

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN KINETIN – INFUSED SOYBEAN SEEDS DURING STORAGE

*PERUBAHAN FISILOGIS AND BIOKHEMIS BENIH KEDELAI YANG DIINFUS
DENGAN KINETIN SELAMA PENYIMPANAN*

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ABSTRACT

Soybean seeds deteriorate rapidly when stored under unfavorable conditions and kinetin infusion prior to storage may reduce the rate of seed deterioration. To answer this hypothesis, soybean seeds were permeated with kinetin at 1.0 mM before storage in Agronomy Laboratory on July 2000 at 15 °C and 30 °C for up to 6 months, and their germination, germination after accelerated aging, speed of germination (T_{50}), electrical conductivity and respiration rate were compared with those of untreated seeds stored under the same conditions. The treatments were arranged in a split plot design with three replications. Treated seeds maintained a higher germination and germination after accelerated aging, showed a more rapid germination, a greater cell membrane integrity and a higher respiration rate than untreated seeds irrespective of either storage temperature or storage period, whereas untreated seeds showed a rapid decline in the five seed quality indicators. By four months of storage at 30 °C, treated seeds retained a high level of germination (>80%), medium level of germination after accelerated aging (65%) and a high speed of germination (around 40 hours), while untreated seeds showed a rapid decline in the three seed quality indicators, germination percentage being less than 80%, germination after accelerated aging being less than 50% and speed of germination being higher than 55 hours. Therefore, it is concluded that kinetin infusion prior to storage, in addition to the acceleration of germination, slowed the rate of seed deterioration irrespective of storage temperature through the improvement of cell membrane integrity and seed respiration.

Key words: soybean, kinetin, infusion, deterioration, storage

ABSTRAK

Benih kedelai mengalami deteriorasi secara cepat dan infusi kinetin mungkin dapat menekan laju deteriorasi tersebut. Untuk membuktikannya, hormon kinetin sebanyak 1,0 mM dirembeskan kedalam benih kedelai sebelum benih tersebut disimpan di laboratorium Agronomi pada bulan Juli 2000 pada suhu 15 °C dan 30 °C selama enam bulan. Setiap dua bulannya, mutunya dievaluasi dan dibandingkan dengan mutu benih yang tidak diperlakukan. Perlakuan ini ditata dalam rancangan Petak Terbagi dengan tiga ulangan. Terlepas dari suhu dan lama simpannya, benih yang telah diperlakukan dapat mempertahankan mutunya tetap tinggi yang tercermin dari nilai daya kecambah (DK), daya kecambah setelah didera (DKSD), daya hantar listrik dan laju respirasinya. Sedangkan benih yang tidak diperlakukan mengalami penurunan mutunya secara tajam. Pada bulan keempat penyimpanan pada suhu 30 °C, benih yang telah diperlakukan tetap memiliki DK yang tinggi (>80%), DKSD yang sedang (sekitar 65%) dan KB yang tinggi (sekitar 40 jam). Sedang benih yang tidak diperlakukan hanya memiliki DK kurang dari 80%, DKSD kurang dari 50% dan KB lebih dari 55 jam pada bulan dan suhu penyimpanan yang sama. Untuk itu, infusi kinetin dalam benih sebelum simpan selain meningkatkan DK, dapat pula memperlambat laju deteriorasi benih melalui perbaikan keutuhan sel membran dan respirasi benih.

Kata kunci: kedelai, kinetin, infusi, deteriorasi, penyimpanan

INTRODUCTION

A humid-tropical conditions characterized by a daily high relative humidity and a high temperature is very conducive to rapid deterioration of soybean [*Glycine max* (L.) Merrill] seed in storage leading to lower its viability and vigor. Chuntirapongsa (1992), Marwanto (2004) and Marwanto *et al.* (2003) reported that eventhough soybean genotypes used in their study deteriorated severely, a significant difference in the rate of seed deterioration under the rather adverse storage conditions was still observed. They further stated that the superior storability of certain genotype was attributed to its slower rate of imbibition as reflected by its low seed coat permeability.

