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High Pressure Treatment Accelerates Germination of Winged Bean Seeds

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Abstract
High hydrostatic pressures (30-200 MPa) have been utilized for accelerating the germination of winged bean (*Psophocarpus tetragonolobus*, 'Urizun') seeds. This pressure treatment promoted rapid germination of seeds and increased the germination percentage; it was especially effective in the germination of hard seeds, To confirm the acceleration mechanism of germination, water absorption parts were investigated under the pressure treatment. Furthermore, the changes in the water absorption content, leakage of solutes from seeds and individual sugar contents were also analyzed. As a result it is assumed that the effect of pressure treatments is at least partially physical leading to the improvement of water penetration, especially through the micropyle and seed coat, with a resulting acceleration of hydrolysis of sugars and other physiological processes associated with germination.

Discipline: Horticulture
Additional key words: head seed, impermeable nature, acceleration mechanism

Introduction

De Vries was the first to observe the effects of hydrostatic pressures on the seed germination of *Oenothra cockenelli*[4]. Thereafter, Davies studied the effects of high hydrostatic pressures (50-200 MPa) on the germination of *Medicago sativa* and *Melilotus alba* seeds, and revealed a relationship between high pressure and seed germination[1-3]. Rivera demonstrated that high pressure treatments were very effective in accelerating the germination of seeds with hard, impermeable seed coats (alfalfa and yellowwood), but were not effective in the case of seeds with dormant embryos (witch hazel and rose)[11]. Thereafter, studies concerned with seed germination using high pressures did not make any progress. In 1991, Hasegawa et al. reported that the dormancy of bulblets of *Allium wakegi* Araki was abruptly broken by forced water infiltration under pressures of 25 and 200 KPa and by the removal of foliage scales[6]. This study was the first to apply pressure for the germination of vegetables. However, high pressures have not yet been used for the germination of vegetable seeds. On the other hand, the methods of application of high pressures have rapidly improved and are currently used in various industrial fields. Especially, several high pressure instruments utilized in the field of food processing and preservation have experienced a remarkable development. Therefore, numerous studies on the use of high pressure treatments in the field of food science and technology have recently been reported[7,8]. We applied this kind of high pressure system for the germination of vegetable seeds for the first time and found that moderate pressure treatments (30 MPa for 5-15 s) stimulated the germination of a few bean seeds and were especially effective in the germination of hard seeds. From these beans we selected winged bean in the current study. In spite of the considerable effort devoted to the introduction and breeding of winged bean, the lower germination rate and the uneven germination associated with the hard seed coat had limited wider cultivation in Japan.

In this study, we directed our attention to the germination of winged bean seeds under high pressure treatments and investigated the effectiveness of high pressures in detail. Furthermore, we also analyzed

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the acceleration mechanism of germination.

Materials and methods

1) Germination test

Winged bean seeds were harvested in 1991 or 1992 in the field of Chugoku National Agricultural Experiment Station. Seeds stored in a desiccator containing silica-gel at 5°C were selected and used for the germination test. Fifteen or 25 seeds of winged bean were placed between a layer of two filter papers moistened with water in a 9-cm petri dish, which were kept in an incubator at 25°C. Each treatment was replicated twice. The number of seeds which had germinated was counted every day, and after each count the germinated seeds were discarded.

2) High pressure treatment

Seeds and then water (about 100 ml) were placed in a sealing bag. This bag was sealed by degassing using a sealer for home use, after which pressures were applied using a high pressure equipment (Mitsubishi Heavy Industries Co., Ltd., MFP-7000). Applied pressures were 30–200 MPa (1 MPa = 9.862 atm); the pressures were kept for 5–120 seconds.

3) Investigation of water absorption parts

Hilum, micropyle, strophiole or these parts together were separately shielded with grease and subjected to a pressure of 30 MPa for 15 s or soaked in water for 2 days. Each pressure treatment applied to 25 seeds of winged bean was replicated twice. The imbibition of seeds was observed immediately after each pressure treatment. Thirty-five seeds were used for the soaking test (no pressure treatment). Seeds were removed as soon as they imbibed.

4) Measurement of leakage of solutes from seeds

UV measurements of the leakage of solutes from winged bean seeds were performed using a Shimazu UV-2100 spectrophotometer. Seeds were soaked in 3 ml of water. After the solutions were diluted to the mark of a 10-ml calibrated flask, the absorbances at 260 and 280 nm, which corresponded to the leakage of nucleic acids and protein[9], were measured. This measurement was repeated every hour for 15 h.

