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Effect of seed moisture content packaging and storage period on mitochondria inner membrane of soybean seed

Aurellia Tatipata *

Department of Agronomy, Faculty of Agriculture, Pattimura University, Ambon, Indonesia.

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A basic mechanism of aging in soybean seeds is a deterioration of the mitochondria inner membrane. The research aims to study the effect of initial moisture content, packaging material and storage period on mitochondria inner membrane. The experimental design was arranged in Factorial in RCB consisting of 3 factors: moisture content, i.e. 8. 10 and 12 percent, packaging materials, i.e. polyethylene, wheat and aluminium foil; storage period i.e. 0, 1, 2, 3, 4, 5, and 6 months. Changes in seed moisture content, phospholipids and protein content of mitochondria inner membrane, germination and coefficient velocity of germination were monthly determined. It was found that seed moisture content was increase and showed positive correlation with electrolyte leakage and showed negative correlation with phospholipids and protein content of mitochondria inner membrane, germination and coefficient velocity of germination. From this experiment, soybean seeds were stored in aluminium foil bags observed highly phospholipids and protein content of mitochondria inner membrane, germination, coefficient velocity of germination and keep moisture content in low level could delay seed deterioration followed by polyethylene and wheat bags.

Key words: Soybean seed, mitochondria, membrane, moisture content

Introduction

The quality of soybean seed is easily decreased, so that it is difficult to keep them for a long time. Environment factors in influencing the life span of seeds are relative humidity, temperature, and initial moisture content of seed.

The problem of soybean seed quality in sub humid tropics like Indonesia is reducing of viability during storage. This is one factor which retarding soybean seed production. Soybean seed may be losing its viability or deterioration even in three months if kept at 14% moisture content at temperature of 30% (Sadjad, 1980). According to Sumarno (1983), viability of soybean seed was 57% even during 6 months if kept at above 13% moisture content at temperature and relative humidity of 20% and 50%, respectively. On the other hand, viability of soybean seed may lose as much as 50% if kept at 27

*Corresponding author: Aurellia Tatipata.; e-mail: lethatatipata@yahoo.com

to 32^{O}C moisture content and 80% relative humidity. When longer storage is needed, seed moisture content should be less than 11% and at 20^{O}C and 50% relative humidity, and its packaging in moisture resistant containers (Wilson and McDonald, 1992). Soybean storage, which is safety enough for 10 months or its deterioration retarded, can be done by using vacuum plastic bag, and the humidity at beginning should be around 8%. Germination of soybean seed which stored at 8% initial moisture content in room during 3 years do not decrease, but it was 60% for 1 month if stored at 12% and 0% after 3 months (Kartono, 2004). Relative humidity of room affect on seed moisture content. Soybean seeds is a hygroscopes seed, so H_2O could be up took from environment.

Polyethylene and aluminium foil materials were moderately effective in preventing moisture uptake and maintaining seed viability, while paper and cloth containers were least effective (Wilson and McDonald, 1992).

Seed deterioration is an inexorable and an irreversible process. One of symptom of seed deterioration is membrane deterioration (Copeland and McDonald, 1985). The primary cause of membrane deterioration is that changes membrane phospholipids. This is the same as mitochondria membrane. Mitochondria contain two membranes, outer membrane and inner membrane. The outer membrane and the inner membrane consisted of phospholipids and protein. The phospholipids and protein of mitochondria inner membrane may be important role of membrane activity. Disorganization of mitochondria inner membrane could be affected to seed viability. However, no detailed studies as available concerning a possible relationship between the mitochondria inner membrane and viability of soybean seed.

The aim of this study were to study the effect initial water content of seed, packaging and storage period on mitochondria inner membrane of soybean seed.

The hypotheses of this study were: (1) soybean seed stored at 8% and 10% of moisture content can maintain high organization of mitochondria inner membrane and seed viability; (2) soybean seed stored using aluminium foil bag can maintain high organization of mitochondria inner membrane and seed viability; (3) interaction of soybean seed stored at 8% and 10% moisture content using aluminium foil bag can maintain high organization of mitochondria inner membrane and seed viability.