In most tropical countries, soybean seeds are generally stored without refrigeration. Under this condition, seed viability as well as vigor drops rapidly

New techniques for altering the longevity of seed in storage have been revealed by Tao and Khan (1984b). They reported that several chemicals in predetermined concentrations can be permeated into seeds and that chemicals reaching the embryo are physiologically active. Their kinetic studies also showed that the extent of penetration of chemicals into seeds depend upon the type of seed, the penetration time and the concentration of solution. Recently, it has been shown that infused chemicals are actively metabolized (Eldan *et al.*, 1984; Sondheimer *et al.*, 1984). In some seeds, the organic solvent itself has been shown to have deleterious effect on germination (Brewer and Wilson, 1985) and protein metabolism (Eldan and Mayer, 1984). In studies with lettuce seeds, acetone was found to increase ³H-uridine uptake by the seed, but inhibited to some extent to the precursor incorporation into RNA. However, when kinetin was permeated via acetone, the deleterious effect of acetone on RNA labeling was not obvious, infact some promotion occurred. It is interesting to note, however, acetone by itself promoted germination to some extent Grand rapids lettuce seeds (Rao *et al.*, 1986) and reduce the amount of

light (Speer, 1984) or gibberellic acid (GA₃) (Rao *et al.*, 1986) required for germination. This suggests that acetone and perhaps other organic solvent may be useful for improving germination by weakening the embryo coverings which may be useful for improving germination of seeds under stress (Rao *et al.*, 1986).

In another report Tao and Khan (1984a) stated that a certain hormone had a positive effect on slowing down the rate of seed deterioration. When lettuce seeds were exposed to an accelerated aging environment of 43 °C and 85% relative humidity, they deteriorated rapidly and by 10 days they had lost their germinability. When seeds were permeated with cytokinins, such as kinetin, via acetone, they germinated better following rapid aging treatment (Tao and Khan, 1984b). The antisenescent properties of cytokinins are well known.

Although kinetin infusion into seed via acetone has been shown to slow down the rate of lettuce seed deterioration during accelerated aging (Tao and Khan, 1984b), its effects in relation to soybean seed storage are not well understood. The objective of this study was to evaluate a selected hormon (kinetin) for its ability to extend the longevity of soybean seeds in the storage. Major emphasis was directed towards the process of permeating soybean seeds with hormon kinetin for the purpose of retarding deterioration during storage at 15 and 30 °C.

METHODOLOGY

The soybean cultivar used throughout the study was Wilis. Soybean seeds were produced specifically for the studies in researcn 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 30 °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

$^{\circ}\text{C}$ 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).

The seed was permeated for four hours at a room temperature with hormone kinetin at a concentration of 1.0 mM dissolved in acetone following the general methods of Khan et al. (1986). Hormone kinetin at 1.0 mM concentration was selected for evaluation on the basis of preliminary experiments. Seeds used for the control or not infused treatment were permeated without kinetin. The solvent was then evaporated by passing air on the treated and untreated seeds for two hours.

After being subjected to the treatment, the treated and control seeds were stored at storage temperatures of 15 $^{\circ}\text{C}$ and 30 $^{\circ}\text{C}$ for six months in Agronomy Laboratory on July 2000 for storability evaluation. The seeds were stored in a plexiglass chamber of about 0.6 m³ capacity. They 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 30 $^{\circ}\text{C}$. Samples of treated and non-treated seeds were withdrawn from the different storage temperatures at two month intervals over six months storage period and were subjected to the following tests: standard germination, accelerated aging, speed of germination, electrical conductivity and respiration rate.

For standard germination test, three replicates of 50 seeds were germinated in rolled paper towel substrata at 30 $^{\circ}\text{C}$ for eight days. Normal seedlings were counted according to the rules for testing seeds and expressed as the percentage of germination.

