Germination of vegetable seeds exposed to very high pressure

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Abstract. Effects of high hydrostatic pressure were investigated on vegetable seeds in the GPa range to examine the potentialities of breed improvement by high-pressure processing. Specimens of several seeds of broccoli (Brassica oleracea var. italica), Turnip leaf (Brassica rapa var. perviridis) and Potherb Mustard (Brassica rapa var. nippoinica) were put in a teflon capsule with liquid high pressure medium, fluorinate, and inserted into a pyrophillite cube. By using a cubic anvil press a hydrostatic pressure of 5.5 GPa was applied to these seeds for 15 minutes. After being brought back to ambient pressure, they were seeded on humid soil in a plant pot. Many of these vegetable seeds began to germinate within 6 days after seeded.

1. Introduction
Concerning the biological studies under high pressures, the techniques for generation of high pressure have firstly been applied industrially to the field of food science [1, 2]. In these studies the pressure was extended to the order of several hundred MPa, where most bacteria and many other organisms die. The high pressure denaturation techniques have been utilized to preserve foods for longer times. The effects of high pressure on living things have also been intensively investigated in the field of deep sea biology [3], where many new species of bacteria and planktons are being found.

On the other hand, there are rather few biological studies under high pressures to the order of several GPa. A several experiments have been conducted by Sharma et al. [4], Margosch et al. [5], and Vanlint et al. [6], where the pressures were extended to a few GPa. The present author’s group have been extended the pressure further to the order of several GPa [7-12]. These experiments have been conducted from bacteria to small animals and plants. It was shown that both small animals (such as tardigrades [7] and Artemia [8, 9]), and mosses (Psychomitrion [10] and Venturiella [11]) in their cryptobiotic state, can survive under high hydrostatic pressure of 7.5 GPa. Apart from elevated temperature, this pressure corresponds to that in the upper mantle, at the depth of 180 km below the earth’s surface. From these series of studies, it becomes clear that the smaller the size of animals and plants is, the stronger the tolerance would be against high pressure.

The study of biological samples under very high pressures could contribute to the development of agricultural applications for the selection of very strong species of vegetables and fruits which can tolerate severe environmental conditions. For the first step to turn the research to such direction, we selected white clover, Trifolium lepens L [12], and found that such a vascular plant could tolerate against 7.5 GPa. Furthermore, it was also shown that though high pressure treated white clovers

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germinated and grew up more slowly than control (not exposed to high pressure), they have stronger resistance and living longer than control.

Considering a possibility for application of our techniques, these experiments have been further extended to some popular winter vegetables to look for improvement of breed.

2. Experimental Procedure
Specimens of seeds of broccoli (*Brassica oleracea* var. *italica*), Turnip leaf (*Brassica rapa* var. *perviridis* (Komatsuna)) and Potherb Mustard (*Brassica rapa* var. *nipposinica* (Mizuna)) were purchased from Takii Seed co., Japan. They were dehydrated by keeping them in a low humidity container of about 20% for a week. Then, they were put in a teflon capsule with an inner diameter and length of 5.0 and 5.0 mm, respectively. As the liquid pressure medium, Fluorinate PC77 (perfluorocarbon, C₈F₁₈, Sumitomo 3M) was adopted and put into the teflon capsule together with 10-20 seeds of the three kinds of vegetables. It was proven that soaking in 100% fluorinate has no practical effect on the life of tardigrades [7], *Artemia* [8, 9], mosses [10, 11] and white clover [12]. The capsule was put in the center of a cube which was made of pyrophyllite with an edge length of 19.0 mm.

To generate hydrostatic pressure up to 5.5 GPa a cubic anvil press was adopted. The pyrophyllite cube was compressed by six tungsten-carbide anvils with a front edge length of 15.0 mm. These anvils were compressed by a 1500-ton press. The pressure was determined by using a calibrated curve of the relation between the press load and the actual pressure established before the experiment. The error of the intensity of pressure was smaller than 0.3 GPa. In this apparatus, the press load was controlled automatically to keep the intensity of the pressure constant during the operation at the maximum pressure.

