KINETIN INFUSION THROUGH AN ORGANIC SOLVENT AS A MEANS OF REDUCING SOYBEAN SEED DETERIORATION DURING HIGH-TEMPERATURE STORAGE

PENGHAMBATAN KEMUNDURAN MUTU BENIH KEDELAI DALAM PENYIMPANAN SUHU TINGGI MELALUI INFUS KINETIN

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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, seeds of three cultivars of soybeans with black seed coat color were permeated for four hours at a room temperature with hormone kinetin at a concentration of 1.0 mM dissolved in acetone before storage at 35 ^{0}C for up to six months in Agronomy Laboratory on July 2000, and their germination, accelerated aging germination and germination speed were compared with those of untreated seeds stored under the same conditions. The treatments were arranged in a split split plot design with three replications. Seeds that had been infused with kinetin prior to storage germinated to a high final percentage (from 98 to 83%) up to four months of storage, maintained a high accelerated aging germination (from 97 to 65%) up to four months of storage and retained a high speed of germination throughout the storage period. By contrast, untreated seeds showed a rapid decline in the three seed quality indicators, and by six months of storage their germination was already less than 30% and their accelerated aging germination declined to less than 20%. The promotive effects of kinetin infusion on reducing the rate of deterioration of soybean seeds were apparently dependent of the cultivar. Among the three cultivars, seeds of Cikuray lost their germination, accelerated aging germination and speed of germination at a slower rate than those of Merapi and Kalitur irrespective of kinetin infusion treatment and storage period. Therefore, it is evident that kinetin infusion prior to storage, in addition to the acceleration of germination, resulted in a delay of soybean seed deterioration.

Key words: soybean, kinetin infusion, seed deterioration, viability, vigor

ABSTRAK

Benih kedelai mengalami deteriorasi (kemunduran mutu) secara cepat dan infusi kinetin mungkin dapat menekan laju deteriorasi tersebut. Untuk membuktikannya, hormon kinetin sebanyak 1,0 mM dirembeskan (diinfuskan) kedalam benih kedelai dari tiga varietas yang berbeda selama empat jam pada suhu kamar sebelum benih tersebut disimpan di Laboratorium Agronomi pada bulan Juli 2000 pada suhu 35 ^{0}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 diinfus dengan kinetin dapat mempertahankan mutunya tetap tinggi. Daya kecambah (DK) sebesar 83% - 98% dan daya kecambah setelah didera (DKSD) sebesar 65% - 97% dapat dipertahankan nilainya sampai bulan keempat penyimpanan, sedang kecepatan berkecambah (KB) yang tinggi dapat dipertahankan hampir selama periode penyimpanan. Sedangkan benih yang tidak diperlakukan mengalami penurunan mutunya secara tajam dan bahkan pada bulan keenam penyimpanan DK nya telah menurun dan nilainya berada dibawah 30%, sedang DKSDnya dibawah 20%. Pengaruh positif perlakuan kinetin terhadap penghambatan laju deteriorasi benih kedelai nampaknya sangat tergantung pada varietasnya. Dari ketiga varietas yang dicoba, benih Cikuray mengalami kehilangan DK, DKSD dan KB nya lebih lambat daripada benih Merapi dan Kalitur. Dengan demikian dapat disimpulkan bahwa selain mempercepat benih berkecambah, infusi kinetin ke dalam benih sebelum disimpan dapat memperlambat kemunduran mutunya.

Kata kunci: kedelai, infusi kinetin, deteriorasi benih, viabilitas, vigor

INTRODUCTION

A humid tropical climate characterized by daily high temperature and relatif humidity is very conducive to rapid deterioration of soybean [*Glycine max* (L.) Merrill] seed in storage. Chuntirapongsa (1992), Marwanto (2004) and Marwanto *et al.* (2003) reported that soybean genotypes differred significantly in their ability to maintain seed quality during storage. 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 such conditions, its vigor, as well as viability, can deteriorate rapidly, making it impossible for farmers to use their own seed for planting in the next season due to their low quality. Therefore, maintenance of their quality during storage to retain their capacity to germinate satisfactorily is an obligatory.