Materials and methods

Sample collection

Soybean seed var. Willis was carried out in March 2007 from Balai Benih Induk Wonosari (BBI) Yogyakarta. After seed processing, water content of seed was collected at 8%, 10%, and 12%. Samples of 500 g were placed in

polyethylene plastic bags, wheat bags and aluminium foil bags for 6 months at room temperature. Storage temperature and relative humidity was monitored throughout the experiment but it was not controlled. One sample from each treatment was removed monthly. All samples were tested for seed moisture content after storage, specific activity of succinate dehydrogenase and cytochrome oxidase, respiration rate, germination, coefficient velocity of germination and abnormal seedlings. Germination and coefficient velocity germination of seed before storage were 100%.

Isolation and purification of mitochondria

Mitochondria was isolated from 3-d-old of 3 g of soybean hypocotyls by the procedure of Day and Hanson (1977), modified by using the centrifugation speeds of Beckman-J6B. Samples were taking placed in refrigerator for 10 minute at 10^{O}C before being ground by hand with a chilled mortar and pestle. The filtered homogenate is centrifuged first at 400 g, 500 g and 600g for 10 minute, respectively. The pellet (containing cell wall, fragments, starch grains, nuclei, and intact plastids) is discarded. The supernatant is then centrifuged at 10 000 g for 20 minute and mitochondria pellet was resuspended in 150 µl of a reaction mixture consisting of 0.4 M sucrose, 0.5% (w/v) bovine serum albumin (BSA), 50 mM Tris pH 7.6 and 10 mM KH_2PO_4. Triplicate aliquots (10 µl) of the resuspension were saved for protein determination (corrected for added BSA). Protein content in the mitochondria suspension dissolved in 1 M NaOH was measured according to Lowry *et al.* (1951). The mitochondria sonicated until the suspension cleared (about 20 second) at 20 kilocycle using sonicator Labsonic U. Mitochondria inner membrane pelleted by centrifugation for 20 minute at 100 000 g using Hitachi SCP 85H ultracentrifuge. The pellet was resuspended in 5 ml of buffer Tris pH 7.6 and stored at 0^{O}C until further processing.

Phospholipids analysis

Phospholipids were separated by column chromatography according to the Maxwell *et al*. (1978) as following: 2 ml of inner membrane mitochondria suspension take place into separate then dissolved in 15 ml of petroleum ether, 5 ml of absolute ethanol, and 1.5 ml ammonia, so there are 2 part of liquid. A bottom liquid was discard and upper liquid was taking place into vial glass which the constant weight. Solute was vapor using rotary vacuum evaporator. Vial glass containing lipid was dried in oven for 10 minutes, then take place in dessicator and weighted. Lipid weight which used in the experiment was calculated as weight of vial glass contain lipid minus vial glass weight. Lipid

was dissolved into 20 ml of methanol-acetic acid 95% and taked place into erlenmeyer and mixed it for 20 minutes, erlenmeyer sealed in aluminium foil and using oven for 1 hour. The non frost sample was taked place into erlenmeyer; frost sample washed with 10 ml of ethanol then mixed them. Solvent was vapor using rotary vacuum evaporator until dry. Dry lipid was dissolved in 3 ml of chloroform and stored at 4^{O}C before further processing. 5 ml of chloroform was take place in column, dry lipid was added to solvent. 10 ml of chloroform and 1.5 ml of methanol-acetic acid 98% (v/v) was added to push out neutral lipid, this eluent was discard. 10 ml of methanol-acetic acid 98% (v/v) was added into column to final eluent elution. Final eluent stored in vial glass with constant weight and dried at T 50^{O}C for 24 hours. Phospholipids content was weight of vial glass containing lipid: weight of lipid sample x 100%.

Protein analysis

Protein content of mitochondria inner membrane was analyzed after membrane protein isolation according to Dickerson *et al*. (1989) as following:40 µl of mitochondria inner membrane, 6 µl of buffer French press (30 mM Tris-SO_4 pH 7.5, glycerol 87%), 4 µl of 0.15 M NaCl, 20 µl of 2M $MgSO_4$, 20 µl of 1% 2 mercaptoethanol, 50 µl of 15 mM EDTA were poured into reaction tube, vortexed and kept them into ice box for 20 minute then centrifuged at 90000 g for 30 minute. The supernatant contain mitochondria membrane protein was to determine protein content of mitochondria inner membrane. Protein content was assayed with the medium (2 ml) containing 1 ml of 2% (w/v) Na_2CO_3, 0.1 N NaOH, 2% (w/v) Na^+K^+tartrate and 1% (w/v) $CuSO_4.5H_2O$ with comparing of 10:0.5:0.5. After that 3 ml of folin-ciocalteau was added, incubated for 10 minutes. Absorbance was measured at 600 nm. The standard BSA was 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 $mgml^{-1}$. Protein content was $mgml^{-1}$.

