Decrease in Germination Activity of Rice Seeds due to Excessive Desiccation in Storage

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Received June 8, 1976

Cool temperature and low moisture has been considered to be favorable and necessary conditions for the long term storage of rice seeds^{1, 4, 5, 8, 11, 14}. However, excessive desiccation may be harmful to the seeds. This paper deals with the results of experiments on the loss and recovery of the germination activity of rice seeds stored over humidity controlling agents in the container and the leakage of electrolytes from desiccated seeds, and discusses the critical level of seed water content in terms of the storage and the germination.

Materials and Methods

The seeds of the paddy rice variety Shiokari, obtained from Hokuren, were stored at 5°C over CaCl_2 in the container until they were used for experiments. This method has been used in our laboratory for the usual storage of rice seeds; the large container (glass desiccator) includes usually a large amount of seeds and a relatively small amount of CaCl_2 ; the water content of seeds is kept at 10-12 percent for more than 2 years; these seeds were used directly as the "controls" for germination tests. The seeds of the red bean (*Phaseolus radiatus* L. var. aurea Prain, variety Kitamishiro) were obtained from the Third Upland Crop Laboratory of the Hokkaido National Agricultural Experiment Station, and stored at room temperature in paper bags.

The water content of the seeds was controlled with CaCl_2 , with saturated aqueous solutions of some salts or with different concentrations of H_2SO_4 aqueous solutions in sealed containers. Table 1 shows the expected air moistures with these moisture controlling agents^{2, 15}. The attained water contents of seeds will be described for individual experiments.

For the test of germination activity, seeds were sterilized with a ten-fold diluted antiformin

solution for 5 minutes after which they were preimbibed at 18°C for 24 hours. Fifty grains were placed on three sheets of wet filter paper in a polycarbonate Petri dish (9 cm in diameter, with air holes on the side) and germinated at 20°C.

Germination was estimated by two different methods; the breaking of the back of the lemma, and 5 mm elongation of the root (or the plumule). The terms G_{50} and E_{50} mean the respective number of days during which the back of the lemma is broken for 50 percent of the tested seeds, and during which the root (or plumule) elongates by 5 mm for 50 percent of the tested seeds. Thus, G_{50} or E_{50} is the reciprocal of germination activity.

The leakage of electrolytes from rice seeds were estimated by the conductivity of the medium water.

Table 1. Control of air moisture with saturated salt solution or H_2SO_4 solution^{2, 15}.

	Air moisture	
	At 18.5°C	At 20°C
CaCl_2 , sat.	35%	32.3%
$\text{Ca}(\text{NO}_3)_2$, sat.	56	54.7
NaNO_2 , sat.	66	
$(\text{NH}_4)_2\text{SO}_4$, sat.	81	81
Na_2CO_3 , sat.	92	90.7
H_2SO_4 67.6%		10%
58.8		20
53.7		30
48.6		40
43.3		50
38.8		60
33.6		70
26.7		80
16.5		90
0		100

Table 2. Effects of storage condition and length of the germination activity of rice seeds.

Storage condition		Storage length (days)		
		11	33	90
Paper bag	5°C	G ₅₀ *	2.3	2.4
		E ₅₀ *	5.0	5.0
	18°C	G ₅₀	2.3	1.9
		E ₅₀	5.1	4.3
Desiccator**	5°C***	G ₅₀	2.6	2.2
		E ₅₀	5.1	5.2
	18°C	G ₅₀	3.4	5.0
		E ₅₀	6.4	9.4

* Refer to "Materials and Methods".

** Sealed glass container with CaCl₂.*** Control (stored over relatively small amount of CaCl₂): Refer to "Materials and Methods".Table 3. Germination activity of rice seeds stored at different temperatures over CaCl₂.