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 ^{3}H-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 to some extent promoted germination of Grand rapids lettuce seeds (Rao *et al.*, 1986) and reduce the amount of light (Speer, 1984) or gibberellic acid (GA_3) (Rao *et al.*, 1986) requirred 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 ^{0}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 aceton 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 hormone (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 hormone kinetin for the purpose of retarding deterioration during storage at 35 ^{0}C.

METHODOLOGY

Seeds of three cultivars of soybeans with black seed coat color (Merapi, Cikuray and Kalitur) were used throughout this study. They were produced specifically for the study in reseacrh 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 ^{0}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 ^{0}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).

Table 1. The effect of kinetin infusion on germination,accelerated aging germination and electrolyte conductivity of soybean seeds stored at 35 ^{0}C for six months.

Treatment	Storage (months)			
	0	2	4	6
	Germination (%)			
Untreated	98.00 a	95.00 a	77.00 b	25.00 d
Treated	98.00 a	95.00 a	83.00 b	39.00 c
	Accelerated aging germination (%)			
Untreated	96.00 a	80.00 b	50.00 d	16.00 f
Treated	97.00 a	84.00 b	65.00 c	26.00 e
	Germination speed (hours)			
Untreated	34.33 d	44.18 c	55.98 b	68.95 a
Treated	33.33 d	42.25 c	43.88 c	51.21 b

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

After cleaning and sizing the seeds were placed in cloth bags and stored at 15 ^{0}C and 65% RH until needed for testing or the storage studies. Its initial seed viability was high (>90%), and its initial moisture content (MC) was about 10% MC (wet weight basis).

Seeds were 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. Seed used for the control or not infused treatment was permeated without kinetin. The solvent was then evaporated by passing air on the treated and untreated seeds for two hours. The treated seeds were then stored for six months at a temperature of 35 ^{0}C for storability evaluation.

For each treatment the seeds were stored in a plexiglass chamber of about 0.6 m^3 capacity for six months in Agronomy Laboratory on July 2000. Seeds of each cultivar were contained in plastic mesh pouches and placed in the chamber. The chamber was positioned in a room with temperature controlled at about 35 ^{0}C. Samples of treated and non-treated seeds were withdrawn from the storage chamber at two month intervals over six months storage period and were subjected to the following tests: standard germination, accelerated aging and speed of germination.

For standard germination test, three replicates of 50 seeds were germinated in a rolled paper towel substrata at 30 ^{0}C for eight days. Only normal seedlings were counted and expressed as the percentage of germination.

The accelerated aging test was conducted using the same procedures as recommended 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 14x18) 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 ^{0}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.

Germination speed of treated and control seeds was evaluated by placing the seeds on germination blotters in 30 cm petri dishes wetted with 20 mL distilled water. Each dish contained 50 seeds, and there were two replicate dishes per treatment. Dishes were put in covered polystyrene boxes lined with moist paper towel. The boxes were enclosed in black polyethelene and placed in a incubator at 30 ^{0}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 or rate of germination (T_{50}) was calculated from the equation:

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

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 germianated 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.

All data were subjected to analysis of variance based on Split Plot Design with kinetin infusion as a main plot, storage period as a sub plot and soybean cultivars 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 evalate the significance of each source of variation.

RESULTS AND DISCUSSION

Analysis of variance shows that kinetin infusion and storage period both as a single factor or as an interaction significantly affected physiological deterioration of soybean seeds as measured by germination (viability), accelerated aging germination (vigor) and germination speed (T_{50}). Kinetin-infused seeds retained better viability, vigor and T_{50} almost throughout storage period than control seeds. The treated seeds were around 5.50% higher than untreated seeds for viability, 7.50% higher for vigor and 8.20 hours earlier for T_{50}. This indicates that the progress of seed deterioration was able to be minimized by the kinetin infusion and this result was in agreement with a report that kinetin treatment maintained seed quality under adverse storage condition (Khan *et al.*, 1986).

The effect of extended storage period was also more pronounced on viability, vigor and T_{50} of control seeds than on those of kinetin infused seeds. As storage was prolonged, a more reduction in seed viability, vigor and T_{50} was noted only in control seeds. No significant differences were observed among seed viability, vigor and T_{50} of treated and untreated seeds either immediately after 0 month or two months of storage (Table 1). Their significant differences were initially observed at six months of storage for seed viability, at four months for seed vigor and two months for T_{50}.

