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ORIGINAL ARTICLE

Physiological Responses of *Synechocystis* sp. PCC 6803 under Clinorotation

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Abstract Photosystem efficiency and the characteristic on oxidative stress were examined to elucidate the metabolic responses of *Synechocystis* sp. PCC 6803 to short-term clinorotation. Results compiled when using clinostat to simulate microgravity for 60 h, showed that clinorotation clearly prohibited the photochemical quantum yield, but promoted the synthesis of chlorophyll and total protein. This may be a compensatory mechanism for the algal cell to maintain its normal metabolism. An increased malondialdehyde (MDA) content of algal cell upon clinorotation, together with an enhanced catalase (CAT) activity was observed during the whole period of clinorotation. One conclusion is that short-term clinorotation acts as a kind of stress, and that these physiological responses may be a special way for an algal cell to adapt itself to a different environment other than earth gravity.

Keywords Clinostat · *Synechocystis* sp. PCC 6803 · Simulated microgravity · Quantum yield · MDA

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Introduction

The influence of microgravity on organisms is a key issue for space-based biological research. Studies performed in space or in earth-based simulations indicate that organisms undergo changes when subjected to environmental factors. These changes include thylakoid membrane structure, mitochondria (Popova 2003; Stutte et al. 2006; Klimchuk 2007; Brykov 2011), gene expression (Visscher et al. 2009), primary (Popova et al. 1989; Stutte et al. 2006) and secondary metabolism (Musgrave et al. 2005; Nechitailo et al. 2008) and so on. Many biological samples cited in the above research were harvested just once after a period of space flight or simulation on a clinostat. To better understand the influence of real or simulated microgravity on life, physiological changes should be continuously sampled and analyzed during the whole period of treatment.

Cyanobacteria, a pioneer of autotrophic colonizers, have a flexible mechanism for accommodating a new environment. Due to their high tolerance against desiccation, cyanobacteria were successfully tested to restore soil suffering from erosion (Hu et al. 2002; Rao et al. 2009). Cyanobacteria were also successfully used for controlling the Lunar and Martian dust, where the dust is similar with Chinese desert (Liu et al. 2008). *Synechocystis* sp. PCC 6803 is a unicellular model strain of cyanobacteria and is expected to be a candidate for primary producer of Controlled Ecological Life Support Systems (CELSS). For these reasons, *Synechocystis* sp. PCC 6803 is the material selected for this study. *Synechocystis* sp. PCC 6803 is used to examine the photosystem efficiency and the anti-oxygenic property under simulated microgravity on a clinostat. It is the intent that the metabolic adaptability of algae in

response to microgravity could be better understood and revealed, hence laying the foundation for further space research.

Materials and Methods

Algal Strain and Cultures

The strain of algae used in this study was the motile and glucose-tolerant *Synechocystis* sp. PCC6803 from Freshwater Algae Culture Collection of Institute of Hydrobiology, Chinese Academy of Sciences.

Microgravity Simulation and Growth Condition

The environment created on earth by a clinostat is referred to as "simulated microgravity" (Klaus 2001). This study used a horizontal one-axis clinostat with a radius of 4 cm (Fig. 1) designed by the Institute of Biophysics, Chinese Academy of Sciences. The solid-agar *Synechocystis* sp. PCC 6803 grown on a culture plate (2.4 cm of radius and 1 cm of thickness) was adhered to the center of clinostat under an average rotation speed of 15 rpm. This created a value of $1.26–1.39 \times 10^{-2}$ g according to Van Loon (2007). All samples used were grown in an illuminator at the temperature of 25°C, under the illumination of 40 μE m^{-2} s^{-1} with a 12:12 light dark cycle. Controls were grown under the same condition; sampling was done every 12 h by scraping the surface of the agar for assay.

Measurement of Photochemical Quantum Yield

The photochemical quantum yield was measured using the plant efficiency analyzer (PEA, Hansatech®, UK). PEA was the instrument in investigating plant photosynthetic efficiency, utilizing the continuous excitation fluorescence measurement principle. Measurement and analysis was performed under the growing condition of the samples.