Seed moisture content test

Determination of seed moisture content after storage was tested as follows: empty bottle was weighed, then put it in oven at 105^{O}C for 3 hours. After that, it was weighed and put it in oven for 1 hour, until reach the constant weighed (a). 5 g of seeds was placed in the bottle and weighed (b) dried at 105^{O}C for 2 hours until reach the constant weight (c). Seed moisture content (SMC) was proposed by Anonym 2004 *cit*. Sutopo (1988) who suggested the following formula:

Weight of the bottle with seed before drying – weight of the bottle with seed after drying.

$$SMC = \frac{\text{Weight of the bottle with seed before drying – weight of the bottle with seed after drying x 100\%}}{\text{Weight of bottle with seed – weight of bottle}}$$

Electrolyte leakage

Electrolyte leakage was determined by McDonald and Wilson (1979) as follow: seeds were measured by adding 100 ml deionized water to each of 100 weighed seeds. After 24 hours at room temperature, the electrical conductivity of the soak water was measured by using HI 8819 conductivity meter. Electrolyte leakage was mS.

Germination and vigor test

All samples were tested for standard germination and vigor (coefficient velocity of germination), removed monthly after storage was performed on four 100-seed replicated planted in the paper, wetted with distilled H_2O. The number of seeds germinated (embryonic axis longer than 1 cm) for 7 days was recorded daily.

Seed germination quantities according to International of Seed Testing Association (1993) as cumulative total of normal seedlings per total of seed tested times 100%. The formula is:

$$\text{Germination} = \frac{\text{Total of normal seedlings}}{\text{Total of seed tested}} \text{ x } 100\%$$

Coefficient velocity germination according to Kozlowski (1972) is:

$$C\ V\ G = \frac{\text{Number of normal seedlings}}{\text{Number of normal seedlings}} + \ldots\ldots + \frac{\text{number of normal seedlings}}{\text{number of normal seedlings}}$$
$$\text{days of first count} \qquad\qquad \text{days of final count}$$

Statistical analysis

The experimental design was layed out using Factorial Randomized Complete Block Design (RCBD) with three factors i.e. initial moisture content (A) which consisting of 3 levels, i.e. 8% (A_1), 10% (A_2), 12% (A_3); packaging materials (B) which consisting of 3 kinds, i.e. polyethylene plastic bag (B_1), wheat bag (B_2), aluminium foil bag (B_3); storage period (C) which consisting of 7 levels, i.e. 0 (control), 1 month (C_1), 2 months (C_2), 3 months (C_3), 4 months (C_4), 5 months (C_5), and 6 months (C_6). Data were analyzed using

analysis of variance of Statistical Analyses System. Record was made on seed moisture content after storage, electrolyte leakage, phospholipids content of mitochondria inner membrane, protein content of mitochondria inner membrane, germination, and coefficient velocity of germination (CVG).

Results

Moisture content and conductivity

Based on the results, it was found that seed storage in wheat bags increased in moisture content faster than that of other packaging materials during storage time. The increase of seed moisture content of seed stored at all levels of initial moisture content was increase after 3 months (Table 1). Seeds stored at higher moisture content of 12% using wheat bag was showed deterioration with an increase in conductivity throughout the storage period. During the first two months of storage, the increase of conductivity from 0.1600 mS (0 month) to 0.2533 mS (3 months) (Table 2) occurred from mainly seed had 12.65% moisture content reflected the highest of seed moisture content (13.58%), because membrane permeable was increased.

Phospholipids and protein of mitochondria inner membrane

Phospholipids of mitochondria inner membrane of seed stored at all levels of moisture content using wheat bag were decreased after 2 months, and faster than that of other packaging (Table 3). Decrease of phospholipids content followed by decrease of protein content (Table 4). The protein content of seed stored at 8%, 10% and 12% moisture content using wheat bag was decreased after 2 months, after 1 month and since 1 month, but if it stored at 8%, 10% and 12% moisture content using polyethylene plastic bag and aluminium foil bag, it was decrease after 4 months, after 4 months and after 3 months, respectively. The results showed that the inner membrane of mitochondria was disorganized.