The pressure was increased from ambient to the maximum pressure of 5.5 GPa at a rate of 0.14 GPa/min⁻¹. The pressure was kept constant at the maximum pressure for 15 minutes, and decreased down to the ambient pressure with the same rate as on increasing. Those high pressure experiments were made at room temperature. Figure 1 shows the pyrophyllite cube and the teflon capsule after exposed to the maximum pressure. Immediately after the pressure was released, the seeds were brought out from the teflon capsule and seeded on a humid sowing soil on a tray. The tray was put on the interior of a room by the side of a bright window, and sometimes brought outside on a fine day in winter.

![Figure 1. A photograph of the pyrophyllite cube, the Teflon capsule with the seeds of Turnip leaf after exposed to 5.5 GPa for 15 minutes.](image)

3. Results and Discussion
A photograph of the tray is shown in figure 2. This photograph was taken two weeks after seeded. It is evident that most of the seeds exposed to high pressure were alive and germinated. Furthermore, the germination rate of the three kinds of vegetables exposed to high pressure was only a little smaller than the controls.

Observed percentages of the germinated seeds for high pressure exposed broccoli, Turnip leaf and Potherb Mustard were 40%, 58% and 83%, respectively, while 58%, 83% and 100% for control, respectively. These germination ratios were almost as large as 80% as indicated by Takii Seed co., except for broccoli. Though the germination ratio of the high pressure exposed seeds were about 20-
30% smaller than control, it was proven that all the three vegetables can tolerate the high pressure of 5.5 GPa.

A   A'  B   B'  C   C'
↓    ↓   ↓    ↓   ↓    ↓

![Figure 2](image1.png)

**Figure 2.** A photograph of sowing soil tray, two weeks after seeded. (A) broccoli and (A') control, (B) Turnip leaf and (B') control, (C) Potherb Mustard and (C') control.

![Figure 3](image2.png)

(a) Broccolis after transferred onto bigger size pots; (a) high pressure exposed group and (b) control.

The germinated vegetables were transferred from the sowing soil tray onto bigger size pots on the day two weeks after seeded. Broccolis just after the transfer were shown in figure 3. A photograph of high pressure exposed Potherb Mustard incubated for two months is shown in figure 4, where some grown up leaves are clearly seen.

![Figure 4](image3.png)

(b) The germinated vegetables were transferred from the sowing soil tray onto bigger size pots on the day two weeks after seeded. Broccolis just after the transfer were shown in figure 3. A photograph of high pressure exposed Potherb Mustard incubated for two months is shown in figure 4, where some grown up leaves are clearly seen.

The average height observed for germinated Potherb Mustard for both high pressure exposed and control was plotted in figure 5 as a function of elapsed days after seeded. In this figure it is seen that the leaves germinated from the high pressure exposed seeds became a little taller than control. The number of the samples shown in figure 5 was 20, and this number is the statistical basis of the present investigation.

Considering the fact that diamond powders industrially used are synthesized under a pressure around 5.5 GPa and at high temperature [13], where graphite changes into diamond structure, it seems difficult, even only counting the pressure, to understand that all of these three winter vegetables survive after being exposed to such a high pressure of 5.5 GPa.
4. Conclusions
It was found from the present study that all the three kinds of seeds of typical winter vegetables, broccoli (*Brassica oleracea* var. *italica*), Turnip leaf (*Brassica rapa* var. *perviridis*) and Potherb Mustard (*Brassica rapa* var. *nippoinica*) germinated after exposed to 5.5 GPa for 15 minutes. Observed percentage of the germinated seeds was 20-30% smaller than control, but they grew up as rapidly and tall as control. The results of the present study may have a possibility to look for a strong species that stands against severe environmental conditions.

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