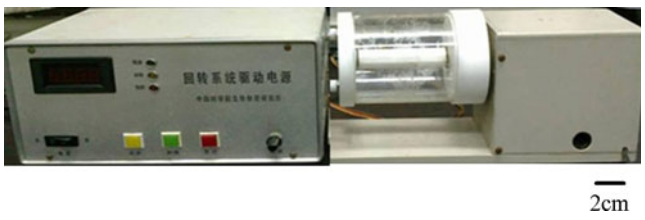

Fig. 1 One-axis horizontal clinostat

Determination of Chlorophyll

Chlorophyll extraction used 95% ethanol at 4°C overnight according to the method of Li (2000). Light absorbance of 665 nm and 649 nm were measured using a UV-2000 spectrophotometer (Beijing Purkinge General Instrument Co., Ltd.). Chlorophyll content was determined using the formula $\text{Chl.}a = 13.95A_{665} - 6.88A_{649}$, $\text{Chl.}b = 24.96A_{649} - 7.32A_{665}$, where for the total chlorophyll content $(\text{mg}\cdot\text{g}^{-1}\text{FW}) = \text{Chl.}a + \text{Chl.}b$

Determination of Total Protein

Total protein was extracted in a 0.5 M NaOH solution under the temperature of 90°C for 1 h. The concentration of total protein was determined using the Bradford method with bovine serum albumin as standard (Bradford 1976).

Measurement of Malondialdehyde (MDA)

A modified thiobarbituric acid method was used for MDA determination (Heath and Packer 1968). After a 30 min boiling water bath with 0.67% TBA, the supernatant was assayed by colorimetry under the wavelength of 450 nm, 532 nm and 600 nm. MDA content was determined using the formula $C\left(\mu\text{mol}\cdot\text{g}^{-1}\text{FW}\right) = \left[6.45\left(A_{532} - A_{600}\right) - 0.56A_{450}\right]\cdot V\cdot g^{-1}$.

Determination of Catalase (CAT) Activity

CAT activity was measured by $KMnO_4$ titration according to Li (2000). The algae sample was grinded in pH7.8 phosphorus buffer and centrifuged. The supernatant was the coarse CAT extracts. H_2O_2 solution was added into the CAT extracts for enzymatic reaction, with the redundant H_2O_2 titrated by $KMnO_4$ standard solution. The enzyme activity unit was $U\cdot mg^{-1}$ Pro, with U representing mg $H_2O_2\cdot min^{-1}\cdot g^{-1}$ FW.

Statistics Analysis

All experiments were done for more than three times. Results were evaluated using the SPSS statistical analysis software with $P < 0.05$ denoting significant difference.

Results and Discussion

Clinostats are used to simulate microgravity on earth. It is not because samples in a clinostat couldn't experience unit gravity (g), but rather the constant rotation of the

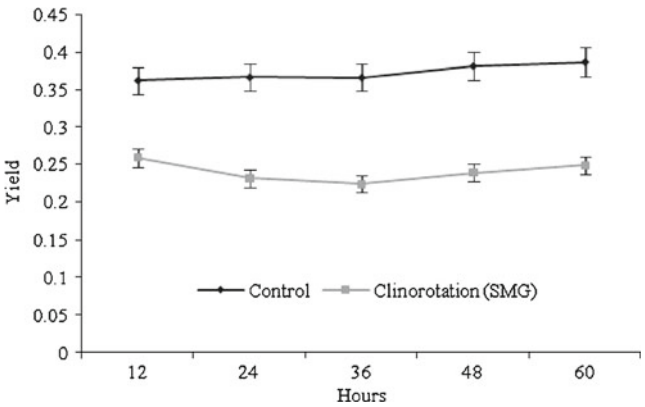

Fig. 2 The decrease of photochemical quantum yield under clinorotation

Table 2 Photochemical quantum yield, chlorophyll, protein, MDA content and CAT activity in *Synechocystis* sp. PCC 6803 at different time points of clinostat treatment with respect to those of ground controls (positive value indicated increase, negative value indicated decrease, "—" indicated no significant difference)

	Yield (%)	Chlorophyll (%)	Protein (%)	MDA (%)	CAT (%)
12 h	−28.49	—	14.88	26.19	19.57
24 h	−38.53	—	21.76	78.79	64.26
36 h	−38.87	23.14	16.09	113	88.75
48 h	−35.71	38.21	21.06	171	117
60 h	−34.38	48.14	29.12	220	124

samples results in the g-vector being time-averaged to near-zero (Klaus 2001). A common property of microgravity and clinorotation is the change in (and under ideal condition neutralization of) sedimentation. Upon clinorotation organisms or cells are exposed to constantly changing acceleration vector, whereas under μg conditions no external acceleration acts on the cells.