The accelerated aging test was conducted using the same procedures as described by Delouche and Baskin (1973). About 150 seeds were placed on a 10.0 x 10.0 x 3.0 cm wire-mesh tray (mesh 14 x 18) in an inverted plastic germination box (11.0 x 11.0 x 3.5 cm) and 40 ml of water was carefully added not to wet the seeds. The plastic germination box was closed with a tight cover to establish 100% RH and placed in an incubator at 42 $^{\circ}\text{C}$ for 48 hr.

After 48 hr the seeds were removed and planted for determination of germination following the method of the standard germination test

Speed of germination of infused and control seeds was evaluated by placing the seeds on germination blotters in 30 cm petri dishes wetted with 25 mL distilled water. Each dish contained 50 seeds, and there were two replicate dishes per treatment. The dishes were put in covered polystyrene boxes lined with moist paper towel. The boxes were enclosed in black polyethylene and placed in an incubator at 30 $^{\circ}\text{C}$. 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 speed of germination (T_{50}) was calculated from the equation:

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

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, twenty five seeds, in three 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 $^{\circ}\text{C}$ for 24 hours after which time the contents of the flasks were gently stirred. The electrical conductivity was measured with a Wescon conductivity meter and reported as mmhos per cm per gram of seed. Respiration rate was measured by oxygen uptake using a Warburg Manometer. To measure it, twenty seeds, in three 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 and 0.2 mL of 15% KOH was added in center well. The reaction flasks were placed in a 25 $^{\circ}\text{C}$ 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.

Table 1. The effect of treated and untreated seeds stored at 15 °C and 30 °C for six months on electrical conductivity of steep water and on seed respiration rate.

Temp (°C)	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
			Infused	0.1590 f	0.803 a
	2		Untreated	0.2120 ef	0.673 c
			Infused	0.1820 f	0.740 abc
	4		Untreated	0.2690 e	0.623 cd
			Infused	0.2463 e	0.710 bc
	6		Untreated	0.4090 cd	0.573 de
			Infused	0.3970 d	0.673 c
30	0		Untreated	0.1613 f	0.813 a
			Infused	0.1627 f	0.802 ab
	2		Untreated	0.2495 e	0.550 e
			Infused	0.2340 e	0.795 ab
	4		Untreated	0.5030 c	0.485 ef
			Infused	0.2840 e	0.667 cd
	6		Untreated	1.0745 a	0.297 g
			Infused	0.6565 b	0.432 f

*: Means not followed by the same letter in each column are significantly different (P<0.05)

