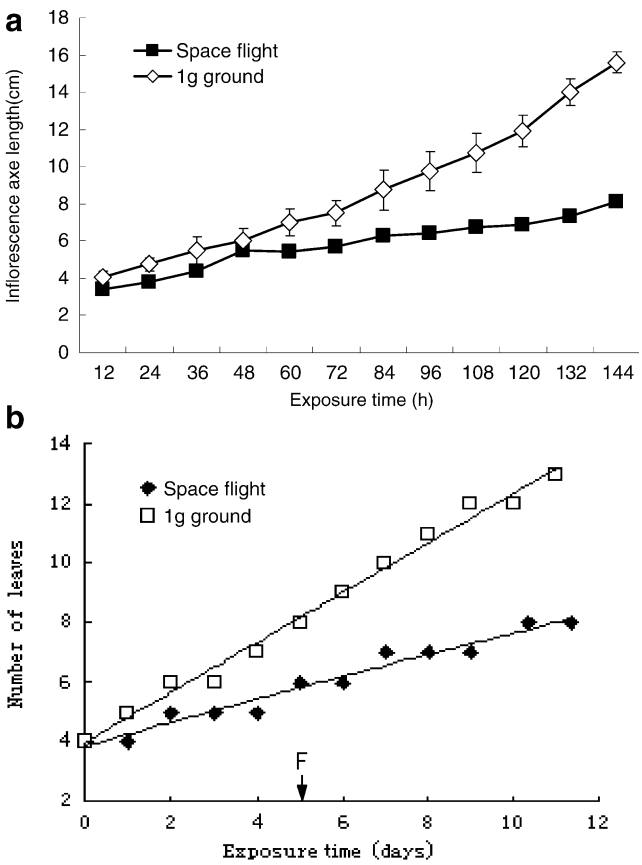

Fig. 4 Time course of development of Chinese cabbage plants under ground and spaceflight conditions. Plant height (**a**), the rate of successive leaves appeared (**b**). *F* Time of flowering

Fig. 5 Different morphology of flowers in different developmental stages during opening under 1 g condition (*top panel*) and examples of flower images(*bottom panel*) took in flight at different time after SJ-8 satellite took off: 0.5 h (**a**), 4 h (**b**), 20 h (**c**), 36 h (**d**), 44 h (**e**), 44.2 h (**f**), 46 h (**g**), 46.2 h (**h**), 48 h (**i**), 52.2 h (**j**), 82 h (**k**), 92.4 h (**l**), 96 h (**m**), 104 h (**n**), 136 h (**o**) and 224 h (**p**). *Bar* 2 mm

and wilted 40–50 h later (Fig. 5 top panel, Fig. 6). In contrast, the flower buds exposed to microgravity opened slowly and wilted without attaining full opening (opening angle about 65°; Figs. 5a–p and 6). The petals of these flowers didn't fully expand before wilted were also observed under microgravity. In addition, clinostat rotation inhibited expansion of petals of Chinese cabbage flowers, but didn't decrease the opening angles in comparison with 1 g control (ESM Fig. 1).

Flower expansion and opening are controlled by environmental factors, such as temperature, light, relative humidity, and an endogenous rhythm, were reported (Ichimura and Suto 1998). Gravity is one of the important environmental factors in regulating the plant reproductive growth and development, but effects of gravity on flower expansion has not yet been investigated in detail (Kuang et al. 2000; Bubenheim et al. 2003). Although morphogenesis of generative organs of Arabidopsis occurs normally in microgravity but neither the male nor the female floral parts fully developed. The anthers which did not dehisce were also observed in spaceflight plants (Kordyum 1998). This is the first study involving the flower expansion and opening in response to microgravity by live imaging techniques.

Effects on Reproductive Structure

Beside the course of flower expansion and opening were inhibited by exposure to spaceflight, anthers of the SJ-8 orbital capsule grown plants did not open before the flower withered were also observed (Fig. 5n–p), but the data about pollen grains presented in the anthers of space flight did not obtained due to the samples were not

