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Estimation of Germinability of Gramineous and Leguminous Seeds in Long-Term Storage by Means of Peroxidase Activity and TTC Reduction

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Introduction

It has been known that the germinability of seeds in storage can be estimated by some germinability indicators such as specific enzymatic activity or specific substance exuded from the seeds, in stead of conducting time-consuming germination tests. Many studies have shown close relationship between germinability and values of these indicators.[2-7] However, these studies were made by using individual species. It has not been made clear whether such close relationship between germinability and its indicators can exist or not among many different species or different genera. In other words, whether a single quantitative correlation is applicable or not to a group of seeds composed of those of different species or genera is not known.

Therefore, the present study was taken up by using the seeds of 76 species (32 genera) of Leguminosae and 56 species (29 genera) of Gramineae, which had been stored for more than 10 years.

Table 1. Color development in the peroxidase activity test and TTC reduction test

Grade of color development*	Pattern of color development							
	Leguminosae seeds				Gramineae seeds			
	Peroxi-act.		TTC		Peroxi-act.		TTC	
	Coty-ledon	Hypo-cotyl	Coty-ledon	Hypo-cotyl	Endo-sperm	Embryo	Endo-sperm	Embryo
0	−	−	−	−	−	−	−	−
1	±	−	±	−	−	−	−	±
2	+	±	+	±	−	−	−	+
3	++	+	++	+	−	−	±	++
4	+++	++	+++	++	±	−	±	+++
5	+++	+++	++++	+++	+++	++	+++	++++

* Grade of color development was regarded to signify the grade of vigor, and used as the standard to determine seed vigor. At the end of the present study the color development observed with all the seeds used in the study was again checked by this standard. Thus, it was confirmed that the standard was consistently applicable.
−; no color, ±; weak pink, +; pink, ++; dark pink or reddish-brown, +++; red or brown, ++++; purplish red or dark brown

Materials and methods

The seeds used in this study are shown in Tables 2 and 3. The total number of seed samples was 312 for Leguminosae, and 282 for Gramineae, including 60 and 4 samples of unclassified genera, respectively. The leguminous seeds were introduced from East Africa in 1972 and 1973, and from South America in 1973, 1974, and 1977. The gramineous seeds were introduced from East Africa in 1972 and 1973. After the introduction, each seed sample was placed in a sealed bottle containing silica-gel, and stored in a storage room (4 °C, 80% R.H. and dark) at Okinawa Branch, Tropical Agriculture Research Center. The seeds appeared well-matured and not damaged.

The germination test and seed vigor assessment by means of peroxidase activity and TTC (Triphenyl tetrazolium chloride) reduction were carried out from January to December 1985. The germination

Table 2. Results of the seed vigor tests of stored leguminous seeds

Country of origin	E. Africa					E. Africa					S. America					S. America					S. America					Total number of seed samples
Year of introduction	1972					1973					1973					1974					1977					
Grade of seed vigor*	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	
Abrus																					1					1
Alysicarpus							1																			1
Canavalia												1														1
Cajanus											2											2	1	1	1	7
Calopogonium																				1						1
Cassia	1	2				1																				4
Centrosema		1				2										1										4
Cicer						1															2					3
Clitoria		1			2			1																		4
Crotalaria			2					1		3				1	1											8
Desmodium		2	2	1	2	2			1	1												2		1		14
Dolichos																				1						1
Galactia																1								1		2
Glycine		1				2	3		1		13	12	5	2	3					2						44
Indigofera			1	4	1	3	3	2	2													1				17
Lablab											1	1									1	1				4
Lathyrus											1															1
Lens						2																1				3
Lupinus																					1	1	2			4
Macrotyloma					1																					1
Macroptilium				1					1					1								1				4
Medicago		3	1					2		5											1	1	1			14
Mimosa										1																1
Mucuna											2		1		1											4
Phaseolus	1									1	12	13	3	2							1	1	1			35
Pycnospora										1																1
Pisum						1	4		1																1	7
Stylosanthes		1	1	1		1			1	1	1	4	1	1	3				1	1						18
Tephrosia										2												2				4
Trifolium	2					5	9	6	2	4																28
Vicia						2																				2
Vigna															3						4			2		9
Unclassfied Leguminosae	6	1	3	2	7	18	5	5	2	10											1					60
Total of seed samples	10	12	10	9	13	40	25	17	11	29	32	31	10	7	11	2		1	4		13	13	5	5	2	(312)

* Determined by peroxidase activity and TTC reduction

test was conducted at 30 °C, with or without light. A usual Petri dish with 2 layers of wetted filter paper or a small glass vessel (dia. 3.5 cm, height 7.5 cm) was used for 20 seeds. As shown later, hydrogen peroxide and thiourea were used to promote germination, when needed. In addition, the scarification treatment for leguminous seeds and dehusking treatment for gramineous seeds were employed.