Photochemical quantum yield represents the photosynthetic fluorescence efficiency of PSII under experimental conditions. The decrease of quantum yield indicated there was stress caused by the environment. In this study, photochemical quantum yields of *Synechocystis* sp. PCC 6803 during different periods of clinorotation were lower (Fig. 2) (Table 1), separately 28.49%, 38.53%, 38.87%, 35.71% and 34.38% lower at indicated sampling time points than those of the ground controls (Table 2). Statistical analysis revealed that yield differences between ground control and clinorotation were significant ($P = 0.03$). Results showed that the constant rotation of clinorotation treatment might act as a kind of stress and influenced the quantum yield of PSII photochemistry in *Synechocystis*. Similar results of decreased photosynthetic fluorescence efficiency in micro-algae and *Brassica rapa* plants in space microgravity condition were also found in Wang et al. (2006) and Kochubey et al. (2004). A decrease in photosystem efficiency may also be the result of mechanical injuries due to clinorotation. Previous studies reported that clinorotation could cause loose arrangements of thylakoids in bunch and significant bends of thylakoids in the membrane system in chlorella (Popova 2006). Clinostat experimentation in our lab also displayed such structural changes in blue-green alga (unpublished). So the decrease in PSII efficiency was the result of all-round influence, including continuous changing gravity vector and mechanical injuries during clinorotation. The decrease of photosynthetic efficiency is likely due to the degradation of pigment in the PSII reaction center and the breakage of the grana lamella by clinorotation.

Chlorophyll plays an important role in harvesting and transforming light energy in the process of photosynthesis, its content has direct influence on photosynthetic efficiency and the metabolic level. Results in the study showed that there was no significant difference on chlorophyll content of *Synechocystis* sp. PCC 6803 between clinorotation and ground control during the early 24 h ($P = 0.42$) (Table 1), but 23.14%, 38.21% and 48.14% higher synthesis was detected during 36 h to 60 h ($P = 0.02$) (Table 2) (Fig. 3). This indicates that the chlorophyll synthesis was influenced later than PSII activity under clinorotation. Previous studies reported that chlorophyll increased in pea and rice seedling under microgravity (Abilov et al. 1986; Aliyev et al. 1987; Jagtap et al. 2011), but decreased in maize, *chlorella* and

Table 1 Physiological characteristics of *Synechocystis* sp. PCC 6803 during 60 h clinostat treatment compared with ground controls

	12 h		24 h		36 h		48 h		60 h	
	Control	Clinostat	Control	Clinostat	Control	Clinostat	Control	Clinostat	Control	Clinostat
Yield	0.36	0.26	0.37	0.23	0.37	0.22	0.38	0.24	0.39	0.25
Chlorophyll	0.20	0.21	0.36	0.32	0.38	0.47	0.48	0.66	0.57	0.85
Protein	29.73	34.16	40.67	49.52	42.50	49.34	48.55	58.77	53.59	69.20
MDA	0.04	0.05	0.03	0.06	0.03	0.07	0.02	0.07	0.02	0.05
CAT	0.09	0.11	0.07	0.12	0.07	0.13	0.05	0.12	0.04	0.08