Temperature		Storage length (days)			
		11	32	68	95
5°C	G ₅₀ *	1.8	3.3	3.2	4.4
	E ₅₀ *	4.8	6.1	5.8	8.1
					(5.7%)**
10	G ₅₀	2.3	4.0	3.2	4.6
	E ₅₀	5.3	6.9	5.7	7.7
					(5.3)
15	G ₅₀	2.4	4.6	3.9	6.0
	E ₅₀	5.4	7.6	6.9	9.5
					(4.3)
20	G ₅₀	2.5	4.3	3.6	4.7
	E ₅₀	5.3	7.1	6.6	8.2
					(4.3)
25	G ₅₀	2.5	4.8	3.3	5.2
	E ₅₀	5.3	7.8	6.2	8.1
					(4.1)
30	G ₅₀	2.2	4.8	2.7	5.2
	E ₅₀	5.3	7.5	5.7	8.2
					(3.8)
35	G ₅₀		4.6	2.5	3.8
	E ₅₀		6.5	5.2	6.9
					(5.2)

*Refer to "Materials and Methods".

**Water content of seeds.

Results

Table 2 shows the results of a preliminary experiment on the effects of storage condition and length on the germination activity of rice

Table 4. Effects of the amount of desiccant on the water content and germination activity of rice seeds*.

Amount of CaCl ₂	G ₅₀ **	E ₅₀ **	Water content
Single***	3.5 days	6.7 days	6.6%
Double	4.4	8.3	5.5
Single	2.4	5.4	6.2
Double	2.8	5.8	4.7
Quadruple	3.2	6.5	4.3
Control**	2.0	4.8	11.0

*At 18°C for 60 days.

**Refer to "Materials and Methods".

***Fixed figures were not measured.

seeds. Storage in paper bags at 5°C and at 18°C, and in a sealed container with CaCl₂ at 5°C did not change the germination activity as far as 90 days. While the germination activity decreased gradually when the seeds were stored at 18°C over CaCl₂ in the container. For elucidating the cause of this decrease, germination activity was estimated for rice seeds stored over CaCl₂ during 95 days at different temperatures. The results are shown in Table 3, indicating that the cause of decrease in the activity was not the temperature, because the decrease occurred over the temperature range from 5 to 35°C. In the next place, the amount of desiccant was examined. Table 4 shows the effect of the amount of CaCl₂ on the water content and germination activity of rice seeds. An increased amount of the desiccant caused the decrease both in water content and in germination activity. Thus, the cause of the decrease in germination activity was concluded to be the excessive desiccation of rice seeds. The difference in activity, found in the first experiment (Table 2), between seeds stored at 5°C and 18°C in the desiccators was due to the different water content of seeds resulted from the difference in the desiccating power of the containers; the 18°C container was smaller and contained more desiccant in relation to the amount of the seeds in comparison with the 5°C container.

For estimating the critical water content of rice seeds for the decrease of germination activity, rice seeds were stored at 18°C over aqueous H₂SO₄ solutions of different concentrations (Table 5), and over aqueous saturated

Table 5. Germination activity of rice seeds stored at 18°C over H₂SO₄ solutions of different concentrations.

Concentration of H ₂ SO ₄		Storage length (days)				
		29	64	98	150	277
16.5%	G ₅₀ *	2.3 days	1.8 days	1.8 days	2.0 days	2.0 days
	E ₅₀ *	5.0	4.5	4.8	5.1	4.9
		(16.7%)**			(17.1%)	(17.0%)
26.7	G ₅₀	2.4	1.9	1.6	2.0	1.8
	E ₅₀	5.0	4.7	4.9	5.2	4.8
		(13.7)			(15.0)	(14.2)
33.6	G ₅₀	2.5	1.7	2.2	2.1	1.9
	E ₅₀	5.2	4.4	4.9	4.5	4.8
		(11.6)			(13.2)	(12.4)
38.8	G ₅₀	2.0	2.0	1.8	2.0	2.3
	E ₅₀	4.8	4.7	4.9	4.7	5.1
		(10.2)			(10.8)	(10.4)
43.3	G ₅₀	2.5	2.0	2.0	2.0	1.9
	E ₅₀	5.2	4.8	5.1	4.4	4.9
		(8.2)			(10.6)	(10.3)
48.6	G ₅₀	2.6	2.4	2.3	2.2	2.2
	E ₅₀	5.4	5.2	5.1	5.1	5.2
		(8.0)			(9.9)	
53.7	G ₅₀	2.8	2.4	2.2	2.4	2.4
	E ₅₀	5.6	5.0	5.1	5.4	5.3
		(7.3)			(7.0)	(6.7)
58.8	G ₅₀	2.8	2.7	2.5	2.6	2.5
	E ₅₀	5.7	5.5	5.4	5.7	5.6
		(6.7)			(5.8)	(5.4)
67.6	G ₅₀	3.9	4.2	3.7	2.8	2.6
	E ₅₀	7.1	7.1	7.0	6.2	5.7
		(5.5)			(5.7)	