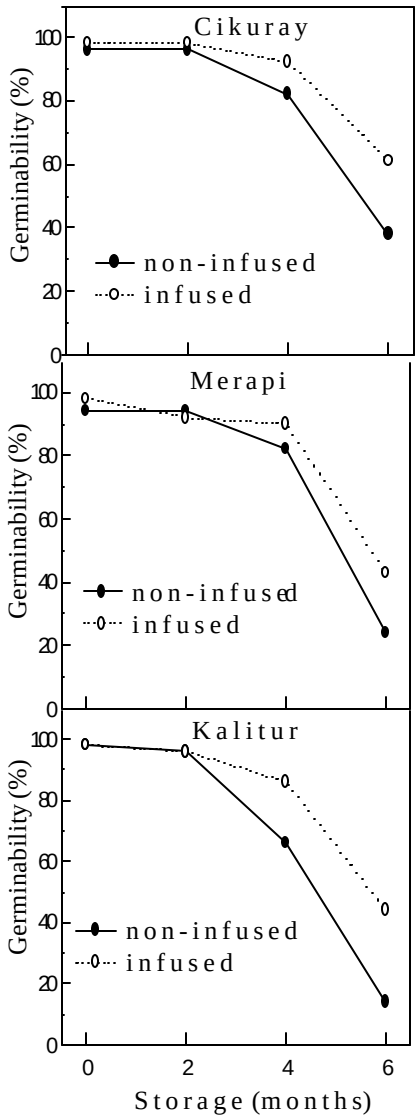

Fig 1. The effect of kinetin infusion on germinability of three soybean cultivars stored for six months.

Kinetin-infused and control seeds were able to retain their high viability (>90%) only until two months of storage. At four months of storage, kinetin-infused seeds still maintained their germination capacity above 80%, while germination capacity of control seeds had dropped to below an acceptable level (< 80%). As storage period was prolonged, germination capacity of both seeds had dropped to far below an acceptable level. The maintenance of a high percentage of

germination in kinetin-infused seeds may indicate the treatment to bring about a delay in the aging process (Khan *et al.*, 1986).

The accelerated aging germination values of both treated and untretaed seeds continously declined at a different rate as storage period was extended and a more rapid decline in the seed vigor was noted in the untreated seeds than in the treated seeds. Until two months of storage, no significant differences in the accelerated aging germination between treated and untretaed seeds were observed. Their significant differences were initially observed at four months of storage and then at six months of storage (Table 1). At four and six months of storage, the vigor of both seeds had dropped to far below an acceptable level. At these two storage periods, a more reduction in vigor was still noted in the untreated seeds than in the tretaed seeds. For example, the accelerated aging germination value of the tretaed seeds was 15% higher than that of the untreated seeds at four months of storage. This indicates that kinetin infusion would therefore be expected to maintain a greater vigor during a high temperature of storage and was succesful to counteract the progress of deterioration. This result was in accordance with the one reported by Khan *et al.* (1986).

Kinetin-infused and control seeds showed a gradual increase in their germination speed (T_{50}) as storage period was extended (Table 1). 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 treated seeds exhibited a slower increase in T_{50} and a more rapid germination than the untreated seeds. This result suggests that kinetin infusion had beneficial effects not only on the maintenance of high seed viability and vigor during high temperature storage but also on the maintenance of high germination speed (lower T_{50}). This result further suggests that high seed viability and vigor was also indicated by the consistently higher speed of germination of kinetin-infused seeds in comparison with untreated seeds.

