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Preservation of Rice Callus by Gas-replacement

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The growth rate of *Chenopodium album* calluses is known to be reduced by immersing them in oil[1]. Immersed calluses could survive after 8 weeks of immersion. The suppression of growth was assumed to be due to the deficiency of oxygen. On the other hand, high concentrations of carbon dioxide lowered the opercular rate and the oxygen consumption of carp (*Cyprinus carpio*)[2]. These facts suggest that the deficiency of oxygen and high concentrations of carbon dioxide reduce the growth rate of cultured plant cells. Consequently, the consumption of nutrients of the culture medium might be reduced and cells might survive for a long time in atmospheres containing low concentrations of oxygen or high concentrations of carbon dioxide compared to air. Therefore, we cultured rice calluses in these atmospheres, and examined the changes in their growth rate and survival rate.

Rice (*Oryza sativa* L. cv Nipponbare) calluses were induced from mature seeds on Linsmaier and Skoog agar (0.9%, w/v) medium[3] with 10^{-5} M of 2, 4-D at 27°C in the dark and subcultured under the same conditions every 2 weeks.

Rice calluses were inoculated on the medium in a flask. The flask was capped with a well-ventilated silicone-cap and put in a desiccator. The atmosphere in the desiccator was removed with a vacuum pump and then the desired gas was put into the desiccator. This gas-replacement was

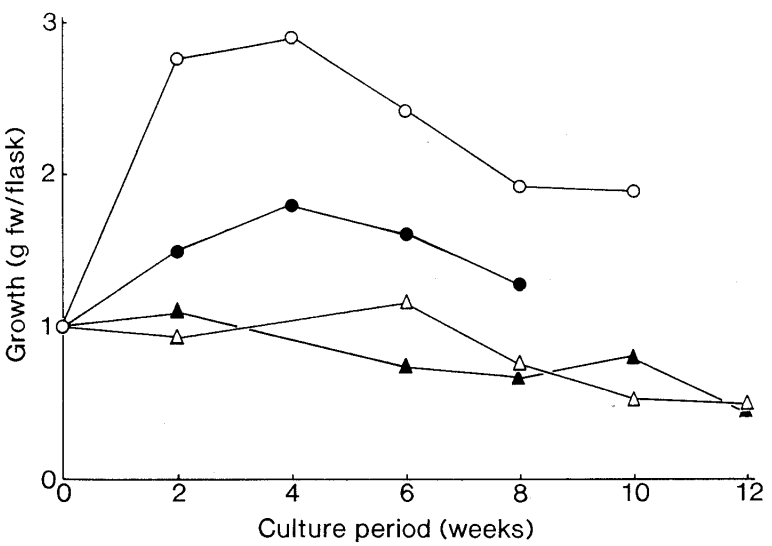

Fig 1. Growth curve of rice calluses in various atmospheres. Calluses (1.0 g fw) were inoculated on medium (25 ml) and cultured in air (O), the gases of $CO_2/O_2=80\%/20\%$ (●), $CO_2=100\%$ (△) or $N_2=100\%$ (△). The means of three measuremets are presented.

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Table 1. Viability of rice calluses cultured in various atmospheres for 8 to 12 weeks.

Period	Atmosphere											
	Air (Control)			CO_2/O_2 (80%/20%)			CO_2 (100%)			N_2 (100%)		
8 weeks	0/8[a]	1/8	0/8	0/8	0/8	0/8	1/7	2/8	4/8	3/8	0/8	1/8
	0/8	1/8					0/8			0/8		
10 weeks	0/8	0/8	0/8	—[b]			5/8	2/7	4/8	0/8	0/8	8/8
	0/8	0/8					0/8			1/8		
12 weeks	—			—			7/7	0/8	7/8	3/8	8/8	0/8
							0/8			0/8		

[a]Number of flasks having cell aggregates with a normal appearance/Total number of flasks used in experiment.
[b]not examined.

After their culture in the various atmospheres, calluses (1. 0 g fw) were inoculated on a medium (25 ml) and cultured in air up to 3 months to examine the regrowth of the stored calluses. The experiments were repeated 3 to 5 times.

done three times with a manometer and a mass flow controller[4]. The gas-replacement did not influence the growth of rice calluses. The calluses in the desiccator having the desired gas were stored at 27°C in the dark. Three types of gases, i. e., 80% (v/v) CO_2+20% (v/v) O_2 and 100% CO_2 and 100% N_2 were used in this experiment. After the storage in the gases, the calluses were transferred onto fresh medium and cultured in air up to 3 months to examine the regrowth of the stored calluses.

The figure shows the growth of rice calluses in various atmospheres. The gas of 80% CO_2+20% O_2 suppressed growth. Rice calluses did not grow in the gases of 100% CO_2 or 100% N_2. In the gases of 100% CO_2 or 100% N_2, some callus cells lost their color and others became brown. However, cell aggregates with a normal appearance were formed from the brown callus cells in the culture in air after 8 to 12 weeks of storage in those gases (Table). The frequency of formation of cell aggregates with a normal appearance was high in the calluses cultured in 100% CO_2 or 100% N_2 as compared to the calluses cultured in air (Table). These cell aggregates exhibited the active growth characteristics of untreated original calluses. In the gas of 80% CO_2+20% O_2, rice calluses formed cell aggregates with a normal appearance in the same manner as did those in air. However, no cell aggregates with a normal appearance were formed from the calluses cultured in the gas of 80% CO_2+20% O_2 for 8 weeks in three separate experiments (Table).

Rice calluses grow vigorously in air. Therefore, we transferred rice calluses to fresh medium every 2 weeks in order to prevent their death from the deficiency of nutrients in the culture medium. If the consumption of nutrients from the culture medium is reduced owing to the suppression of growth, we will be able to transfer them at longer intervals. The high concentration of carbon dioxide (the mixed gas of 80% CO_2+20% O_2) suppressed the growth of rice calluses (Figure). However, the survival rate of rice calluses in the gas was similar to that in air (Table). Rice calluses might take up nutrients from the culture medium in the flask for the slow growth in the gas, and might die from the deficiency of nutrients in the culture medium. The gases of 100% CO_2 or 100% N_2 suppressed the growth of rice calluses very strongly compared with the mixed gas (Figure). This strong suppression might prevent the deficiency of nutrients in the culture medium during storage in these gases. Therefore, some callus cells would survive even after storage for 12 weeks. These results showed that the growth of rice calluses was suppressed by the gas-replacement, and thus the consumption of culture medium nutrients might be reduced and rice calluses could survive for a long time in the gases of 100% CO_2 or 100% N_2 compared with air. The strong suppression of growth by the gas-replacement made it possible to prolong the interval of transplan-

tation of cultured plant cells. Low temperatures also suppress the growth of cultured plant cells[5]. However, because the desiccator used for the gas-replacement is a very simple apparatus, this method is favorable for transportation of rice calluses. (Accepted October 16, 1990)

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《和文要約》

ガス置換によるイネカルスの保存

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空気中培養の場合には10週間植えかえずに培養したイネカルスでは，新しい培地に植えかえ再培養しても再増殖しなかった．しかし，CO_2 (100%) ガス中や N_2 (100%) ガス中では生育が抑制され，12週間置いたカルスからも新しい培地に植えかえ空気中で再培養すると，元の無処理のカルス同様に増殖する細胞塊が形成された．