Table 1. The effect of initial moisture content packaging materials and storage period on seed moisture content (%).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	8.00 q-r	8.00 q-r	8.00 q-r
	C_1 (1)	8.03 q-r	8.67 p-r	8.00 q- r
	C_2 (2)	8.63 p-r	9.24 m-q	8.63 p-r
	C_3 (3)	8.70 p-r	9.20 m-q	8.70 o-r
	C_4 (4)	8.87 o-r	11.23 b-i	8.84 o-r
	C_5 (5)	8.98 o-r	11.40 b-h	8.92 o-r
	C_6 (6)	11.24 b-i	11.96 b-e	9.20 m-q
10 (A_2)	C_0 (0)	10.00 h-p	10.00 h-p	10.00 h-p
	C_1 (1)	10.15 f-n	10.35 f-n	10.00 h-p
	C_2 (2)	10.23 f-n	10.63 e-m	10.18 l-p
	C_3 (3)	10.66 e-m	11.00 b-k	10.22 f-n
	C_4 (4)	10.72 c-l	11.48 b-f	10.33 f-n
	C_5 (5)	10.75 d-l	11.60 b-j	10.60 e-m
	C_6 (6)	11.81 b-e	12.40 b	11.25 b-i
12 (A_3)	C_0 (0)	12.00 b-e	12.00 b-e	12.00 b-e
	C_1 (1)	12.12 b-d	12.42 b	12.00 b-e
	C_2 (2)	12.22 b-c	12.64 a-b	12.14 b-d
	C_3 (3)	12.25 b-c	13.03 c	12.18 b-c
	C_4 (4)	12.36 b	13.26 a	12.24 b-c
	C_5 (5)	12.42 b	13.42 a	12.26 b-c
	C_6 (6)	12.50 a-c	13.58 a	12.28 b

Note: number followed by the same alphabet do not significant different at 5% of DMRT k - p = klmnop

Germination and Coefficient Velocity of Germination (CVG)

The soybean seed germination that stored at all levels moisture content using wheat bag was decreased faster than the used of polyethylene plastic bag and aluminium foil bag. The germination of seed stored at 12% moisture content using wheat bag for 6 months was low (87.75%), followed by that stored using polyethylene plastic bag (89.25%) and aluminium foil bag (90.75%) (Table 5).

CVG of soybean seed stored at all levels of seed moisture content using all packaging materials decline before germination (Table 6).

Table 2. The effect of initial moisture content, packaging materials and storage period on electrolyte leakage (mS).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	0.1500 p	0.1633 m-p	0.1533 o-p
	C_1 (1)	0.1767 j-p	0.2067 d-p	0.1733 k-p
	C_2 (2)	0.1800 i-p	0.2167 b-o	0.1833 h-p
	C_3 (3)	0.1933 e-p	0.2233 b-n	0.2167 b-o
	C_4 (4)	0.1967 e-p	0.2464 a-h	0.2169 b-o
	C_5 (5)	0.2333 a-k	0.2467 a-h	0.2367 a-k
	C_6 (6)	0.2367 a-k	0.2467 a-h	0.2433 a-i
10 (A_2)	C_0 (0)	0.1600 n-p	0.1600 n-p	0.1630 n-p
	C_1 (1)	0.1867 g-p	0.1633 m-p	0.1800 i-p
	C_2 (2)	0.1900 f-p	0.2133 c-p	0.2000 e-p
	C_3 (3)	0.2000 e-p	0.2333 a-k	0.2067 b-o
	C_4 (4)	0.2267 a-m	0.2367 a-k	0.2200 b-n
	C_5 (5)	0.2300 a-l	0.2367 a-k	0.2500 a-g
	C_6 (6)	0.2400 a-j	0.2567 a-e	0.2533 a-f
12 (A_3)	C_0 (0)	0.1667 l-p	0.1600 n-p	0.1600 n-p
	C_1 (1)	0.1633 m-p	0.1867 g-p	0.1733 k-p
	C_2 (2)	0.2133 c-p	0.2233 b-n	0.2033 d-n
	C_3 (3)	0.2300 a-l	0.2533 a-f	0.2167 b-o
	C_4 (4)	0.2533 a-f	0.2667 a-d	0.2367 b-k
	C_5 (5)	0.2667 a-d	0.2733 a-c	0.2400 a-j
	C_6 (6)	0.2900 a	0.2800 a-b	0.2533 a-f