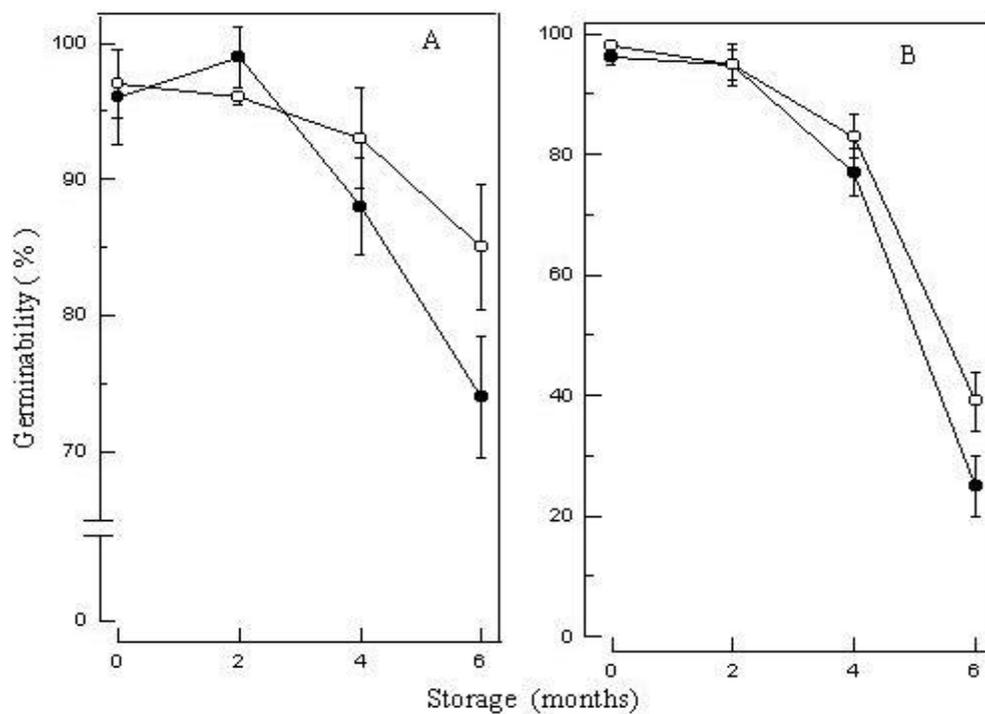


Fig. 1. Final germination of untreated (—●—) and kinetin - infused (—○—) soybean seeds stored at 15⁰C (A) and 30⁰C (B) as a function of the duration of storage. Vertical bars = s.e.

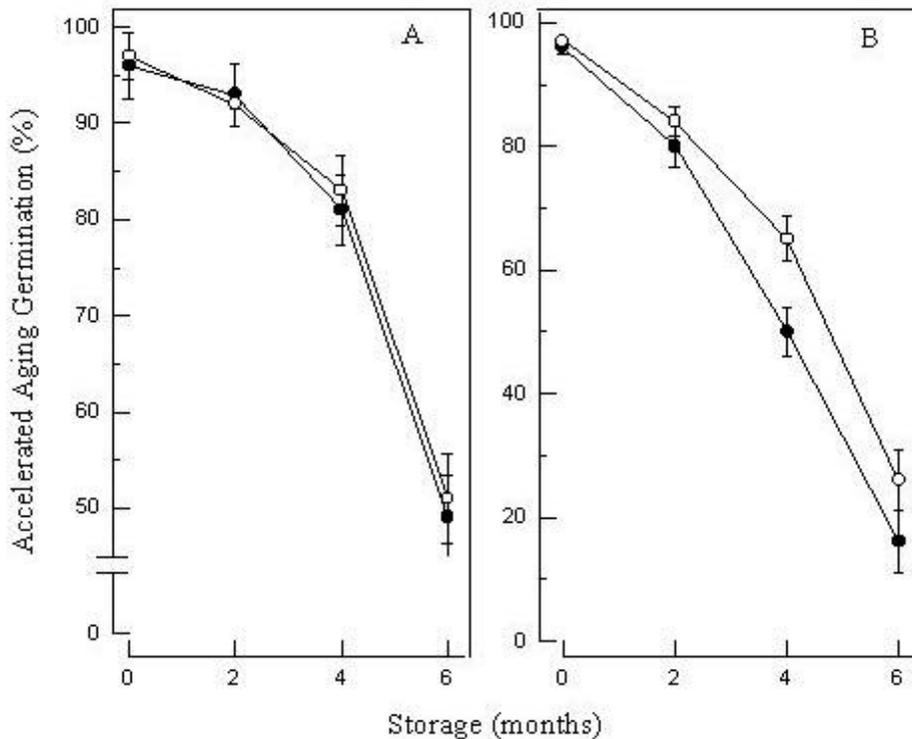


Fig. 2. Accelerated aging germination of untreated (—●—) and kinetin-infused (—□—) soybean seeds stored at 15°C (A) and 30°C (B) as a function of the duration of storage. Vertical bars = s.e.

All data were subjected to analysis of variance based on Split Plot Design with storage temperature as a main plot, kinetin infusion as a sub plot and storage period as a sub-sub plot. A 3-way ANOVA was used to test for storage temperature, kinetin infusion and storage period effects, as well as their interactions. F test at 5% level was carried out to evaluate the significance of each source of variation.