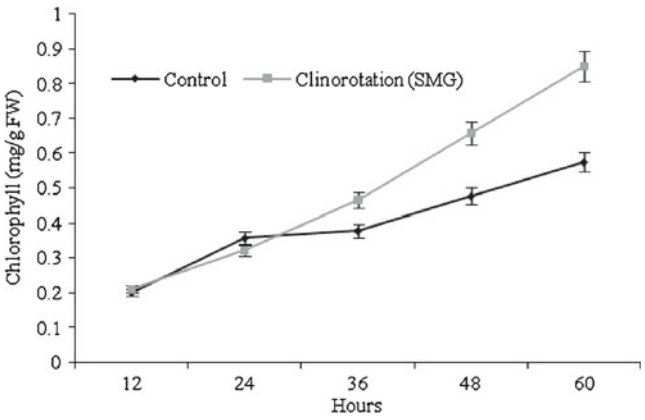

Fig. 3 Change of chlorophyll content under clinorotation

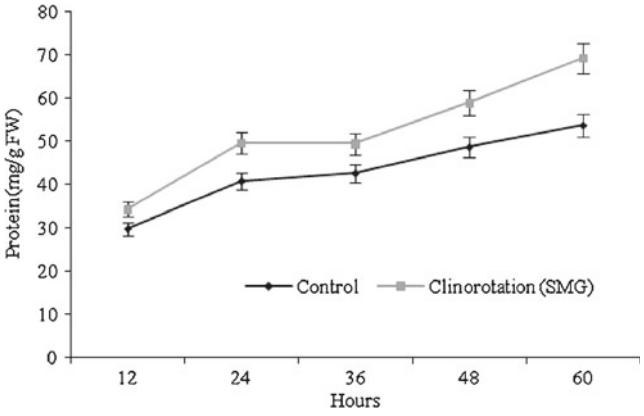

Fig. 4 Change of total protein content under clinorotation

oat leaf segment (Rumyantseva et al. 1990; Moleshko et al. 1991; Miyamoto et al. 2001). Except for species differences, the period of microgravity treatment might also be the cause of metabolic discrepancy (Jagtap et al. 2011). Decreased chlorophyll content is often exhibited in long-time microgravity studies (e.g. studies greater than 10 days). This shows that microgravity or clinorotation duration may influence chlorophyll content by inhibiting chlorophyll synthesis or accelerating its degradation (Zhao et al. 2007). The increase of chlorophyll content found in this study may be a compensatory effect of the decrease in PSII photochemical efficiency upon clinorotation. The process was presumed to be that: after the decline in PSII efficiency, algal cell had to synthesize more chlorophyll to compensate for the decreased photochemical efficiency to stabilize photosynthetic carbon fixation and to maintain its normal growth demand. This explains why changes in chlorophyll content followed changes in PSII efficiency.

Protein synthesis played a crucial role in plant acclimation process (Jagtap et al. 2011). Protein contents of *Synechocystis* sp. PCC 6803 during the whole period of clinorotation were increased significantly ($P = 0.04$) (Table 1) (Fig. 4), 14.88%, 21.76%, 16.09%, 21.06% and 29.12% higher than those of ground controls (Table 2). It showed that either clinorotation could induce more protein synthesized, or algal cells had to produce more protein to accommodate to clinorotation. Most proteins act as enzymes in defense or metabolic reactions, and the increased protein contents may indicate an enhanced physiological metabolism. Previous reports showed that the micro-environment of cells is balanced in 1G, indicating that the output and the input of the cells are balanced on earth gravity. When exposed to microgravity, the balanced micro-environment might be destroyed and the cell needs to increase the activity of proteins to establish a new state of balance to stabilize the environment (Kordyum 1994). The increased synthesis of protein may provide energy dissipation during the period of clinorotation. In addition, particular proteins would be produced under unusual environment condition (Scandalios 1990). The accumulation of heat shock proteins (*HSPs*) may protect the cell from harmful circumstance (Vierling 1991), thus the increased level of *HSP* in altered gravity and microgravity condition may indicate the extent of stress on the cell, for example *HSP70* and *HSP90* (Kozeko and Kordyum 2006, 2009). Li et al. (2002) also showed that protein synthesis plays a role in regulating osmotic potential in indirect water stress caused by clinorotation. So the increased protein synthesis could be a strategy for algal cells to protect and to adapt themselves to clinorotation.