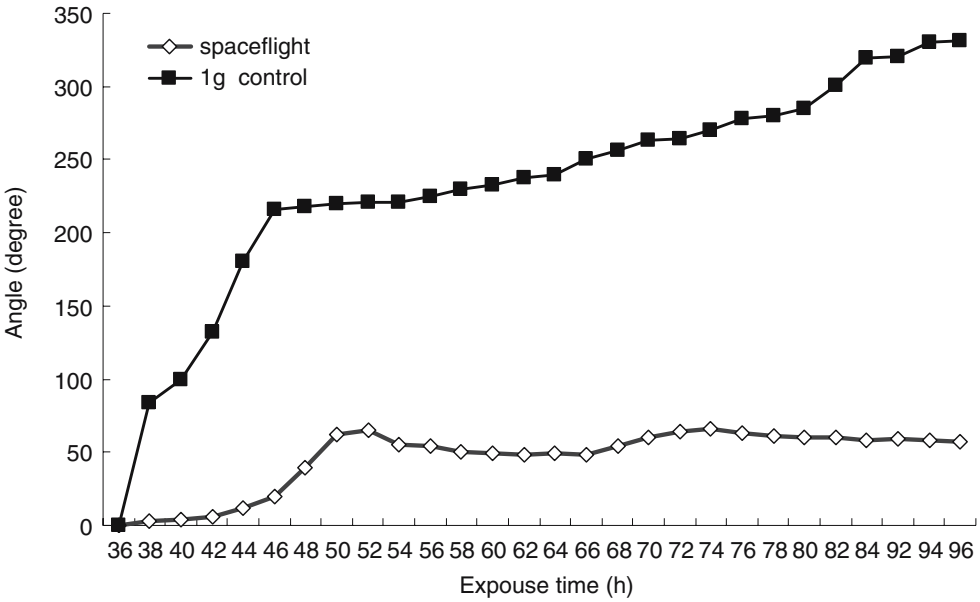

Fig. 6 Comparison of the flower opening angles between "SJ-8" plant and its control on ground. Flower buds (Fig. 5a) at 2–3 days before the flower opening were selected and imaged repeatedly at 2 h intervals in real time under both ground and space flight conditions (the part of images shown in Fig. 5). The angle between the opposite petals was determined in real time. Values are means of two flowers of space flight samples and five flowers of ground controls

recovered. In order to further know effects of gravity on flower development in detail, we determined whether or not the flower attributes of plant grown on 3D clinostat were altered compared to those in stationary control (1 g). The plant was set on the clinostat with flower buds, but without open flower as the samples used in space flight experiment. Seven days after clinostat rotation under the period of 12 h light/12 h dark illumination, the plant had four opening flowers. Clinostat rotated plants produced flowers with smaller petals and shorter stamens than control plants ($P<0.01$), but no other attributes were apparently affected (Table 1, ESM Fig. 1).

Pollen grain size was not altered, but pollen production in this plant appears to be sensitive to the clinostat rotated treatment (Table 1). The number of pollens per anther apparently declined ($P<0.01$) after treated by clinostat treatment. The number of viable pollens also considerably decreased in the flowers in clinostat-rotated samples in comparison with stationary controls (data not shown).

Table 1 Descriptive statistics (mean ± SD) for flower attributes of control and clinostat rotated plants of Chinese cabbage (means of four replicates, ± SD)

Attribute	Control	Treatment	*P* value[a]
Pollen diameter (μm)	22.73±1.49	23.00±1.34	ns
Long stamen (mm)	7.07±0.28	6.52±0.34	<0.001
Short stamen (mm)	5.33±0.52	4.35±0.36	0.003
Petal length (mm)	10.28±1.03	9.11±0.93	0.007
Petal width (mm)	4.67±0.43	4.27±0.39	ns
Pistil length (mm)	6.17±0.76	6.03±0.29	ns
Pollen production (grains/anther)	22,440±6,690	7,440±4,197	<0.001

ns not significant
[a] Significant at 1% level of probability by *t* test

Conclusions

This study presents automated image sequence analysis for detailed study of Chinese cabbage plant growth and flower opening influenced by microgravity in space. This method is suitable for the closed cultured plant growth experiments in manned space lab or unmanned satellite and can monitor the aggregate growth of a whole plant in larger size as well as a organ in smaller size over several days to several months.

Plant growth and flower opening are visually accessible but analysis in space is complicated because their growth is very dynamic, both temporally and spatially. To analyse the spatial distribution of growth during flower opening, a meshwork was used for fixation of the flower base. This technique allows macroscopic expansion growth to be mapped with high spatial and temporal resolution in plant leaves and flowers. This is the first technique reported that provides a high spatial and temporal resolution in plant biology space experiment.

In addition, appropriation flight conditions, in biological and technological reasons, other than microgravity, related with the modification of the physical environment of plant growth should be considered. Much additional work is needed in studying of plant responses to the environment of spaceflight and a detail analysis should be done with more strictly controls, included 1 g centrifugation control in space, in the future space experiment.

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