*Refer to "Materials and Methods".

**Water content of the seeds.

solutions of different salts (Table 6). The decrease in germination activity was observed clearly at the water content of 5–6 percent; this result was in accord with those obtained in other experiments (Table 3, 4, 9, 10 and 11); thus, the critical water content was estimated at 6–7 percent. Table 5 shows the high water content of 17 percent was not injurious as far as 277 days of storage. As for the experiment shown in Table 6, the germination activity of the seeds stored over the saturated NaNO₂ solution declined much faster than the seeds of other plots, and did not reach any equilibration of the activity; this might be due to some adverse effect of this salt, e.g. the release of injurious vapour. Seeds of 10–14 percent water content kept their activity as far as 777 days. While the activity of seeds contained water

more than 19 percent much declined at and after 461 days of storage.

It has been known for beans (lima bean^{9,10} and soybean³) that the initial water content of the seeds affects the germination activity. Tables 7 and 8 show the results of experiments for red bean on the germination activity of the seeds stored at 18°C over saturated salt solutions. Desiccation was injurious to their germination, and the critical content of water was 12–13 percent. Rehumidification of desiccated seeds by storing over the saturated Ca (NO₃)₂ solution was effective for the recovery of germination activity, when the damage was not serious.

Experiments were made for rice seeds to test whether or not the rehumidification is effective for the recovery of germination activity as in

Table 6. Germination activity of rice seeds stored at 18°C over saturated salt solutions.

Moisture control agent		Storage length (days)					
		33	77	110	182	461	777
Na ₂ CO ₃ , sat.*	G ₅₀ **	2.3 days	1.8 days	1.6 days	2.1 days	(33:36%)**	
	E ₅₀ **	4.7	4.5	4.8	5.4	(33:0%)	
Water content		20.8%			23.0%	23.9%	
(NH ₄) ₂ SO ₄ , sat.	G ₅₀	1.8	1.8	1.7	2.1	11.4	(6:42%)
	E ₅₀	4.4	4.5	4.8	5.6	(26:42%)	(6:0%)
Water content		19.8			19.0	19.0	17.9%
NaNO ₂ , sat.	G ₅₀	1.7	2.2	2.8	7.8	(26:38%)	
	E ₅₀	4.0	4.9	6.2	16.0	(26:0%)	
Water content		16.1			15.8	16.0	
Ca(NO ₃) ₂ , sat.	G ₅₀	2.1	1.7	1.8	1.9	2.1	2.2 days
	E ₅₀	4.6	4.2	4.7	4.7	4.7	4.9
Water content		14.6			13.7	14.5	13.3
CaCl ₂ , sat.	G ₅₀	2.0	2.1	1.8	1.8	1.8	2.2
	E ₅₀	4.6	5.2	4.9	4.9	4.8	4.9
Water content		11.5			10.3	10.8	10.2
CaCl ₂	G ₅₀	4.5	4.5	5.3	4.3		
	E ₅₀	7.8	7.7	9.0	7.8		
Water content		5.8			5.8		
Control**	G ₅₀					2.3	1.8
	E ₅₀					5.2	4.5
Water content		13.4			12.2	11.1	10.8

*Saturated solution.