Abnormal physiological responses in space environment were attributed to oxygen radical accumulated in organism (Ray 1991). Prior studies reported that clinorotation caused the intensification of the lipid peroxidation process in pea chloroplasts. The increased level of lipid peroxidation was interpreted as an indicator of the elevated production of reactive oxygen species (ROS) (Baranenko 2001). MDA was used to examine the level of lipid peroxidation of cells under stress. MDA contents of *Synechocystis* sp. PCC 6803 during the whole period of clinostat treatment were promoted (Fig. 5) (Table 1), separately 26.19%, 78.79%, 113%, 171% and 220% higher than those of ground controls ($P = 0.02$) (Table 2). Increased lipid peroxidation level was also detected in micro-algal cells under clinorotation (Li et al. 2002). The produced MDA could damage membranes by increasing membrane permeability and thus affecting the catalytic function of membrane enzymes. This is particularly true for a unicellular organism without gravity receptor, which arises a series

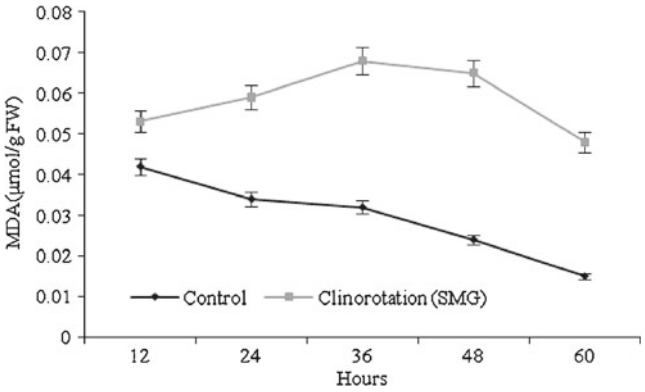

Fig. 5 The increase of MDA content under clinorotation

of metabolic changes (Slenzka and Kordyum 1996). Decreased MDA accumulation represented a higher protective capability of scavenging free radicals. During the early clinorotation, the ascending trend of MDA content indicated the membrane was continually suffering, however after 36 h the MDA content began to decrease. CAT activity closely correlated with the lipid peroxidation of the algal cell and exhibited a similar content trend with respect to MDA. The stronger decrease in MDA content and CAT activity in our 1 g controls is due to the fact that it often takes some time for cyanobacteria to accommodate to a new culture environment after inoculation. Xiao et al. (2010) reported that cyanobacteria showed a significant decrease in growth and metabolism until the 4th day after inoculation. Due to the relative shorter sampling period in this study, this effect can be observed in the 1 g reference samples. CAT activity in *Synechocystis* sp. PCC 6803 during different periods of clinostat treatment were higher (Fig. 6) (Table 1), respectively 19.57%, 64.26%, 88.75%, 117% and 124% upon clinorotation ($P = 0.03$) (Table 2). CAT is an antioxidant enzyme developed

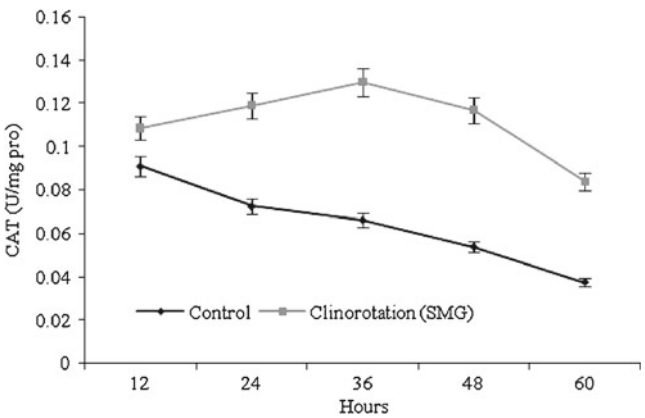

Fig. 6 The increase of CAT activity under clinorotation

to scavenge the oxidative stress caused by ROS. CAT was also reported to be increased in Anabaena cells by clinorotation (Li et al. 2004). Higher CAT activity could help to scavenge toxicants in cell (Zhao et al. 2003). Changes of CAT activity and MDA content were similar in this study. Both displayed a descending trend after 36 h, indicating that either there was a decrease in harm to the cell or the damaged membrane might be partially in repair during a period of accommodation. Zhao et al. (2003) in previous work found that physiological changes in response to microgravity could disappear gradually with time. The antioxidant enzyme behavior in this study may provide a defense response to accommodate unfavorable conditions.