Note: number followed by the same alphabet do not significant different at 5% of DMRT
m - p = mnop

Discussion

The used of wheat bag was found out that don't proved any moisture and oxygen properties, so seed moisture content was increase. The increase of moisture contents was due to increase of phospholipase, so phospholipids hydrolyzed. Consequently, phospholipids content of cellular membrane in general and that of mitochondria inner membrane in particular were decrease. This event causes disorganization of membrane, so loss of membrane integrity or loss of selective permeability. It was indicated by leakage. Early loss of selective membrane permeability leading to ultimates overt of membrane damage is a consistent feature of most form of cell injury. Cell injury is associated with a decrease in the content of membrane phospholipids because of degradation likely due to activation of endogenous phospholipase (Anonym, 2008). On the other hand, the increase of membrane permeability and activation of hydrolytic enzymes disrupt the cell structure and compartments (Anonym, 2007).

The increase leakage associated with aging might be the result of a more permeable membrane or of a larger pool of electrolytes (Copeland and

McDonald, 1985). Abdul Baki and Anderson (1972) concluded that leakage is an index of seed vigor. Loss of membrane integrity which have direct relationship with low standard germination and vigor (Wilson and McDonald, 1992. In such case, leakage might be due to loss of metabolic energy for membrane transport mechanism and maintenance of cellular integrity or the result of autolytic damage to membranes. Membrane damage may affect the mitochondria, the plasma membrane and other cellular membrane damage (Anonym, 2008). The phospholipase hydrolyzes the phospholipids and thus destroys the structure of mitochondria inner membrane (Anonym, 2008).

Table 3. The effect of initial moisture content, packaging materials and storage period on phospholipids content of mitochondria inner membrane (%).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	5.58 a-b	4.55 b-i.	6.11 a
	C_1 (1)	4.98 b-e	3.47 i-p	5.72 a-b
	C_2 (2)	4.81 b-f	3.52 i-p	5.54 a-c
	C_3 (3)	4.79 b-f	3.26 k-r	5.47 a-c
	C_4 (4)	4.66 b-h	3.26 k-r	5.23 a-d
	C_5 (5)	4.45 b-j	3.03 l-r	4.79 b-f
	C_6 (6)	4.35 b-k	2.45 p-r	4.66 b-k
10 (A_2)	C_0 (0)	3.89 e-m	4.21 d-k	5.16 a-d
	C_1 (1)	3.79 f-n	3.47 i-p	4.78 a-d
	C_2 (2)	3.64 h-p	3.14 k-r	4.71 b-f
	C_3 (3)	3.45 i-q	2.89 l-r	4.53 b-i
	C_4 (4)	2.68 n-r	2.77 m-r	4.47 b-i
	C_5 (5)	2.65 n-r	2.64 n-r	3.92 e-m
	C_6 (6)	2.63 n-r	2.52 o-r	3.89 e-m
12 (A_3)	C_0 (0)	3.79 f-n	3.57 h-p	3.89 e-m
	C_1 (1)	3.57 h-p	3.04 l-r	3.34 k-r
	C_2 (2)	3.14 k-r	2.83 m-r	3.20 k-r
	C_3 (3)	3.04 l-r	2.46 q-r	3.08 l-r
	C_4 (4)	2.52 o-r	2.37 q-r	2.64 n-r
	C_5 (5)	2.48 q-r	2.29 q-r	2.60 n-r
	C_6 (6)	2.46 q-r	2.21 r	2.58 n-r

Note: number followed by the same alphabet do not significant different at 5% of DMRT e-m = elm

Phospholipids, which are part of cell membranes including mitochondria inner membrane, come into close contact with other cell constituents, including the macromolecules or enzyme and other proteins. Defective mitochondrial function results in decrease of phospholipids synthesis, which affects all the cellular membranes, including the mitochondria themselves. At the same time, increase of cytosolic calcium associated with ATP depletion results in increased uptake of Ca^{2+} into mitochondria activating phospholipase and leading to breakdown of phospholipids. The net results depleted of phospholipids from the

mitochondria and other cellular membranes. The loss of phospholipids membrane content may changes the shape of proteins embedded in the lipid bilayer of the membrane.

