The senescence of oat leaf segments is promoted under simulated microgravity condition on a three-dimensional clinostat

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Introduction

Plants have evolved on the earth, indicating the morphology, growth and development, and life cycle of plants are highly influenced by gravity as well as other environmental stimuli. Indeed, simulated microgravity on a clinostat or hypergravity on a centrifuge has recently been reported to change the growth and development of plants (Hoson *et al.* 1992, 1993, 1995, Rasmussen *et al.* 1994, Kasahara *et al.* 1995).

Senescence is a final drastic phenomenon in life cycle of plants, which is characterized by the loss of total chlorophyll and protein, and/or the formation of the abscission (Osborne 1973, Thimann 1977, Addicott 1982). Many environmental stimuli as well as the qualitative and quantitative changes of plant hormones have been reported to affect plant senescence. Among those stimuli, light is the most important factor to regulate plant senescence (Leopold 1964). Dark condition promotes leaf senescence due to the decrease in endogenous level of cytokinin and/or the increase in that of abscisic acid or ethylene (Tetley and Thimann 1974, Gepstein and Thimann 1980). However, there are few reports concerning the effect of gravity on leaf senescence. Strenuous effort to learn leaf senescence under microgravity condition has been done using a three-dimensional (3-D) clinostat. In this paper, we report that simulated microgravity condition on a 3-D clinostat promoted the senescence of oat leaf segments in the dark. A possible mechanism of microgravity

condition on promoting the senescence is also discussed.

Materials and methods

The senescence of oat leaf segments under simulated microgravity on a 3-D clinostat was studied according to the methods previously reported with modifications (Ueda and Kato 1980, Hoson *et al.* 1992).

Oat (*Avena sativa* L. cv Victory) seeds were germinated in vermiculite under continuous white fluorescent light of 5 W/m² at 25 °C for 9 days on 1 xg condition. The first leaves were harvested and 3-cm segments of the distal portion of the leaves were excised. The set of 15 segments was placed on 2 layers of filter papers moistened with 5 ml of water or 10⁻⁵ M kinetin solution in a disposable plastic Petri dish (Iwaki Glass Co., Ltd. Japan). Petri dishes were covered with wet paper towel and aluminum sheet and then incubated under simulated microgravity condition on a 3-D clinostat in the dark or under 1 xg dark condition at 25 °C for appropriate period. As shown in Fig. 1, the water content of filter papers in a Petri dish reduced significantly during the incubation on 3-D clinostat condition, indicating the relative humidity in a Petri dish is very low. Therefore, oat leaf segments in a Petri dish were covered with an additional another filter paper to prevent evaporation under simulated microgravity condition on a 3-D clinostat and also in dark control. At the end of the incubation, the leaf segments were harvested and total chlorophyll were extracted with boiling 80 % ethanol. Absorbance of the extracts was measured at 665 nm with a Shimadzu UV-1200 spectrophotometer. Results were expressed as percentages of the initial value.

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Fig. 1 Water content of filter papers in a Petri dish kept oat leaf segments on 1 xg condition or under simulated microgravity condition on a 3-D clinostat at 25 °C in the dark.

Fig. 2 Chlorophyll retentions of oat leaf segments in the presence or absence of 10^{-5} M kinetin on 1 xg condition or under simulated microgravity condition on a 3-D clinostat at 25 °C in the dark. Chlorophyll retentions are expressed as percentages of the initial value. Bars indicate standard error of the mean (n=4).

Fig. 3 Effects of 10^{-5} M abscisic acid (ABA) and jasmonic acid (JA) on senescence of oat leaf segments in the presence or absence of 10^{-5} M kinetin on 1 xg condition or under simulated microgravity condition on a 3-D clinostat at 25 °C in the dark. Chlorophyll retentions of the segments are expressed as percentages of the initial value. Bars indicate standard error of the mean (n=4).

Results and discussion

It has been well known that cytokinins are effective on inhibiting or delaying the senescence of excised leaf segments, and abscisic acid, jasmonic acid and its methyl ester promote senescence under 1 xg condition (Ueda and Kato 1981). In this assay system, auxins and gibberellins, however, have almost no effect (data not shown). As shown in Figs. 2 and 3, kinetin at 10^{-5} M was effective on inhibiting or delaying senescence, and abscisic acid at 10^{-5} M and jasmonic acid at 10^{-5} M promoted the senescence, suggesting the modification of the experimental condition to cover oat leaf segments with an additional filter paper is suitable for the investigation for the effect of microgravity condition on a 3-D clinostat.

Fig. 2 shows the chlorophyll retention of oat leaf segments under simulated microgravity condition on a 3-D clinostat in the presence or absence of 10^{-5} M kinetin. In the absence of kinetin, the chlorophyll contents of the segments reduced dramatically just after the start of the incubation. The chlorophyll retention of the segments was slightly low under simulated microgravity condition on a 3-D clinostat in the absence of kinetin at day 2. In the presence of kinetin, the promoting effect of microgravity condition on the loss of chlorophyll (senescence) of the segments was observed at day 3. The chlorophyll retention of the segments under simulated microgravity on a 3-D clinostat in the presence of kinetin reduced to about 20% of the initial value at the end of the experiment. The color of the segments completely turned to yellow. These facts indicate that the simulated microgravity condition on a 3-D clinostat promotes the senescence of excised plant tissues.

Abscisic acid (10^{-5} M) and jasmonic acid (10^{-5} M) significantly promoted the loss of chlorophyll of the segments in the absence of kinetin under 1 xg condition at day 1 and 2 (Fig. 3). Since the loss of chlorophyll of the segments was substantially promoted under simulated microgravity condition on a 3-D clinostat, the promoting effect of abscisic acid or jasmonic acid was not observed at day 2. Similar results were found in the presence of 10^{-5} M kinetin at day 3 and

day 5.

The activation of several degradation enzymes which might be newly biosynthesized after the perception of the external signals was observed during plant senescence (Drivdahl and Thimann 1977, 1978). If the synthesis of enzymatic proteins is prevented, it is expected that the loss of chlorophyll of the segments is partially nullified. As shown in Fig. 4, cycloheximide at 10^{-5} M was significantly effective on preventing the loss of chlorophyll of the segments on both 1 xg and a 3-D clinostat conditions at day 2 and day 3.

Based on the data described in this paper,

Fig. 4 Effect of cycloheximide on the senescence of oat leaf segments on 1 xg condition or under simulated microgravity condition on a 3-D clinostat at 25 °C in the dark. Chlorophyll retentions of the segments are expressed as percentages of the initial value. Bars indicate standard error of the mean (n=4).

simulated microgravity condition affects the life cycle of plants due to several changes in the dynamics of plant hormones. As mentioned above, plant senescence causes the induction of some hydrolytic enzymes including serine proteinase (Thimann 1977), suggesting the expressions of genes encoded these enzymatic proteins might be accelerated by microgravity conditions. Measurements of the endogenous levels of plant hormones together with the analysis of the changes in molecular aspects of leaf tissues are remaining as the further problem. The data described in this paper provide the important problem in a research field of space botany in relation to the production of cultivated plants in space. Detailed studies of plant senescence will be also required under microgravity conditions in space in near future.

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