Conclusion

Results of this study showed that 60 h clinorotation brought a series of physiological changes in *Synechocystis* sp. PCC 6803. MDA content, a sign of peroxidation of membrane lipid, increased under clinorotation. When MDA increased, the protective enzyme CAT was activated to protect the algal cell. Mechanical harm of clinorotation on the membrane system especially on the thylakoid membrane was the most likely reason for the decrease in PSII photochemical efficiency. To stabilize a normal growth demand, there should be a compensatory synthesis of chlorophyll upon clinorotation. The increased protein synthesizing system also played a crucial role in the clinorotation acclimation processes as seen with the activation of antioxidant enzyme system and other metabolic reactions. In conclusion, the change of physiological metabolic reactions provided a specific way for *Synechocystis* sp. PCC 6803 to adapt to short-term clinorotation. In future experiments we will consider faster acceleration speeds and check the sedimentation behavior of the algal cells.

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References

Abilov, Z.K., Alekperov, U.K., Mashinskiy, A.L., Aliyev, A.A.: The morphological and functional state of the photosynthetic system of plant cells grown for varying periods under space flight conditions. USSR Space Life Sci. Digest. **8**, 15–18 (1986)

Aliyev, A.A., Abilov, Z.K., Mashinskiy, A.L., Ganiyeva, R.A., Ragimova, G.K.: The ultrastructure and physiological characteristics of the photosynthesis system of shoots of garden