The peroxidase activity was measured using 3% *O*-methoxyphenol (guajacol) and 30% hydrogen peroxide based on the theory of Kanazawa et al.[1] The TTC reduction test was carried out using 3% tetrazolium salt solution.[4] About 10 seeds for each seed sample were longitudinally cut into 2 parts in such a manner to divide an embryo into halves and soaked separately in these test solutions. Then the color development was examined.

Experimental result

A preliminary experiment to know the pattern of color development in the peroxidase activity test and TTC reduction test was carried out. The result, given in Table 1, indicates that the seed vigor as shown by the color development can be classified into 6 grades from 0 to 5. This classification was employed throughout all experiments.

Table 2 shows the result of the seed vigor test for stored leguminous seeds. About 30% of the total leguminous seeds exhibited high vigor with the score of 4 or 3. Seeds of plants which belongs to the genus *Crotalaria, Desmodium,* and *Indigofera* showed

Table 3. Results of the seed vigor test of stored gramineous seeds

Country	E. Africa										Total number of seed samples
Year of introduction	1972					1973					
Grade of seed vigor*	0	1	2	3	4	0	1	2	3	4	
Andropogon						5					5
Anthephora	1										1
Aristida						4					4
Beckeropsis						1					1
Bothriochloa	1					6					7
Brachiaria						2					2
Capillipedium						1					1
Cenchrus	2					5					7
Chloris	14					21					35
Cynodon	5					4					9
Dactyloctenium	4					2					6
Digitaria	2					4					6
Echinochloa						4					4
Eleusine	6					5					11
Enteropogon						1					1
Eragrostis	7					12					19
Heteropogon	1										1
Hyparrhenia	9					21					30
Leersia						3					3
Panicum	22					29	1				52
Paspalum	5					1					6
Penniselum	16					4					20
Schizachyrium	1										1
Setaria	1					6					7
Sorghum	18	1				3	2	1	1	1	27
Sporobolus	2										2
Themeda	2					3					5
Urochloa	2										2
Zea	1				1		1				3
Unclassfied Gramineae	1					3					4
Total of samples	123	1			1	150	4	1	1	1	(282)

*Determined by peroxidase activity and TTC reduction

high vigor. On the other hand, as given in Table 3, most gramineous seeds were zero in the score of seed vigor. Only some seeds of *Zea mays* and *Sorghum* sp. showed high vigor.

The relationship between seed vigor and seed germinability in the stored leguminous seeds is shown in Table 4, in which the results of the experiment are grouped into 4 cases. In the case A, seeds which showed high vigor gave high percent germination. The scarification treatment failed to promote germination due to mold infestation. In the case B, high vigor of seeds was associated with high percent germination. The scarification treatment was effective in promoting germination to some extent. In the case C, seeds treated with scarification showed that high vigor is associated with high percent germina-

Table 4. Relationship between seed vigor and germinability of stored leguminous seeds

Case	Grade of seed vigor*	Germination (%)			Species of leguminosae
		Non-treat.	Scarification-treat.	Thiourea-treat.(10^{-1}M)	
A	4—3	70—100	10—50		*Cajanus cajan, Mucuna pruriens*
	2	20—50	0—30		*Glycine max*
B	4—3	50—80	60—100		*Calopogonium mucunoides, Macrotyloma axillare, Phaseolus vulgaris, Pycnospora lutescens, Tephrosia* sp., *Trifolium repens, Vigna unguiculata, V. mungo*
	2	20—30	30—50		*Lupinus mutabilis, Phaseolus* sp., *Pisum sativum, Trifolium* sp.,
C	4—3	10—50	60—100		*Clitoria ternatea, Crotalaria incana, C. juncea, C. intermedia, C.* sp., *Desmodium distortum, D. triflorum, Dolichos axilaris, Galactia striata, Glycine wightii, Indigofera hirsuta, I. spicata, Macroptilium atropurpureum, Medicago* sp., *Mimosa invisa, Stylosanthes guyanensis, S. himilis, S.* sp.
	2	0—20	30—50		*Clitoria ternatea, Crotalaria intermedia, Desmodium* sp., *Glycine wightii, Indigofera* sp., *Macroptilium* sp., *Stylosanthes gracilis*
D	4—3	0—10	10—20	50—90	*Medicago* sp., *Trifolium pratense.*
	2	0	0—10	20—40	*Medicago lupulina, Trifolium semipilosum*

* Determined by peroxidase activity and TTC reduction

Table 5. Relationship between seed vigor and germinability of stored gramineous seeds

Grade of seed vigor*	Germination (%)				Species of gramineae
	Non-treat.	Dehusking	Hydrogen peroxide treat. (1%)	Dehusking +Hydrogen peroxide treat. (1%)	
4	30		100		*Zea mays*
4	0	10	70	90	*Sorghum* sp.
3	0	0	50	65	*Sorghum* sp.
2	0	0	30	45—55	*Sorghum* sp.
1	0		20—30		*Zea mays*
1	0	0	0	10—20	*Sorghum* sp.
1	0	0	0	5	*Panicum* sp.