**Refer to "Materials and Methods".

***The 36 percent of the tested seeds germinated by the 33th day of incubation.

red bean (Tables 9 and 10). The activity recovered with the recovery of water content in some experiments. In the cases of more intensive desiccation, however, the activity did not recover though the water content of the seeds recovered.

Table 11 shows the leakage of electrolytes from rice seeds into the medium water. Much electrolytes leaked out immediately after the soaking, and then the velocity of leakage gradually decreased. The amount of leakage from the excessively desiccated rice seeds was roughly double as compared with that from control seeds.

Discussions

It is a generally accepted idea that cool temperature and low moisture content are favorable and necessary conditions for the long term storage of rice seeds^{1,4,5,8,11,14}. On the other hand, NAKAJIMA^{6,7} reported about 50 years ago that the excessive desiccation of rice seeds injured the germination activity. These papers, however, have been in obscurity and

the injury by excessive desiccation has not been recognized for a long time. For example, ROBERTS' equation¹¹ for the relationship between temperature, water content and the viability of rice seeds was applied to the range of water content below 6 percent, by extrapolation^{4,5,8}; thus for rice seeds of 2 percent water content, the half-value period of germination percentage was calculated at approximately 2,000 years at 5°C⁴.

However, the present author experienced through his experiments that the germination activity of rice seeds stored over CaCl₂ in sealed containers sometimes declined unexpectedly. The former half of this report clarified that the cause of this decline in germination activity is due to the excessive desiccation of the seeds. The critical water content is 6–7 percent. These findings confirm NAKAJIMA's reports. Besides, this paper showed that the germination activity of desiccated rice seeds can be recovered by rehumidification, completely or partly according to the degree of the injury and to the

degree of rehumidification. NAKAJIMA^{6,7)} also found the similar fact. In the case of serious desiccation, however, the activity did not recover even after the water content increased sufficiently.

When rice seeds are soaked into water, electrolytes leak out from the seeds. This paper

Table 7. Germination activity of red bean seeds stored at 18°C over saturated salt solutions (1).

Moisture control agent	Storage length (days)	
	78	126
Na ₂ CO ₃ sat.*	G ₅₀ **	1.5 days
	E ₅₀ **	0.9 days
(NH ₄) ₂ SO ₄ , sat.	G ₅₀	3.4
	E ₅₀	2.3
NaNO ₂ , sat.	G ₅₀	1.0
	E ₅₀	0.8
Ca(NO ₃) ₂ , sat.	G ₅₀	2.3
	E ₅₀	2.2
CaCl ₂ , sat.	G ₅₀	0.6
	E ₅₀	0.6
CaCl ₂	G ₅₀	1.7
	E ₅₀	1.6
Ca(NO ₃) ₂ , sat.	G ₅₀	0.6
	E ₅₀	0.8
CaCl ₂ , sat.	G ₅₀	2.6
	E ₅₀	2.0
CaCl ₂	G ₅₀	1.6
	E ₅₀	1.6
CaCl ₂	G ₅₀	3.5
	E ₅₀	3.7
CaCl ₂	G ₅₀	(6:0%)*
	E ₅₀	(5:0%)*
CaCl ₂	G ₅₀	(6:0%)*
	E ₅₀	(5:0%)*
CaCl ₂	G ₅₀	(5:0%)*
	E ₅₀	(5:0%)*
→Ca(NO ₃) ₂ , sat.‡	G ₅₀	(5:0%)*
	E ₅₀	(5:0%)*

*Saturated solution.