5) Measurement of sugar contents

Winged bean seeds were roughly ground in a minimill (Sibata, SCM-40A) and then more finely ground with a small amount of quartz sand using a mortar. The ground solids were extracted with 80% ethanol (10 ml) at around 80°C for 30 min. This extraction procedure was repeated three times. After the extracted solutions were combined and centrifuged (4,000 × g, 10 min), the supernatant was collected, filtered and evaporated to dryness. About 1 ml of water was added, and then the suspension was passed through Sep-pak Plus C18 cartridges (Waters). Polymers, especially proteins were removed by centrifugal filtration of the eluent (4,000 × g, 60 min) with Ultracent-30 (Tosoh) to give a clear solution. This solution was passed through a 0.45-μm membrane filter (Millipore) and used for HPLC analysis of sugars. HPLC was performed on a Tosoh 8010 system with a refractive index detector. A column (Shodex Ionpak KS-801, 8ϕ × 30 cm) and a precolumn (Shodex Ionpak KS-800P, 6ϕ × 5 cm) were installed in a column oven and then heated at 60°C. Sugar samples (100 μl) were injected and eluted with distilled water at 0.5 ml/min.

Results and discussion

1) Effect of high pressure treatment on germination

Table 1 shows the results of the germination test of winged bean seeds (harvested in 1991) subjected to various pressure treatments. The germination percentage of the control seeds was about 80%, with approximately 20% of ungerminated seeds referred to as hard seeds. Pressure treatments of 30 and 50 MPa resulted in a higher total germination percentage than that of the control, though 200 MPa treatment reduced it. The longer the exposure time and the higher the pressure, the lower the germination percentage. Furthermore, days to 50% germination were shorter than in the control, indicating that the pressure treatment accelerated germination. However, the pressure treatment also caused abnormal germination. The seeds with abnormal germination were partially injured around the micropyle so that the growth of the germ was delayed. These injured seeds were prone to solute leakage and became moldy. In the pressure treatments with 50 and 200 MPa, all of the ungerminated seeds displayed degenerative changes. This germination test indicates that lower pressures (30–50 MPa) and shorter exposures (5–30 s) are effective in the germination of winged bean seeds. Exposure for 5 s under a pressure of 30 MPa gave optimum results.

Table 2 shows the results of the germination test of winged bean seeds, which were harvested in 1992 and contained about 50% hard seeds. In this test,

Table 1. Germination of winged bean seeds[a] subjected to various hydrostatic pressure treatments[b]

Pressure (MPa)	Exposure (s)	Number of germinated seeds[c]						Percentage of germination	Days to 50% germination
		2	3	4	5	6 (days)	Total		
Control			5	8	6	2	21	84.0	3.9
30	5		11	10 (1)	4 (1)		25	100.0 (8)	3.2
30	15		10 (1)	13 (5)	1	1 (1)	25	100.0 (28)	3.2
30	30		5	16 (6)	2 (1)	1 (1)	24	96.0 (32)	3.5
30	60		5	9 (3)	7 (3)		22	88.0 (24)	3.8
50	30		5 (1)	17 (5)	2		24	96.0 (24)	3.4
50	120		9	8 (1)	2	3 (3)	22	88.0 (16)	3.4
200	120		6	11 (4)	2		19	76.0 (16)	3.6

a): Harvested in 1991.
b): Twenty-five seeds were used in each treatment.
c): Mean number of duplicates.
Number or percentage of abnormal germination is indicated in parentheses.

Table 2. Germination of winged bean seeds[a] subjected to various hydrostatic pressure treatments[b]

Pressure (MPa)	Exposure (s)	Number of germinated seeds[c]						Percentage of germination	Days to 50% germination
		2	3	4	5	6 (days)	Total		
Control			2	2	1	2	7	46.7	
30	15	1	7 (1)	2	1		11	73.3 (6.7)	2.9
30	30		3	7 (1)	2		12	80.0 (6.7)	3.6
50	30	1	6	2	1	3	13	86.7	3.3

a): Harvested in 1992.
b): Fifteen seeds were used in each treatment.
c): Mean number of duplicates.
Number or percentage of abnormal germination is indicated in parentheses.

