Notes Plant Tissue Culture Letters, 7(2), 117-119 (1990)

Low and Super Low Temperature Storage of Green Chlorophyllous Spores of *Equisetum arvense*

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In vitro culture system of *Equisetum arvense* spore and gametophyte is useful for studies of cell polarity,[1] growth and development,[2] sexual organ differentiation[3-5] and apogamous plant production.[6] However, green chlorophyllous spores of *E. arvense* lose their viability in a short period.[7] Therefore, if the spores are used as experimental material, the problem of seasonal limitation occurs. To overcome this problem, extensive studies have been made by Castle[8] and Wollersheim.[9] Both found that a small percentage of spores retain the viability if stored at 4℃ in sterile nutrient solution. Similar results were obtained in ten species of *Equisetum* by Duckett.[3] However, in his study, none of spores germinated after more than 150 days storage at 4℃. In this paper, the availability of low and super low temperature storage of *E. arvense* spores is described.

Spores of *E. arvense* were harvested and sterilized as we reported previously.[2] Dried spores which were kept in a desiccator were transferred into a glass vial (10 mm×20 mm) with a screw cap and then stored at room temperature, 4℃, −30℃ or −196℃ (in liquid nitrogen). After 7 months of storage, spores were directly sowed in Murashige and Skoog's[10] liquid medium containing 3% sucrose and cultured at 26℃ under continuous light (1,000 lux). Germination rate was determined after 7 days of culture.

Table 1 shows the germination rate of *E. arvense* spores stored at different temperatures. More than 50 per cent of spores stored at −30℃ or in liquid nitrogen retain the ability to germinate while all spores stored at room temperature or 4℃ had lost the viability. **Figure 1** shows the morphology of spores stored at room temperature and in liquid nitrogen at different culture stages. Immediately after sowing in culture medium, morphological difference was barely recognized between the two cases as far as light microscopic observation (**Fig. 1 A** and **D**). However, after 1 day of culture, room temperature stored spores had shrunk 70-80 per cent of their original diameter and their color changed from yellowish green to brown (**Fig. 1 B** and **C**). These behaviors identical to dead spores were observed partly even in the spores stored at −30℃ or in liquid nitrogen, which had lost germination capacity. Viable spores without shrinkage became dark green (**Fig. 1 E**) and germinated in 2 to 3 days in culture (**Fig. 1 F**). Although some of spores which did germinate died during further culture, the others normally developed to young gametophytes.

Green chlorophyllous spores of *E. arvense* could be kept in viable condition for 7 months by storage at −30℃ or in liquid nitrogen. Since chlorophyllous spores have a high respiratory activity, it was naturally thought that they metabolized stored reserves in a shorter period of time than nongreen spores.[11] Probably, some physiological changes occur during storage at room or chilling temperature and lose the viability of spores. The temperature below −30℃ delay or almost stop this process.

Significant difference in morphology was not recognized between alive and non-germinatable spores immediately after sowing in culture medium as far as light microscopic observation. However, at this stage, there might have already been ultrastructural and physiological difference in spores stored at different temperatures.

(Received March 6, 1990)

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Table 1. Germination rate of *E. arvense* spores stored at different temperatures.

Storing temperature (℃)	Germination rate (%)
Room temperature	0
4	0
−30	55
−196	58

At least 1,000 spores were counted in each determination.

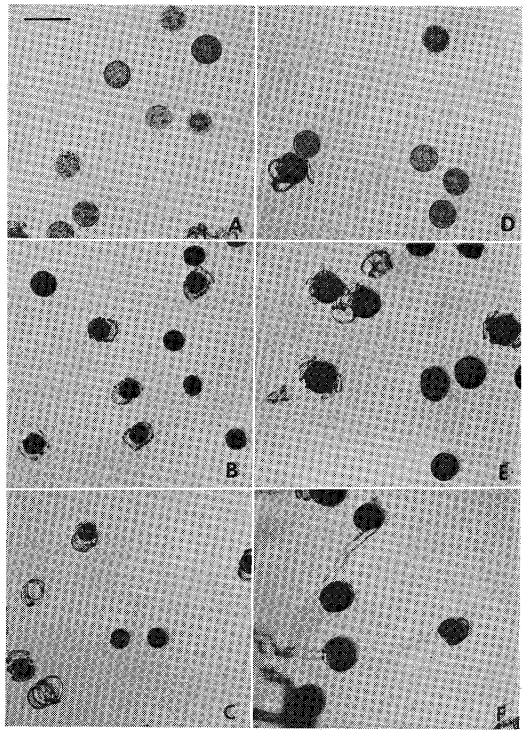

Fig. 1. Morphological changes of *E. arvense* spores during early days in culture after storage of different temperature. Bar=100 μm. A-C: Stored at room temperature. D-F: Stored at −196℃ (in liquid nitrogen). A and D: Immediately after sowing. B and E: 1 day after sowing. C and F: 3 days after sowing.

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スギナ (*Equisetum arvense*) 胞子の低温，超低温保存について

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長期保存が困難であるスギナ (*Equisetum arvense*) 胞子の保存法の改良のために，乾燥した胞子の保存温度と生存率の関係について調べた．室温または 4℃ で7カ月保存した場合，すべての胞子が発芽能を失っていたのに対し，−30℃ または液体窒素中（−196℃）で保存した胞子の半数以上は発芽能を保持していた．