**Refer to "Materials and Methods".

***No germination occurred by the 6th day of incubation.

‡ Kept over saturated Ca(NO₃)₂ solution for 7 days after the storage over CaCl₂ for 126 days.

showed that the amount of the leakage was much higher in excessively desiccated seeds with less germination activity than in control ones. The result combined with the following reports suggests that rice seeds are likely injured at cell membranes when seed water content lowered below critical point. The occurrence of increased leakage of some substances from desiccated bean seeds has been reported: 264 mμ absorbing compounds (probably nucleotides) for lima bean^{9,10)}, sugar and ninhydrin positive materials for soybean³⁾, electrolytes and protein for pea¹³⁾, and electrolytes for castor bean¹³⁾. The cases for lima bean and soybean are in parallel with the decrease in germination activity and the cause of this increase in the leakage is attributed to the loss of integrity of the cell membrane^{12,13)}.

For the long term storage of rice seeds, therefore, the harmful effect of excessive desiccation should be taken in consideration. The seed water content is preferred to be kept above 6–7 percent, though there is still a possibility of longer storage at the highly desiccated state if the recovery of germination activity can be attained effectively by some devices in rehumidification.

Summary

The germination activity was estimated at 20°C for rice seeds stored over humidity controlling agents. Rice seeds of 10–14 percent water content were stored at 18°C in good condition for more than 2 years. While the seeds of water content over 19 percent declined the

Table 8. Germination activity of red bean seeds stored at 18°C over saturated salt solutions (2).

Moisture control agent and storage length	G ₅₀ *	E ₅₀ *	Water content
Ca(NO ₃) ₂ , sat.** for 32 days	0.8 days	2.3 days	13.7%
CaCl ₂ , sat. for 32 days	2.2	4.8	11.5
CaCl ₂ for 32 days	(6:0%)*	(6:0%)*	6.9
CaCl ₂ for 23 days			
Ca(NO ₃) ₂ , sat. for 9 days	3.9	(6:6%)*	8.2
CaCl ₂ for 12 days			
Ca(NO ₃) ₂ , sat. for 20 days	1.8	3.9	11.4

*Refer to "Materials and Methods".

**Saturated solution.

***No germination occurred by the 6th day of incubation.

Table 9. Effect of rehumidification on the germination activity of rice seeds desiccated over CaCl_2 .

Treatment			Treated			Control*		
Desiccation**	Humidification***		G_{50} *	E_{50} *	Water content	G_{50}	E_{50}	Water content
I	157 days 157	33 days	2.8 days 2.3	6.3 days 4.7	5.4% 12.6	2.3 days	4.7 days	11.7%
II	97 97	92	2.9 2.0	6.4 5.5	4.5 13.6	2.4	5.1 5.7	11.3 11.1
III	302 302	57	9.0 9.6	(17:26%) § (16:36%)	3.4 18.2	1.7 1.8	4.2 4.2	10.4 11.2

*Refer to "Materials and Methods".

At 18°C over CaCl_2 .*At 18°C over saturated solution of $\text{Ca}(\text{NO}_3)_2$ for I and II, and saturated solution of $(\text{NH}_4)_2\text{SO}_4$ for III.

§ The 26 percent of the tested seeds germinated by the 17th day of incubation.

Table 10. Effect of rehumidification on the germination activity of rice seeds desiccated over H_2SO_4 solution.

H_2SO_4 concentration		Desiccated for 90 days*	Desiccated for 195 days	Desiccated for 349 days, rehumidified for 18 days**	Desiccated for 349 days, rehumidified for 57 days
17.1%	G_{50} *** E_{50} ***	1.5 days 4.0 (16.4%)§	1.7 days 3.8 (16.2%)	2.5 days 5.0 (13.8%)	
34.2	G_{50} E_{50}	1.5 3.9 (10.9)	1.6 4.1 (11.3)		1.7 days 4.2 (13.3)
43.3	G_{50} E_{50}	1.7 4.4 (9.4)	1.6 4.0 (9.6)	1.6 4.3 (12.5)	
53.2	G_{50} E_{50}	1.5 4.2 (7.3)	1.8 4.2 (7.6)		1.5 4.2 (13.3)
68.4	G_{50} E_{50}	2.2 4.8 (4.9)	2.4 5.0 (5.2)	1.7 4.6 (12.5)	
76.0	G_{50} E_{50}	2.4 5.2 (4.2)	2.8 5.7 (4.2)		1.8 4.5
85.5	G_{50} E_{50}	2.6 5.4 (4.0)	2.9 6.2 (3.8)	2.5 6.0 (12.5)	
95.0	G_{50} E_{50}	3.0 5.7 (3.9)	4.8 8.8 (3.4)		2.6 6.0 (14.1)
Control***	G_{50} E_{50}			1.8 4.5 (10.8)	1.8 4.1 (11.2)

