

Osmoconditioning and Deterioration of Soybean Seeds During Storage

Osmoconditioning dan Deteriorasi Benih Kedelai Selama Penyimpanan

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ABSTRACT

Soybean seeds deteriorate quite rapidly when stored at high humidity and temperature and osmoconditioning prior to storage may reduce the rate of seed deterioration. To answer this hypothesis, soybean seeds were osmoconditioned with PEG 6000 solutions at -11.9 bars osmotic potential before storage at 15 and 35°C for up to 6 months, and their germination, T_{50} , electrical conductivity and respiration rate were compared with that of untreated seeds stored under the same conditions. Untreated seeds stored at 15°C maintained a high level germination (from 100-70%) throughout storage period, while untreated seeds stored at 35°C showed a rapid decline in germination and by 4 months of storage its seed viability was already less than 50%. At storage temperature of 35°C , osmoconditioned seeds retained higher germination, germinated more rapidly and showed greater cell membrane integrity and higher respiration rate than untreated seeds irrespective of storage period. Therefore, it is concluded that osmoconditioning prior to storage slowed the rate of seed deterioration especially at high temperature of storage.

Keywords: soybean, osmoconditioning, deterioration, storage

INTRODUCTION

Quality of planted seeds was regarded as the starting point of the characteristic behavior of seed during germination. Soybean seed inherently deteriorates quite rapidly leading to lower its quality during storage. Therefore, maintenance of its quality during storage to retain its capacity to germinate satisfactorily is of particular relevant to soybean.

In most tropical countries, soybean seeds are generally stored without refrigeration. Under this condition, seed viability as well as vigor drops quite rapidly. Priming or osmoconditioning have been shown to reduce the rate of seed deterioration and to improve the germination of seed (Bradford, 1986). Previous works also revealed that osmoconditioned seeds of different species have been reported to either retain their germinability (Atherton and Faroque, 1983) or

the promotive effect of osmoconditioning was retained for up to 2 months (Perl and Feder, 1981). However, its effects in relation to soybean seed storage are still ill-defined. If osmoconditioning is to be of practical application on germination, it is important that any promotive effects on germination should be retained throughout storage. The purpose of the research was to compare the rate of deterioration of primed and untreated soybean seeds during storage at low and high temperature.

MATERIALS AND METHODS

The soybean seed cultivar used throughout the study was Wilis. The seeds were planted in research plots at Agriculture Faculty, Bengkulu University on February 2000. At harvest maturity (13 to 15% seed moisture), the seeds were harvested by hand stripping of the

Pods. The pods were dried with heated air at 35°C to reduce the moisture content to 10-12% for threshing. The dried pods contained in jute bags were threshed by flailing and the seeds were separated from the pod walls and other plant parts by sieving. After cleaning and sizing the seeds were placed in paper bags and stored at 15°C until needed for the storage studies. Its initial seed viability was high (>90%), and its initial moisture content (MC) for the varieties was about 10% MC (wet weight basis).

Seeds were osmoconditioned with PEG 6000 solutions (Sigma) at -11.9 bars osmotic potential (30 g PEG 6000 dissolved in 100 ml water) on three layers of filter paper in a 5 l plastic box saturated with 1 l of PEG solution at 15°C in the dark for 5 days. OC with PEG 6000 at -11.9 bars was selected for evaluation on the basis of preliminary experiment. At the end of period of OC the seeds were removed from the plastic box, rinsed in a stainless steel stainer under running tap water for 5-10 sec., and blotted with paper germination towels to remove surface water. The osmoconditioned seeds were dried in a forced air dryer at 35°C to their original moisture content.

After OC, the treated and control seeds were stored at a temperature of 15 and 35°C for six months at RH of 85%. The seeds were stored in a wood box of about 0.6 m³ capacity lined with plastic with saturated sodium chloride at the bottom to maintain a RH of 85%. Seeds were contained in plastic mesh pouches and placed in the chamber. The chamber was positioned in a room with temperature controlled at about 15 and 35°C. Samples of treated and non-treated seeds were withdrawn at two months interval over six months of storage period for quality evaluation. To evaluate their quality, the seeds were subjected to the following tests: standard germination, rate of germination (T_{50}), electrical conductivity, and respiration rate.

For standard germination test, two replicates of 50 seeds were germinated in rolled paper towel substrata at 30°C for eight days. Normal seedlings were counted according to the rules for testing seeds (AOSA, 1978) and expressed as the percentage germination.