RESULTS AND DISCUSSION

Treated seeds storage at 15°C retained their germination throughout storage period and no significant differences were observed among their final germination, either immediately after 0 month or after six months (Fig. 1). Treated seeds germinated 97% at 0 month of storage and 85% at six months of storage, while control seed 96% at 0 month of storage and 74% at six months of storage. Meanwhile, germination of treated and

control seeds were affected and significantly reduced by subsequent storage at 30 °C for six month periods. For instance,

germination percentage of treated seeds had dropped from 98% at 0 month to 39% at six month period of storage, while control seeds from 96% at 0 month storage to 25% at six month storage. At four months of storage, treated seeds retained their high germination (>80%), while control seeds had dropped to a medium seed quality level (<80%). Therefore, storage at 30 °C may have accelerated the deterioration of both kinetin-infused and control seeds, as seen from the loss of germinability and increased number of abnormal seedlings. However, the detrimental effect of high temperature was able to be minimized by the kinetin treatment. These results were in agreement with reports that kinetin treatments extended seed longevity and maintained seed quality under adverse storage conditions (Marwanto, 2005; Tao and Khan, 1984b).

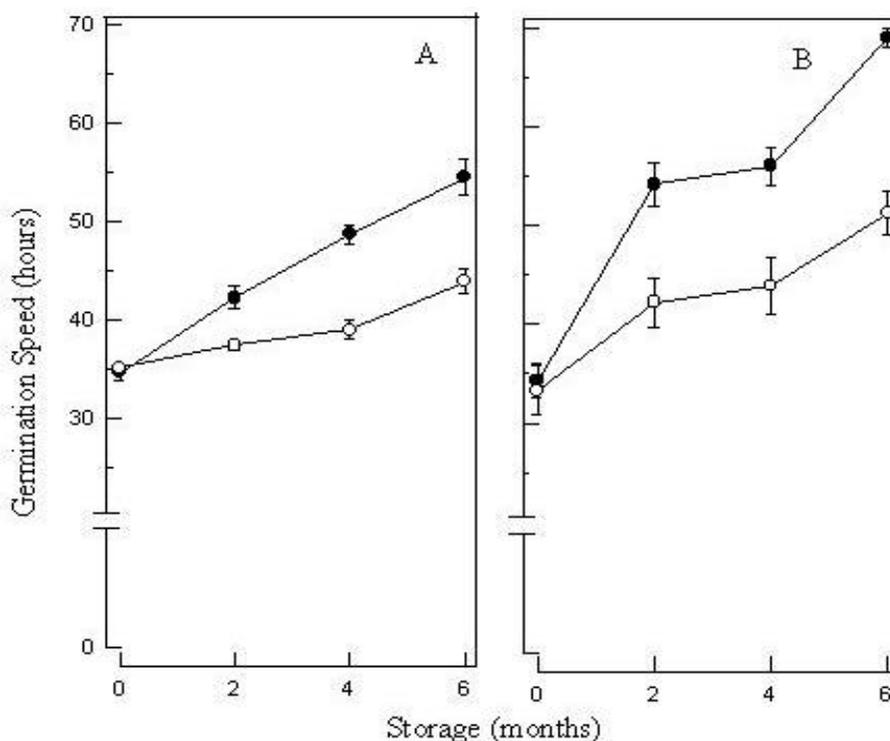


Fig. 3. Germination speed of untreated (—●—) and kinetin-infused (—□—) soybean seeds stored at 15⁰C (A) and 30⁰C (B) as a function of the duration of storage. Vertical bars = s.e.

The effects of storage temperature on the progress of soybean seed deterioration stored for six deterioration than those storage at 30⁰C over the entire six month periods of storage. Significant differences were observed among germination (viability), germination after accelerated aging (vigor), speed of germination (T_{50}), electrolyte conductivity, and respiration rate on both treated and control seeds storage at 15⁰C or at 30⁰C.