pea grown for 29 days on the 'Salyut-7' space station. USSR Space Life Sci. Digest. **10**, 15–16 (1987)
Baranenko, V.V.: Pea chloroplasts under clino-rotation: lipid peroxidation and superoxide dismutase activity. Adv. Space Res. **27**, 973–976 (2001)
Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248–254 (1976)
Brykov, V.: Clinorotation affects the ultrastructure of pea root mitochondria. Microgravity Sci. Technol. **23**, 215–219 (2011)
Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. **125**, 180–198 (1968)
Hu, C., Liu, Y., Song, L., Zhang, D.: Effect of desert soil algae on the stabilization of fine sands. J. Appl. Phycol. **14**, 281–292 (2002)
Jagtap, S.S., Awhad, R.B., Santosh, B., Vidyasagar, P.B.: Effects of clinorotation on growth and chlorophyll content of rice seeds. Microgravity Sci. Technol. **23**, 41–48 (2011)
Klaus, D.M.: Clinostats and bioreactors. Gravit. Space Biol. Bull. **14**, 55–64 (2001)
Klimchuk, D.O.: Structural and functional features of mitochondria in statocytes of soybean root under microgravity conditions. Cytol. Genet. **41**, 25–29 (2007)
Kochubey, S.M., Adamchuk, N.I., Kordyum, E.I., Guikema, J.A.: Microgravity affects the photosynthetic apparatus of *Brassica rapa* L. Plant Biosyst. **138**, 1–9 (2004)
Kordyum, E.L.: Biology of plant cells in microgravity and under clinostating. Int. Rev. Cyt. **171**, 1–78 (1994)
Kozeko, L., Kordyum, E.: The stress protein level under clinorotation in context of the seedling developmental program and the stress response. Microgravity Sci. Technol. **18**, 254–256 (2006)
Kozeko, L., Kordyum, E.: Effect of hypergravity on the level of heat shock proteins 70 and 90 in pea seedlings. Microgravity Sci. Technol. **21**, 175–178 (2009)
Li, H.S.: Experiment Principle and Technology of Plant Physiology and Biochemistry (in Chinese), pp. 194–197. High Education Press, Beijing (2000)
Li, G., Wang, G., Song, L., Liu, Y.: Lipid peroxidation in microalgae cells under simulated microgravity. Space Med. Med. Eng. **15**, 270–272 (2002)
Li, G., Liu, Y., Wang, G., Song, L.: Reactive oxygen species and antioxidant enzymes activity of *Anabaena* sp. PCC 7120 (Cyanobacterium) under simulated microgravity. Acta Astronaut. **55**, 953–957 (2004)
Liu, Y., Cockell, C.S., Wang, G., Hu, C., Chen, L., De Philippis, R.: Control of Lunar and Martian dust-experimental insights from artificial and natural cyanobacterial and algal crusts in the desert of Inner Mongolia, China. Astrobiology **8**, 75–87 (2008)
Miyamoto, K., Yuda, T., Shimazu, T., Ueda, J.: Leaf senescence under various gravity conditions: relevance to the dynamics of plant hormones. Adv. Space Res. **27**, 1017–1022 (2001)
Moleshko, G.I., Anton'yan, A.A., Sycheyev, V.N., Solontsova, I.P., Shetlik, I., Doukha, Y.: The effects of space flight factors on the pigment system of one-celled algae. USSR Space Life Sci. Digest **31**, 43–45 (1991)
Musgrave, M.E., Kuang, A., Tuominen, L.K., Levine, L.H., Morrow, R.C.: Seed storage reserves and glucosinolates in *Brassica rapa* L. grown on the international space station. J. Am. Soc. Hortic. Sci. **130**, 848–856 (2005)
Nechitailo, G.S., Yurov, S., Cojocaru, A., Revin, A.: Spectrophotometric analysis of tomato plants produced from seeds exposed under space flight conditions for a long time. In: 37th COSPAR Scientific Assembly, p. 2191 (2008)
Popova, A.F.: Comparative characteristic of mitochondria ultrastructural organization in *Chlorella* cells under altered gravity conditions. Adv. Space Res. **31**, 312253–312259 (2003)
Popova, A.F.: Structural changes of chloroplasts and galactolipid contents in Chlorella cells during clinorotation. Tsitol. Genet. **40**, 39–43 (2006)
Popova, A.F., Sytnik, K.M., Kordyum, E.L.: Ultrastructural and growth indices of Chlorella culture in multicomponent aquatic system under space flight conditions. Adv. Space Res. **9**, 79–82 (1989)
Rao, B., Liu, Y., Wang, W., Hu, C., Li, D., Lan, S.: Influence of dew on biomass and photosystem II activity of cyanobacterial crusts in the Hopq Desert, northwest China. Soil Biol. Biochem. **41**, 2387–2393 (2009)
Ray, E.K.: Introduction: are aging and space effects similar? Exp. Gerontol. **26**, 123–129 (1991)
Rumyantseva, M.N., Merzlyak, M.N., Mashinskiy, A.L., Nechitaylo, G.S.: Effect of space flight factors on the pigment and lipid composition of wheat plants. Kosm. Biol. Aviakosm. Med. **24**, 53–56 (1990)
Scandalios, J.G.: Response of plant antioxidant defence gene to environmental stress. Adv. Genet. **28**, 1–41 (1990)
Slenzka, K., Kordyum, E.: Gravity cellular membranes and associated processes: an introduction [J]. Adv. Space Res. **17**, 141–142 (1996)
Stutte, G.W., Monje, O., Hatfield, R.D., Paul, A.L., Ferl, R.J., Simone, C.G.: Microgravity effects on leaf morphology, cell structure, carbon metabolism and mRNA expression of dwarf wheat. Planta **224**, 1038–1049 (2006)
Van Loon, J.J.W.A.: Some history and use of the random positioning machine, RPM, in gravity related research. Adv. Space. Res. **39**, 1161–1165 (2007)
Vierling, E.: The role of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**, 579–620 (1991)
Visscher, A.M., Paul, A.-L., Kirst, M., Alling, A.K., Silverstone, S., Nechitailo, G., Nelson, M., Dempster, W.F., Van Thillo, M., Allen, J.P., Ferl, R.J.: Effects of a spaceflight environment on heritable changes in wheat gene expression. Astrobiology **9**, 359–367 (2009)
Wang, G.H., Chen, H.F., Li, G.B., Chen, L.Z., Li, D.H., Hu, C.X., Chen, K., Liu, Y.D.: Population growth and physiological characteristics of microalgae in a miniaturized bioreactor during space flight. Acta Astronaut. **58**, 264–269 (2006)
Xiao, Y., Liu, Y., Wang, G., Hao, Z., An, Y.: Simulated microgravity alters growth and microcystin production in *Microcystis aeruginosa* (cyanophyta). Toxicon **56**, 1–7 (2010)
Zhao, H.C., Zhu, T., Wu, J., Xi, B.S.: Effect of simulated microgravity on aged pea seed vigour and related physiological properties. Colloids Surf. B: Biointerfaces **27**, 311–314 (2003)
Zhao, G.Q., Ma, B.L., Ren, C.Z.: Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Sci. Soc. America **47**, 123–131 (2007)