Protein embedded in the phospholipids bilayer can selectively take up and expel molecules that otherwise could not penetrate the membrane. In doing so, these proteins function as pores and sites for active transport, membrane-bound proteins also function as enzymes and receptors that defect signals from the environment or from other cells (Anonym, 2006). Consequently, mitochondria inner membrane was disorganized which indicated the mitochondria structure disorganization or mitochondria deteriorate. As the mitochondria deteriorate, they are not able to produce as much ATP.

ATP is a higher energy phosphate which used to germinate seed, seedling growth and protein synthetic. Energy conservation in mitochondria has focused on the structural relationship of the lipid and protein components within the mitochondria inner membrane (Green and Baum, 1970 and Racker, 1970). On the other hand decrease of phospholipids and protein of mitochondria inner membrane causes in the loss of membrane fluidity. The fluidity of cellular membranes decrease with age, a change that may be attributed in part to oxidation of plasma and mitochondrial membrane lipid components. The increase of the fluidity of cellular membranes decreased of membrane permeability, so more leakage out of cell. Consequently, germination and vigor indicated by the coefficient velocity of germination (CVG) were decrease. Alterations to membrane fluidity could seriously impair the ability of mitochondria to meet cellular energy demand (Anonym, 2007). Mitochondria from the old seeds have a less organized membrane and less tightly bonded fatty acids in the membrane than that of the new seeds (Abu Shakra and TeMay Ching, 1967). In aged seeds caused either by a long period of storage or a short time under unfavorable storage conditions, germination is not greatly impaired, but the vigor or growth rate of seedlings is often markedly reduced (Ching, 1973). Disorganization of mitochondria inner membrane caused in decrease of respiratory enzyme activity such as succinate dehydrogenase and cytochrome oxidase (Tatipata 2006). Mitochondria activity has been clearly demonstrated to be the primary source of energy during seed germination (Attuci *et al*., 1991). The general decrease in the seed lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the germinating seed, so seed deteriorated Deterioration is evidenced by decline in germination or seedling growth of soybean seeds (Fabrizius *et al*., 1999).

Table 4. The effect of initial moisture content, packaging materials and storage period on protein content of mitochondria inner membrane ($mgml^{-1}$).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	5.58 a-b	4.55 b-i.	6.11 a
	C_1 (1)	4.98 b-e	3.47 i-p	5.72 a-b
	C_2 (2)	4.81 b-f	3.52 i-p	5.54 a-c
	C_3 (3)	4.79 b-f	3.26 k-r	5.47 a-c
	C_4 (4)	4.66 b-h	3.26 k-r	5.23 a-d
	C_5 (5)	4.45 b-j	3.03 l-r	4.79 b-f
	C_6 (6)	4.35 b-k	2.45 p-r	4.66 b-k
10 (A_2)	C_0 (0)	3.89 e-m	4.21 d-k	5.16 a-d
	C_1 (1)	3.79 f-n	3.47 i-p	4.78 a-d
	C_2 (2)	3.64 h-p	3.14 k-r	4.71 b-f
	C_3 (3)	3.45 i-q	2.89 l-r	4.53 b-i
	C_4 (4)	2.68 n-r	2.77 m-r	4.47 b-i
	C_5 (5)	2.65 n-r	2.64 n-r	3.92 e-m
	C_6 (6)	2.63 n-r	2.52 o-r	3.89 e-m
12 (A_3)	C_0 (0)	3.79 f-n	3.57 h-p	3.89 e-m
	C_1 (1)	3.57 h-p	3.04 l-r	3.34 k-r
	C_2 (2)	3.14 k-r	2.83 m-r	3.20 k-r
	C_3 (3)	3.04 l-r	2.46 q-r	3.08 l-r
	C_4 (4)	2.52 o-r	2.37 q-r	2.64 n-r
	C_5 (5)	2.48 q-r	2.29 q-r	2.60 n-r
	C_6 (6)	2.46 q-r	2.21 r	2.58 n-r

Note: number followed by the same alphabet do not significant different at 5% of DMRT a-b = ab

Respiration within the mitochondria is a function of "unit membranes" and loss of mitochondria membrane integrity would presumably alter the functional relationships of the membrane-bound components of the respiratory chain. Some workers have suggested that mitochondria of older seeds are progressively uncoupled (Wilson and McDonald, 1992). This could easily be reconciled with loss of membrane integrity (especially if the transmembrane proton gradient or electron motive force is crucial for coupling). These processes responsible of low polymer synthesis might be associated with energy (adenosine triphosphate) production. TeKrony and Egli (1990) suggested that the decrease in mitochondria respiration during storage may be associated with the peroxidative in mitochondria lipids and that these changes occurred prior to loss in seed vigor.