Table 3. Germination of hard seeds[a] of winged bean subjected to various hydrostatic pressure treatments[b]

Pressure (MPa)	Exposure (s)	Number of germinated seeds[c]						Percentage of germination	Days to 50% germination
		2	3	4	5	6 (days)	Total		
Control		0	0	0	0	0	0	0.0	
30	15	5	7	2 (2)			14	93.3 (13.3)	3.4
30	30	2	11	2			15	100.0	2.5

a): Harvested in 1991.
b): Fifteen seeds were used in each treatment.
c): Mean number of duplicates.
Number or percentrage of abnormal germination is indicated in parentheses.

we analyzed only the treatments with 30 or 50 MPa pressure and 15 or 30 s exposure, that were effective in the germination in the above first test. All of the three pressure treatments strikingly increased the total germination percentage and accelerated germination, compared to the control. Furthermore, the percentage of abnormal germination remarkably decreased. This beneficial effect of the pressure treatment on the germination suggests that hard seeds, which accounted for about 50%, became permeable by the pressure treatment. To confirm this assumption, only hard winged bean seeds that did not germinate in the control were subjected to the pressure treatment. The results are shown in Table 3. There was a significant difference between the germination percentages in the control and the pressure treatments. These results certainly confirm that the lack of permeability to water of hard seeds can be alleviated by exposure to high pressures.

Winged bean seeds require relatively longer days to germinate and do not show a uniform germination because they have usually harder seed coats[5,10]. Until now, some methods have been applied to improve the permeability of hard seed coat; for example, scarification of seed coat with a needle or treatment of seeds with hot water or a strong corrosive such as concentrated sulfuric acid. In addition to these methods, high pressure treatment may be effective in improving the permeability of winged bean seeds. Naturally, the conditions of pressure treatments must be investigated before application and be slightly modified depending on the seed conditions.

2) Confirmation of the acceleration mechanism of germination

(1) Investigation of water absorption parts

We investigated the water absorption parts subjected to the pressure treatment (30 MPa for 15 s) and the soaking treatment in water for two days (no pressure treatment) by separately shielding the hilum, micropyle and strophiole of seeds. To confirm that water was absorbed through the seed coat, the three parts were shielded together. Under the pressure treatment seeds imbibed immediately after the treatment, while in the absence of the pressure treatment seeds imbibed gradually and hardly imbibed after two days. Table 4 shows the swelling rates in both treatments.

In the absence of pressure treatment the swelling rates showed that water could be absorbed throughout every part. The swelling rate of the three shielded parts of seeds was the lowest. Most seeds that absorbed water through the seed coat tended to require a longer soaking time than that through the other parts (results not shown). These observations suggest that it is difficult for seeds to absorb water through the seed coat. However, virtually there was no significant difference among the swelling rates. On the other hand, in the pressure treatment there was an apparent difference among the swelling rates. The hilum- or strophiole-shielded seeds exhibited almost the same high swelling rate as the control seeds, while the micropyle- or three shielded parts of seeds displayed a lower swelling rate. These

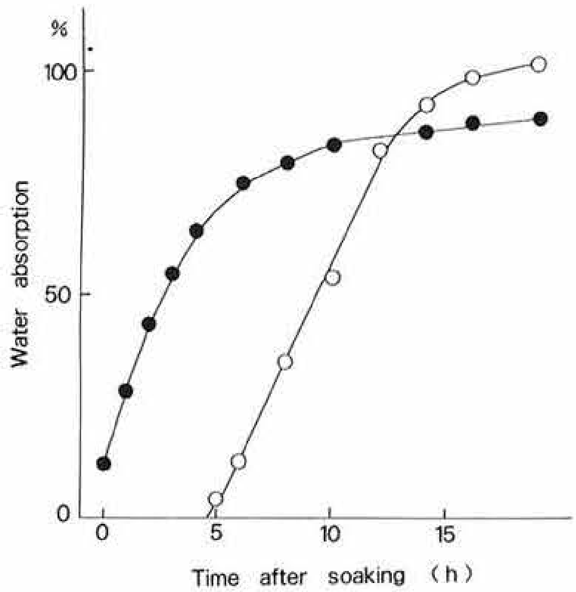

Fig. 1. Water absorption processes in the winged bean seeds not subjected to the pressure treatment (—○—) and in those subjected to moderate pressure treatment (30 MPa for 15 s., —●—)