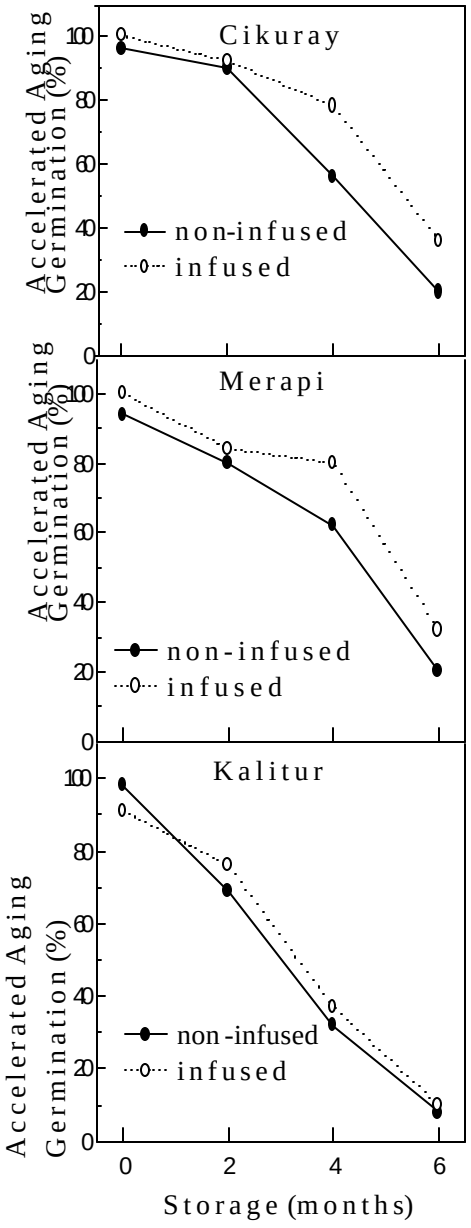

Fig.2. The effect of kinetin infusion on accelerated aging germination of three soybean cultivars stored for six months.

Varietal differences were significantly observed on the maintenance of viability, vigor and T_{50}. In addition, in each cultivar kinetin-infused seeds maintained higher viability, vigor and T_{50} throughout storage period than untreated seeds (Fig. 1, 2 and 3). Among the three cultivars, seed viability, vigor and T_{50} of Cikuray was less

affected by time in storage than those of Merapi and Kalitur. A significant difference on the three seed quality indicators among the cultivars was initially observed at a storage period of four months. At this storage period, viability, vigor and T_{50} of seed of Cikuray showed higher than those of Merapi and Kalitur in both treated and untreated seeds.

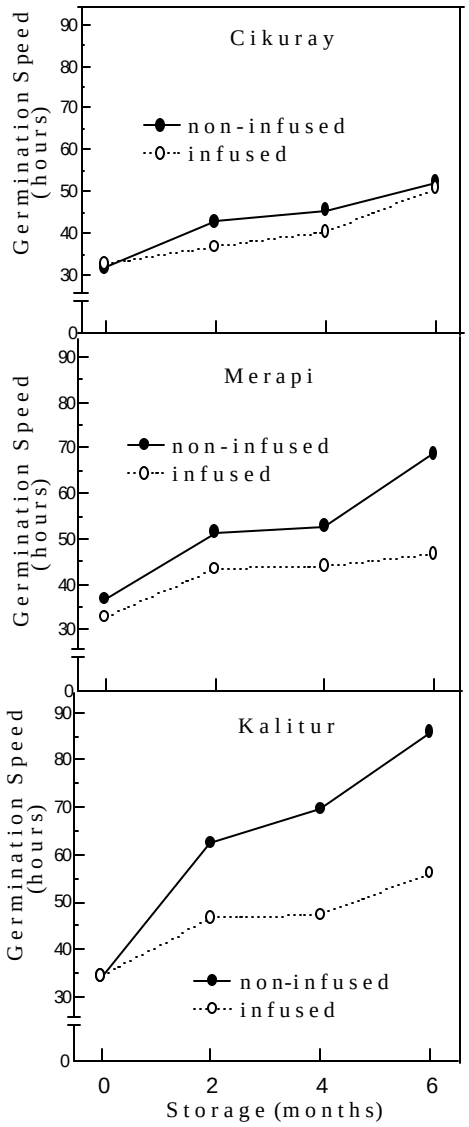

Fig3. The effect of kinetin infusion on germination speed (T_{50}) of three soybean cultivars stored for six months.

CONCLUSIONS

When stored at 35 ^{0}C, kinetin-infused seeds maintained higher viability as measured by germination percentage, higher vigor as measured by accelerated aging germination and hgher speed of germination than untreated seeds throughout the entire storage period. The promotive effects of kinetin infusion on soybean seed germination are apparently dependent of the cultivar. Among the three cultivars, the progress of deterioration kinetin-infused seed of Cikuray was less affected by time in storage

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