The rate of germination of primed and control seeds was evaluated by placing the seeds on germination blotters in 30 cm petri dishes wetted with 4.8 ml distilled water. Each dish contained 50 seeds, and there were two replicate dishes per treatment. Dishes were placed in an incubator at 30°C. Radicle protrusion of 2 mm was scored as germination. Counts of the number of germinated seeds were made at six-hour intervals until no further germination was observed. The germination rate (T_{50}) was calculated from the equation:

$$T_{50} = t_i + \frac{[(N + 1)/2 - n_i]}{(n_j - n_i)} \times (t_j - t_i)$$

where n_i is the number of germinated seeds at time t_i ; n_j is the number of germinated seeds at time t_j ; N is the total number of germinated seeds; t_i is the time needed for the seeds to germinate at number n_i ; and t_j is the time needed for the seeds to germinate at number n_j .

For electrical conductivity test, 25 seeds, in two replications, were placed in a 100 ml flask and 25 ml of deionized water was added. The flasks were placed in an incubator at a constant temperature of 30°C for 24 hours after which time the contents of the flasks were gently stirred. The electrical conductivity of seed steep water was measured with a Cole Palmer conductivity meter (Cole Palmer, Chicago, IL) and reported as mmhos per cm per gram of seed.

Respiration rate was determined by oxygen uptake and was measured by a Warburg Manometer. Twenty seeds, in two replications, were weighed, imbibed in 50 ml distilled water for three hours and placed with 2 ml water in the main compartment of a reaction flask, 0.2 ml of 15% KOH was added in center well. The reaction flasks were placed in a 25°C water bath and were shaken at 78 oscillations minute⁻¹. Readings were taken three times at an interval of 30 minute after equilibrating the system for 30 minute. Respiration rate was reported as micro litres of oxygen per seed per minute at standard temperature and pressure.

All data were subjected to analysis of variance based on Split Plot Design with storage temperature as a main plot, storage period as a sub plot and osmoconditioning as a sub-sub plot. F test at 5% level was carried out to evaluate the significance of each source of variation.

RESULTS AND DISCUSSION

Analysis of variance shows that osmoconditioning, storage temperature and storage period both as a single factor or as an interaction significantly affected physiological deterioration of soybean seeds as measured by germinability (total germination percentage and T_{50}), electrical conductivity of seed steep water, and respiration rate. Untreated seed stored at 15°C retained a high level of germinability throughout the entire storage period, total germination being consistently between 80 and 96% (Fig. 1A) and T_{50} (the time to 50% germination) between 33 – 45 hours. By contrast, untreated seed that was stored at 35°C showed a significant decline in germinability during storage that was particularly marked when storage period was at 4 and 6 months (Fig. 1B). The total germination fell from

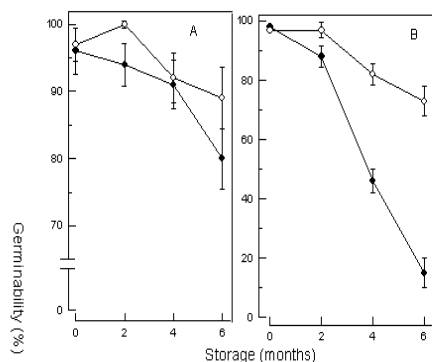


Fig. 1. Final germination of untreated (●) and osmoconditioned (◊) soybean seeds stored at 15°C (A) and 35°C (B) as a function of the duration of storage. Vertical bars = s.e.

96% before storage to 91% after 4 months and to 86% after 6 months of storage at 15°C and from 98% before storage to 46% after 4 months and to 15% after 6 months of storage at 35°C. The T_{50} increased from 33 hours before storage to 41 hours after 4 months and to 45 hours after 6 months storage at 15°C (Fig. 2A) and from 36

without refrigeration. On the other hand, the hours before storage to 50 hours after 4 months and to 69 hours after 6 months of storage at 35°C (Fig. 2B). This indicates that soybean seed deterioration occurs rather quickly at high temperature of storage, especially in tropical countries where the seeds are generally stored maintenance of high seed germination at low temperature of storage indicates low temperature to bring about a delay in the deterioration process.

Storage at 35°C brought about a rapid decline in both the final germination percentage and the rate of germination of untreated seeds. After four months of storage, the number of seeds germinating was only 46 per cent of the total. After six months of storage, the viability was less than 20 per cent. By contrast, osmoconditioned seeds retained much greater capacity to germination. After six months of storage at 35°C, the final germination remained above 70%. The maintenance of a high percentage of total germination throughout the storage period in the seeds osmoconditioned prior to storage indicates priming to bring about a reduction of the rate of deterioration during storage at 35°C. Basu and Pal (1980) also reported the beneficial effect of priming on seed viability maintenance.