The germination values of treated and control seeds after accelerated aging (AA) at four months of storage at 30⁰C ranked them very well in terms of storage potential. The germinative responses of treated and control seeds to AA at four months of storage at 30⁰C were 65% and 50%, respectively (Fig. 2). After four months of storage, a marked decline in germination after AA continuously took place. Meanwhile, the germination values of treated and control seeds after AA storage at 15⁰C at the same storage period remained high (>80%). As storage period

was prolonged, they continuously declined though not as quickly as storage at 30⁰C. As reviewed by Khan *et al.* (1986) that the antisenescence properties of kinetin were well known. Kinetin infused seeds would therefore be expected to maintain greater viability and vigor during storage and the results indicate that kinetin treatment was successful to counteract the progress of deterioration that had occurred at 30⁰C or even at 15⁰C. Storage temperature and storage period alone or in interaction affected and significantly reduced T_{50} of treated and control seeds (Fig. 3). Also, kinetin infusion treatment significantly reduced the T_{50} of both seeds storage at 30⁰C but not at 15⁰C. This might due to the deteriorative senescence of the seeds during storage especially at 30⁰C, which would require a longer imbibition period prior to germination for repair and replacement of membranes, organelles, and enzymes (Burgess and Powell, 1984). The kinetin infusion reduced the T_{50} approximately 15 and 20% of the control

values when treated seeds kept at 15 °C and 30 °C, respectively. Therefore, the kinetin treatment was more effective in reducing the T_{50} storage at 30 °C than at 15 °C. At six months of storage at both storage temperature, treated seeds still had essentially the same advantage over control seeds.

Control seeds were more adversely affected by high storage temperature than those of treated seeds; therefore, the detrimental effect of high temperature was even more evident on them. At 30 °C, the T_{50} of treated and control seeds increased significantly relative to the corresponding values at 15 °C after two months of storage. The T_{50} of control seeds stored at 30°C increased continuously with time and began to differ significantly from those of treated and control seeds stored at 15 °C at the second month of storage.

Determination of the conductivity of seed leachates from treated and control seeds provided good information on their storage potential. The results show that regardless of storage temperature, the values of conductivity for treated and control seeds increased as deterioration progressed (Table 1). A significant increase in leaching was observed only for treated and control seeds storage at 30 °C and the values of conductivity for treated seeds were significantly lower than those of control seeds. Meanwhile, storage at 15 °C made a slight increase in the values of conductivity for treated and control seeds and throughout six month periods the values of conductivity for treated seeds were also lower than those of control seeds. The electrical conductivity test measures the amount of electrolytes which leach from seeds. Poor membrane structure and leaky cells are usually associated with deteriorating and low vigor seeds. Stewart and Bewely (1980) reported that leaky membrane occurred in deteriorated seeds. The promotive effects of kinetin infusion on a delay in aging process of soybean seeds were apparently manifested by improving cell membrane integrity as well as by maintaining higher germination, germination after AA and speed of germination. The results obtained from this study suggests that

the conductivity might also be developed into powerful techniques for evaluating the storability of soybean seeds as also recommended by Turnipseed (1987) for soybean..

Many researchers had demonstrated that various steps are involved during seed deterioration. One of them is the decline in respiration activity. The respiration rate as measured by the evolution of O_2 had been shown to relate with seed deterioration. The statistical analysis show that storage temperature affected and significantly reduced the respiration rate (Table 1). Storage at 30 °C made treated and control seeds deteriorate rapidly and significantly reduced the values of respiration rate as storage period was extended. The detrimental effect of high storage temperature on respiration activity was more evident on control seeds. The respiration rate for treated seeds had dropped from 0.802 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at 0 month to 0.432 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at six months of storage; while control seeds from 0.813 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at 0 month to 0.297 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at six months of storage. While at 15 °C, treated and control seeds deteriorated at lower rate than those at 30 °C and was able to maintain their germination capacity. Since they retained their germination capacity, storage at 15 °C made only a slight decrease in the respiration activity for both seeds. The values of respiration rate for treated and control seeds were not significantly different at immediately after 0 month and after six months of storage.