Table 4. Swelling rate of winged bean seeds[a)] separately shielded with grease and subjected or not subjected to the pressure treatment

Shielded parts	Swelling rates (%)	
	Treatment[b)]	No treatment[c)]
Control	86	51
Hilum	90	34
Micropyle	56	29
Strophiole	86	34
Hilum + Micropyle + Strophiole	52	23

a): Harvested in 1991.

b): Twenty-five seeds were subjected to a pressure of 30 MPa for 15 s in each treatment. The imbibition of seeds was observed immediately after each treatment. Mean percentage of treatments applied twice.

c): Thirty-five seeds were soaked in water for 2 days in each treatment. Seeds were removed as soon as they imbibed.

observations indicate that water can be mainly absorbed through the micropyle and seed coat and that water absorption is accelerated by the pressure treatment. The lack of permeability of hard seed coat is probably alleviated by water absorption through the micropyle and seed coat. These aspects are currently investigated in detail.

(2) Water absorption process

Fig. 1 shows a representative example of the water absorption processes of winged bean seeds not subjected to the pressure treatment (control) and subjected to a moderate pressure treatment (30 MPa for 15 s). In the former, once water absorption started, the water content almost linearly increased over about 7 h, and eventually reached a plateau with about 100% increase. The latter also showed almost the same tendency, though water absorption started immediately after the pressure treatment. The amount of water absorbed increased by about 12% immediately after the onset of the moderate pressure treatment. This amount corresponded well to the very early stage of the water absorption process in the control, suggesting that immediately after the moderate pressure treatment water was not absorbed significantly to injure seeds.

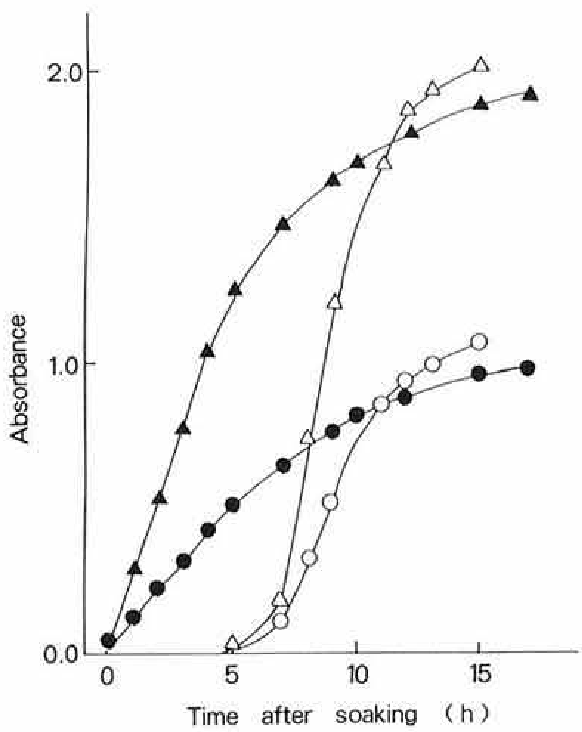

Fig. 2. Changes in the leakage of solutes from the winged bean seeds not subjected to the pressure treatment (260 nm, —△—; 280 nm, —○—) and in those subjected to moderate pressure treatment (30 MPa for 15 s, 260 nm, —▲—; 280 nm, —●—) with the progression of soaking

(3) Leakage of solutes from seeds

The leakage of solutes from winged bean seeds was examined by measuring the absorbances at 260 and 280 nm, which corresponded to the leakage of nucleic acids and protein. Fig. 2 shows the changes in the leakages of solutes in relation to the soaking time using the pressure-treated and -untreated seeds (control). All the curves were very similar to those of the water absorption process, in short, once the solutes started to leak, the amount leaked increased linearly. This suggests that the leakage of solutes increased according to the increase of the amount of water absorbed; prolonged soaking in water was not beneficial for seed germination due to the excessive loss of nutrients. In order to examine the imbibition damage induced by the pressure treatments in seeds, the absorbances at 260 and 280 nm were measured immediately after the onset of the moderate pressure treatment (30 MPa for 15 s). The absorbances (about 0.15 at 260 nm and about 0.07 at 280 nm) corresponded to the very initial stage of the changes in the leakage of solutes under the soaking treatment, indicating that no significant leakage was observed from the pressure-treated seeds. This finding demonstrates that the imbibitional damage of the seeds in the moderate pressure treatment was negligible.