Irrespective of storage temperature, primed and untreated seeds showed a gradual increase in the rate of germination (T_{50}) as storage period was extended (Fig. 2A, B). This might due to the deteriorative senescence of the seeds

during storage, which would require a longer imbibition period prior to germination for repair and replacement of membranes, organelles, and enzymes (Burgrass and Powell, 1984). However, the primed seeds exhibited a slower increase in T_{50} and a more rapid germination than the untreated seeds. This indicates that metabolic events required for germination were activated by priming and retained in the seeds during dehydration.

Osmoconditioning prior to storage significantly promoted the rate of germination of soybean seeds especially under storage at 35°C as indicated by the consistently higher rate of germination (lower T_{50}) of pre-storage-primed seeds in comparison with untreated seeds (Table 1). The T_{50} for primed seeds rose from

Table 1. The effect of untreated and primed seeds soted at ambient condition for six months on electrical conductivity of steep water and on seed respiration rate.

Storage		Treatment	Electrical Conductivity (mmhos g ⁻¹ cm ⁻¹) ^x	Respiration Rate (μL O ₂ g ⁻¹ min ⁻¹) ^x
Temp. (°C)	Period (months)			
15	0	Untreated	0.1623 f	0.812 a
		Primed	0.1590 f	0.803 a
	2	Untreated	0.2120 ef	0.673 c
		Primed	0.1820 f	0.740 abc
	4	Untreated	0.2690 e	0.623 cd
		Primed	0.2463 e	0.710 bc
35	6	Untreated	0.4090 cd	0.573 de
		Primed	0.3970 d	0.673 c
	0	Untreated	0.1613 f	0.813 a
		Primed	0.1627 f	0.802 ab
	2	Untreated	0.2495 e	0.530 e
		Primed	0.2340 e	0.795 ab
	4	Untreated	0.3030 c	0.485 ef
		Primed	0.2840 e	0.667 cd
	6	Untreated	1.0745 a	0.297 g
		Primed	0.6565 b	0.432 f

^x: Means not followed by the same small letter in each column are significantly different

34.hours before storage at 35°C to 50 hours after six months of storage. By contrast, the T_{50} for untreated seeds increased from 36 hours prior storage to 69 hours after six months of storage at 35°C. At storage temperature of 35°C, the promotive effect of priming on the T_{50} was manifested immediately after two months of storage, whereas at storage temperature of 15°C was not manifested until six months of storage. Georghiou et al. (1987) and Thanos et al. (1989) also reported the similar promotive effect of priming on the increasing rate of germination.

The membrane permeability of the seeds as evaluated by the electrical conductivity of

seed-steep water was significantly affected by storage temperature and extended storage period. Irrespective of storage temperature, primed and untreated seeds showed a gradual increse in the membrane permeability as storage period was extended (Table.1). The increase in the membrane permeability due to aging or deterioration during storage was more in the untreated than in the primed seeds especially storage at 35°C. The electrical conductivity for primed seeds rose from 0.1627 before storage at 35°C to 0.6565 mmhos g⁻¹cm⁻¹ after six months of storage. By contrast, the electrical conductivity for untreated seeds increased from 0.1613 prior storage to 1.0745 mmhos g⁻¹cm⁻¹ after six months of storage at 35°C. This indicates that during aging, degradation of cell membrane took place more progressively in untreated than in primed seeds. The involvement of cellular repair system in correcting age-induced biochemical lesions during seed hydration may explain the more sound cell membrane integrity of the primed seeds (Burgrass and Powell, 1984).

Seed respiration rate as measured by O₂ evolution was significantly affected by storage temperature and extended storage period. Irrespective of storage temperature, primed and untreated seeds showed a gradual decrease in the respiration rate as storage period was extended (Table 1). A considerable reduction in the respiration rate was noted especially in the untreated than in the primed seeds stored at 35°C.

The oxygen uptake for primed seeds declined from 0.802 before storage at 35°C to 0.432 μL O₂ g⁻¹ min⁻¹ after six months of storage. By contrast, the oxygen uptake for untreated seeds decreased from 0.813 prior storage to 0.297 μL O₂ g⁻¹ min⁻¹ after six months of storage 35°C. This data confirm that metabolic events required for germination were impaired by deterioration but activated by priming as described extensively elsewhere (Dell'Aquila and Taranto, 1986; Dell'Aquila and Tritto, 1990).

The results clearly indicate that priming of soybean seeds prior to storage had a very important advantage in that it slowed down the rate of seed deterioration at 35°C, indicated by a higher total germination, lower T_{50} , lower

electrical conductivity, and higher seed respiration. The beneficial effect of osmoconditioning that was not negated for as long as 6 months is unique and possibly had a practical use in seed storage.

CONCLUSIONS

Osmoconditioning prior to storage reduced the rate of seed deterioration during storage especially at 35°C. The action of osmoconditioning in counteracting the progressive seed deterioration was via the preservation of cell membrane integrity and the improvement of metabolic activity, e.g., seed respiration.

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