Table 5. The effect of initial moisture content, packaging materials and storage period on germination (%).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	98.50 a-d	98.00 a-f	99.25 a-b
	C_2 (2)	97.75 a-f	97.50 a-g	98.75 a-c
	C_3 (3)	97.75 a-f	97.50 a-g	97.75 a-f
	C_4 (4)	97.00 a-g	96.00 b-i	97.00 a-g
	C_5 (5)	95.75 b-i	95.50 c-i	96.75 a-h
	C_6 (6)	95.50 c-i	94.50 e-i	96.00 b-i
10 (A_2)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	98.00 a-f	98.00 a-f	98.50 a-d
	C_2 (2)	97.75 a-f	96.75 a-h	98.00 a-f
	C_3 (3)	97.75 a-f	96.00 b-i	97.50 a-g
	C_4 (4)	95.75 b-i	95.25 c-i	97.00 a-g
	C_5 (5)	95.50 c-i	95.00 c-i	95.50 c-i
	C_6 (6)	92.50 i-k	92.50 i-k	95.25 c-i
12 (A_3)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	98.00 a-f	98.00 a-f	98.25 a-f
	C_2 (2)	97.50 a-g	94.75 e-i	97.25 a-g
	C_3 (3)	97.00 a-g	93.25 h-j	96.75 a-h
	C_4 (4)	94.25 f-i	92.75 i-j	96.50 a-h
	C_5 (5)	94.00 g-j	92.75 i-j	92.50 i-k
	C_6 (6)	89.25 k-l	87.75 l	90.75 j-l

Note: number followed by the same alphabet do not significant different at 5% of DMRT a-f = abcdef

Seed germination and seedling growth are an energy-requiring process must rely on respiratory metabolism to supply this energy. Thus, a decrease in the rate of respiration of germinating seeds has been shown to precede a decline in the rate of seedling growth (Wilson and McDonald, 1992). Delayed seedling emergence is among the first noticeable symptoms, followed by a slower rate of seedling growth and development and decreased germination. Seed vigor which indicated by coefficient velocity of germination was much more sensitive to high temperature and relative humidity than germination (Spears *et al*., 1997 and Egli *et al*., 2005). These findings are consistent with the concept that vigor decline before germination and during seed deterioration (Byrd and Delouche, 1971).

Table 6. The effect of initial moisture content, packaging materials and storage period on coefficient of germination (CVG).

Initial moisture content (%)	Storage period (month)	Packaging materials		
		Polyethylene (B_1)	Wheat (B_2)	Al.foil (B_3)
8 (A_1)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	98.00 a-c	97.50 a-c	98.75 a-b
	C_2 (2)	97.50 a-c	96.75 a-b	97.75 a-c
	C_3 (3)	96.75 a-c	96.00 b-g	97.00 a-d
	C_4 (4)	95.50 b-h	95.50 b-h	97.00 a-d
	C_5 (5)	95.38 c-h	95.50 b-h	96.00 b-g
	C_6 (6)	95.00 c-i	91.50 j-k	95.00 c-i
10 (A_2)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	97.63 a-c	97.50 a-c	98.00 a-e
	C_2 (2)	97.13 a-d	95.63 b-h	97.50 a-c
	C_3 (3)	96.88 a-d	95.50 b-h	97.00 a-d
	C_4 (4)	96.50 b-e	95.00 c-f	96.75 a-b
	C_5 (5)	95.50 b-h	95.13 c-i	95.50 b-h
	C_6 (6)	92.50 h-j	92.50 h-j	95.50 b-h
12 (A_3)	C_0 (0)	100.00 a	100.00 a	100.00 a
	C_1 (1)	97.13 a-d	96.00 b-h	97.50 a-c
	C_2 (2)	97.00 a-d	95.25 d-j	97.00 a-d
	C_3 (3)	96.75 a-b	92.75 g-j	96.75 a-b
	C_4 (4)	93.13 f-j	92.75 g-j	95.75 e-j
	C_5 (5)	92.75 g-j	92.00 i-j	93.25 e-j
	C_6 (6)	89.00 k-l	87.75 l	89.13 k-l

Note: number followed by the same alphabet do not significant different at 5% of DMRT
a-d= abcd

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