(4) Changes in sugar contents

In this report we directed our attention to sugars to examine the changes in the chemical constituents of the pressure-treated seeds in the process of germination. Figs. 3 and 4 show the changes in total sugar and individual sugar contents, respectively. The total sugar content included stachyose, raffinose, sucrose, glucose and fructose. The sugar content in the leakage of solutes form seeds, which was minimal under the moderate pressure treatment as described previously, could be neglected here. With the progression of germination, the total sugar content and the contents of stachyose and raffinose decreased, while those of sucrose and glucose + fructose increased. This tendency was almost the same as that in the control, although the changes were more rapid under the pressure treatment which accelerated germination. The increase in the sucrose and glucose + fructose contents and the decrease in the stachyose and raffinose contents indicate that a metabolic reaction (hydrolysis) occurred in the process of germination. Furthermore, the total sugar content and the contents of stachyose and raffinose already

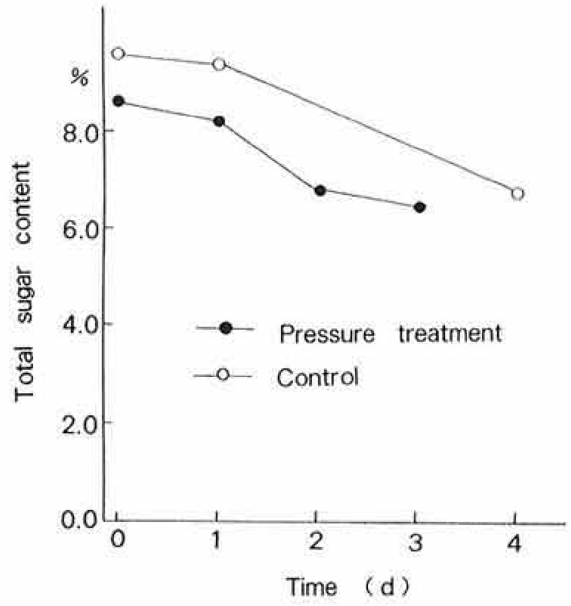

Fig. 3. Changes in the total sugar content of winged bean seeds with the progression of germination

Applied pressure was 30 MPa, and the pressure was kept for 15 s.

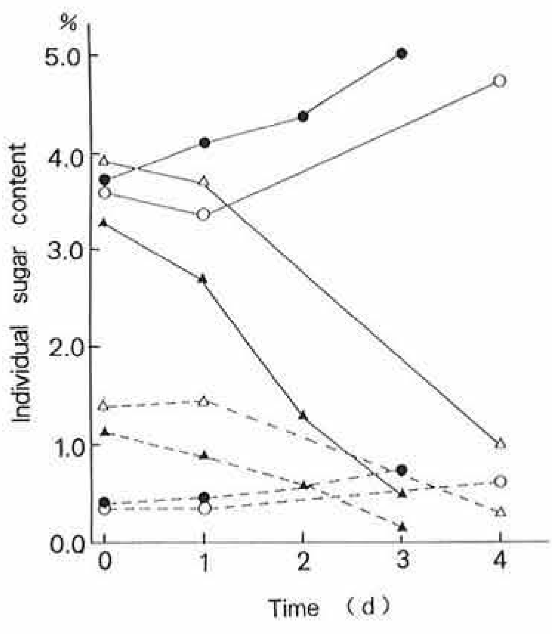

Fig. 4. Changes in individual sugar content of winged bean seeds with the progression of germination

Applied pressure was 30 MPa, and the pressure was kept for 15 s.

Stachyose (C, —△—; PT, —▲—); Raffinose (C, ---△---, PT, ---▲---); Sucrose (C, —○—; PT, —●—); Glucose + Fructose (C, ---○---, PT, ---●---).

C, Control; PT, Pressure treatment.

decreased even immediately after the pressure treatment (time (days): 0) in comparison with the control. These facts suggest that the changes in total and individual sugar contents, in other words, the metabolic reaction of sugars, already started immediately after the pressure treatment.

Based on the results obtained, it is assumed that the effect of pressure treatment is at least partially physical leading to the improvement of the penetration of water, especially through the micropyle and seed coat, with a resulting acceleration of hydrolysis of sugars and other physiological processes associated with germination. This acceleration mechanism can be further confirmed by studying the changes in enzyme or hormone activity.

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