Though not significantly different, the treated seeds had higher values of respiration rate than those of control seeds. At 15 °C, the values of respiration rate for treated seeds ranged from 0.803 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at 0 month to 0.673 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at six months of storage, while control seeds from 0.812 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at 0 month to 0.573 $\mu\text{L } O_2 \text{ g}^{-1} \text{ minute}^{-1}$ at six month storage. The results were in agreement with Ram and Wresner (1988) working with rapid deterioration in wheat in that a positive correlation was observed between germination capacity and respiration rate.

CONCLUSIONS

Seeds that had been infused with kinetin prior to storage maintained a higher level of germination and germination after accelerated aging, showed a more rapid germination, a greater cell membrane integrity and higher respiration rate than untreated seeds irrespective of either storage temperature or storage period, whereas untreated seeds showed a rapid decline in the five seed quality indicators. The action of kinetin-infusion on the maintenance of soybean seed quality during storage was indicated by counteracting the progressive seed deterioration through the improvement of metabolic activity, e.g., seed respiration and the preservation of cell membrane integrity.

REFERENCES

- Brewer, P.E. and R.E. Wilson. 1985. Dichloromethane: Variability in penetration and resulting effects on seed germination and CO₂ evolution. *Bot. Gaz.* 136:216-218.
- Burgrass, R.W. and A.A. Powell. 1984. Evidence for repair processes in the invigoration of seeds by hydration. *Ann. Bot.* 53:753-757.
- Chuntirapongsa, S. 1992. Effects of seed coat color on storability of soybean seeds. Dissertation (Ph.D.) Miss. State Univ., Miss. State, MS.
- Delouche, J.C. and C.C. Baskin. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Sci. Technol.* 1: 427-452.
- Eldan, M. and A.M. Mayer. 1984. Acid invertase in germinating lettuce seeds: Evidence for *de novo* synthesis. *Phytochemistry* 13:389-395.
- Eldan, M., A.M. Mayer and A. Poljakoff-Mayber. 1984. Permeation of dry lettuce seeds with acetic anhydride and with amino acids, using dichloromethane. *Seed Sci. Technol.* 2:317-322.
- Khan, A.A., J.W. Braun and K.L. Tao. 1986. New methods for maintaining seed vigor and improving performance. *J. Seed Tech.* 21:33-57.
- Marwanto. 2004. Soybean seed coat characteristics and its quality losses during incubator aging and storage. *JIPI.* 6(2):57-65.
- Marwanto, 2005. Kinetin infusion through an organic solvent as a means of reducing soybean seed deterioration during high-temperature storage. *JIPI.* 7 (1):1-7.
- Marwanto, Marlin and M. Marlinda. 2003. The relationship between seed coat lignin content and seed quality of soybeans during storage. *JIPI* 5(1):12 – 17.
- Ram, C. and L.E. Wiesner. 1988. Effects of artificial aging on physiological and biochemical parameters of seed quality in wheat. *Seed Sci. Technol.* 16:579-587.
- Rao, V.S., J.W. Braun and A.A. Khan. 1986. Promotive effects of organic solvent and kinetin on dark germination of lettuce seeds. *Plant Physiol.* 57:446-449.
- Sondheimer, E., E.C. Galson, T. Tinelli and D.C. Walton. 1984. The metabolism of hormones during seed germination and dormancy. IV. The metabolism of (S)-2-¹⁴C-abscisic acid in ash seed. *Plant Physiol.* 54:803-808.
- Speer, H.L. 1984. Some aspects of the function of the endosperm during the germination of lettuce seeds. *Can. J. Bot.* 53:1117-1121.
- Steward, R.R.C. and J.D. Bewely. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.* 65:245-248.
- Tao, K.L. and A.A. Khan. 1984a. Permeation of plant hormones into dry seeds by means of organic solvent. *Plant Physiol.* 53:30-40.
- Tao, K.L. and A.A. Khan. 1984b. Penetration of dry seeds with chemicals applied in acetone. *Plant Physiol.* 54:956-958.
- Turnipseed, E.B. 1987. Evaluation of methods for measuring and monitoring field deterioration in soybean seed. Thesis (MS). Miss. State Univ., MS