* Determined by peroxidase activity and TTC reduction

tion, but this relationship was not clearly shown with non-treated control seeds. In the case D, only the seeds treated with thiourea gave high percent germination associated with high vigor.

These results indicate a consistent trend that the leguminous seeds showing high vigor gave the germination higher than 50%, with or without the scarification and thiourea treatments. When the germinability was not fully manifested due to hard seed coat and seed dormancy, these treatments are needed.

Table 5 shows the relationship between seed vigor and germination of stored gramineous seeds. As most of the gramineous seeds have already lost their vigor, the result obtained with seeds of *Zea mays* and others is shown. Here again, the same trend as shown with leguminous seeds was recognized. Namely, the seeds with high vigor showing the score of 4 and 3 gave the germination higher than 50%, when their germinability was fully manifested by treatments* of dehusking and hydrogen peroxide application (Plate 1).

The seed vigor and germinability of fresh seeds harvested from plants which were grown in 1985

Plate 1. Germinated Sorghum seeds in the glass vessel containing 1% hydrogen peroxide added to 2 layers of filter paper.

Table 6. Seed vigor and germinability of fresh leguminous and gramineous seeds

Sci. name	Number of samples	Grade of seed vigor	Germination percentage (%)			
			Non-treat.	Scarification treat.	Thiourea (10^{-1}M)	Hydrogen peroxide (1%)
(Leguminosae)						
Cajanus	5	5	95—100	95—100		
Calopogonium	1	5	95	90		
Cassia	2	5	10— 30	85—100		
Clitoria	3	5	10— 20	90—100		
Crotalaria	5	5	80	100		
Desmodium	2	5	10— 20	95—100		
Glycine max	3	5	100	100		
Glycine wightii	2	5	20— 30	95—100		
Indigofera	2	5	10— 20	85— 90		
Macroptilium	3	5	100	100		
Macroptilium sp.	1	5	0— 10	100		
Medicago	3	5	0— 5	80— 90	0—5	
Phaseolus	2	5	95—100	100		
Vigna	5	5	90—100	95—100		
Total of seed samples (Gramineae)	39					
Zea	1	5	90			85

* Treatments with 10^{-1}M–10^{-4}M of KNO_3, $CaNO_3$, $CaCO_3$, and 2,4-dinitrophenol were also conducted, but promoted germination was not observed.

from the stored seeds, originally introduced, are shown in Table 6. All the seeds examined showed high vigor with the score of 5, and almost 100% of germination with the treatments of scarification, etc.

Discussion

There have been many studies indicating a close relationship between germinability of seeds in long-term storage and specific enzymatic activity.[3,5,7] However, such a relationship was recognized only with individual species. In the present study, a consistent relationship between seed vigor, as expressed by peroxidase activity and TTC reduction activity, and seed germinability was confirmed with seeds of many different genera of Leguminosae and Gramineae. Namely, taking all these seeds as a whole, a single quantitative correlation between percent germination and seed vigor was proved to exist.

In the germination test of leguminous seeds, it was observed that seeds of most genera were not able to manifest their germinability to a full extent due to hard seed coat or seed dormancy. Therefore, it happened quite often that seeds with high vigor showed very low percent germination. In such a case, the scarification treatment or thiourea treatment must be applied. On the other hand, for gramineous seeds, dehusking or 1% hydrogen peroxide treatment was very effective in promoting germination. As a matter of fact, without these treatments, almost all seeds were not able to germinate, being severely infested with mold. These facts suggest that it is necessary to pay attention on the problems of seed dormancy, hard seed coat, germination inhibitor, etc. in conducting germination tests.

Summary

The relationship between seed vigor and germinability of the seeds in long-term storage was examined using a large number of seed samples including 76 species (32 genera) of Leguminosae and 56 species (29 genera) of Gramineae. Based on the preliminary experiment, the seed vigor as represented by peroxidase activity and TTC reduction was rated into 6 grades from 0 to 5.

A consistent trend that seeds with high vigor showing the vigor grade* of 4 and 3 can give the germination higher than 50% was recognized, irrespective of different species and different genera of Leguminosae as well as Gramineae. Namely, taking all these seeds as a whole, a single qualitative correlation that high vigor is associated with high percent germination was proved to exist.

In the germination tests, seeds of most genera were not able to manifest their germinability to a full extent due to hard seed coat, seed dormancy, etc. The scarification treatment and thiourea application for leguminous seeds, and dehusking and hydrogen peroxide application for gramineous seeds were effective in promoting germination in such cases.

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* Grade 5 was not shown by stored seeds.