*Desiccated at 18°C.

**Rehumidified at 18°C over saturated $\text{Ca}(\text{NO}_3)_2$ solution.

***Refer to "Materials and Methods".

§ Water content of the seeds.

Table 11. Leakage of electrolytes from rice seeds desiccated over CaCl_2 at 18°C .

Soaking	Excessively desiccated seeds*		Control**	
	Leakage***	Leakage/h	Leakage	Leakage/h
0~ 1 h	13.30 $\mu\text{S}/\text{cm}$	13.30 $\mu\text{S}/\text{cm}/\text{h}$	6.95 $\mu\text{S}/\text{cm}$	6.95 $\mu\text{S}/\text{cm}/\text{h}$
1~ 2	4.25	4.25	1.65	1.65
2~ 3	2.55	2.55	1.25	1.25
3~24	14.30	0.681	9.35	0.445
24~48	7.25	0.302	4.20	0.175
48~72			3.60	0.150
G_{50} **	4.9 days		2.4 days	
E_{50} **	9.4		5.7	
Water content	4.0%		11.1%	

*Desiccated over CaCl_2 at 18°C for 427 days.

**Refer to "Materials and Methods".

***Ten grains were soaked in 10 ml distilled water.

germination activity after approximately 1 year. For the seeds of water content at 5–6 percent, the germination activity was much lower, though the activity was not changed through 1 to 9 months of storage.

The decreased activity of desiccated rice seeds was recoverable by the rehumidification of the seeds, completely or partly depending on the degree of desiccation and on the degree of rehumidification. In the case of serious desiccation, however, the activity did not recover though the water content recovered.

The leakage of electrolytes was much higher in excessively desiccated rice seeds than in the seeds of proper water content, indicating the loss of integrity of cell membranes by desiccation.

The results showed the necessity of caution for the use of desiccants in the storage of rice seeds.

Acknowledgements

The author wishes to express his thanks to the Third Upland Crop Laboratory, Hokkaido National Agricultural Experiment Station for providing the seeds of red bean.

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〔和 文 摘 要〕

貯蔵中の過度の乾燥によるイネ種子の発芽活性の低下

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(農林省北海道農業試験場)

湿度調節剤を入れた容器の中に貯蔵されたイネの種子の発芽活性が 20°C で測定された。含水量 10～14 パーセント程度の種子は 18°C で 2 年以上にわたって良好な状態に保存された。含水量 19 パーセント以上の種子は約 1 年をすぎると発芽活性が低下した。含水量 5～6 パーセント以下の種子の発芽活性はかなりひくかったが、1～9 カ月の間変化しなかった。

乾燥により低下した種子の発芽活性はふたたび含水量を増加させることによりある程度回復する。その程度は乾燥および再加湿の程度によるが、はなはだしく乾燥した種子においては含水量が十分にたかくなっても活性が回復しなかった。

乾燥した種子を浸漬すると電解質が漏出してくる。過剰乾燥種子では通常乾燥のものよりも電解質の漏出が多量であった。このことは細胞膜の構造が乾燥により破壊されていることを意味する。

以上の諸事実は、イネ種子の長期貯蔵にあたって乾燥剤の使用法に注意して過度の乾燥をさける必要があることをしめしている。