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Original Research Article

Effect of accelerated aging on mungbean (Vigna Radiata L. Wilczek) seed vigour

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Seed is one of the basic inputs in agriculture. Standard seed germination which usually conducted under optimum growing conditions, may not serve as an accurate vigour indicator for a seed lot. A vigour test, however, would offer the scope and possibility to determine vigour of a seed lot so that its field and storage performance can be assessed. Thus, this study aims to evaluate the effectiveness of accelerated aging test as a vigour test for mungbean seeds. Accelerated aging procedure was done to obtain seed samples varying in vigour levels. Seeds were exposed to high temperatures (41, 43 and 45°C) with high humidity environment (R.H.≈95%) for different periods of time (48 and 96 hours). Seed moisture content, electrical conductivity of seed leachate and standard germination test were performed on the aged seeds and the results were compared to the non-aged seeds. Current results revealed that accelerated aging treatments statistically ($p<0.05$) affected all the twelve traits evaluated. After aged at 45°C for 96 hour, seed moisture content increased from 6.84% to 23.56% while seed leachate conductivity increased from 9.95 µS/cm/seed to 14.28µS/cm/seed. All the germination percentage, germination index, seedling vigour index and seedling growth parameters showed drastic reduction after accelerated aging. The seven resultant seed lots ranged in germination percentage and seedling vigour index from 95 % and 19.68, respectively (for non-aged seeds) to 59.5 % and 5.73 (for seeds aged at 45°C for 96 hours).The findings demonstrated that high vigour seeds outperformed low vigour seeds in all traits evaluated.

Key words: accelerated aging, mungbean, seed vigour, seed quality.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important pulse crops grown in South and Southeast Asia (Nair et al., 2013). About 90% of mungbean production originated from Asia country, especially from India, China, and Thailand (Lambrides and Godwin, 2007). Mungbean is a short duration cash crop in various intercropping and crop rotation systems to provide additional income to farmers. It provides essential nutritents in human and animal diet as the grains are rich in protein and micronutrients. Besides that, it helps the symbiotic association of *Rhizobium* species to fix the atmospheric nitrogen in order to improve soil fertility (Somta et al., 2007). Mungbean also used as excellent green manure. These advantages thus lead to increase of demand in mungbean supply.

Seed vigour test is aimed to provide information about the planting value in a wide range of environments and/or the storage potential of a particular seed lot. Accelerated aging was initially developed as a test to estimate the longevity of seed in storage and have been evaluated as a

seed vigour indicator in a wide range of crops. The accelerated aging stress test exposes seeds for short periods to high temperature and high relative humidity (≈95 %). During the test, the seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed aging (ISTA, 2015). High vigour seed lots will withstand these extreme stress conditions and age more slowly than low vigour seed lots. Thus, after aging treatment, high vigour lots retain high germination while low vigour lots significantly decline in the capacity to germinate.

Seeds progressively gain the germination ability and capacity on their mother plants during seed development (Bewley et al., 2013). Seed vigour gradually increases and reaches the maximum level at physiological maturity. Beyond that, seeds start to age and seed vigour begins to decrease with time. During seed deterioration, a series of deleterious events such as cellular membrane degradation, lipid peroxidation, and DNA degradation will occur in the seed. As seed vigour decreases, seed longevity decreases. Ultimately, seed losses its viability and fail to germinate. The rate and extent of seed aging and deterioration mainly depend on the initial vigour of the particular seed lot. Seed vigour not only affects crop growth and yield, but also the seed storage potential.

Most of the seed producers keep harvested seeds in stock for months or even years before selling or next sowing. Rapid seed aging and deterioration during storage is one of the most critical constraints confronting farmers. One of the important factors that influences life span of seed is vigour. The decline of seed vigour and viability over time is usually illustrated by a sigmoid survival curve, whereby the viability loss is parallel with vigour loss (Harrington, 1972). Seed vigour and viability moderately decline during the early period of storage, followed by a sharp decrease and finally a gradual reduction in vigour level. Seed aging progressively weakenes the seed which eventually causes the seeds unable to germinate (Shaban, 2013). Robust seeds deteriorate slower, thus have better potential than low vigour seed.

A standard germination test is widely used for evaluating the quality of seeds. It is conducted under optimum growth conditions for a particular species (Julio, 2015). Ordinarily, they show no association with field performance. In some case studies, seed lots with similar germination percentages differ greatly in field performance values. Poor yield production was obtained even the laboratory germination test showed a high germination percentage. Similarly, despite the seed lots having similar germination percentage, they may show different rates of deterioration and storage life (Khan et al., 2007). The variations could be due to the suboptimal field and storage conditions which differed from the optimum growth conditions during the standard germination test. These situations thus indicate the incompleteness of the standard germination test to assess the seed lot field performance and storage potential.

Mungbean is one of the important sources of dietary protein, thus it is of utmost importance to understand the effect of aging on mungbean seed vigour. This could enhance the seeds storability, increase productivity, benefit farmers and national economies. Accelerated aging is a very responsive test in identifying seed quality and vigour (Khan et al., 2007; Kibinza et al., 2006). Regarding the relation between vigour and seed quality, this study was initiated with the objective to evaluate the effectiveness of accelerated aging test as a vigour test for mungbean seeds.

MATERIALS AND METHOD

Seed Materials

Certified mungbean (*Vigna radiata*) seeds were purchased from SSH Green Eagle Seeds International Co. Ltd., Bangkok, Thailand. Working samples were obtained by using the principle of extraction and combination using a seed divider (Model Inosaw 6716 - Boerner Type). Only seeds with uniform size were selected to form a composite working sample. Seeds were with moisture content of 10-12 %) when received. They were sealed in air-tight glass jar and stored at 5°C in the dark until used (Khan et al., 2007).

Accelerated Aging Method

Traditional accelerated aging was carried out followed the modified set up as demonstrated by Grabe (1965) as shown in Figure 1. A single layer of seeds for each treatment was placed on a screen tray which inserted into an aluminium tin filled with 150 ml distilled water. A piece of baking paper was used to cover the tray to prevent the dropping of condensed vapour from the lid onto the seeds. The lid of the aluminium tin was closed tightly. All tins were placed into the oven (Model Binder) and aged at a high temperature of 41°C, 43°C and 45°Cwith humid environment (R.H.≈95 %) for 48 and 96 hours respectively. Measurement of moisture content of treated seeds, electrical conductivity of seed leachate as well as germination test were carried out.

Seed Moisture Content Measurement

Forced air oven method of moisture content determination was used (ISTA, 2015). Four replications of 15 intact seeds were evenly distributed and slipped into the folded aluminium foil. The folded aluminium foil was weighed using analytical balance (Model Mettler Toledo) before and after the insertion of seeds. Then, the folded foils with seeds were placed in an oven (Model Binder) at 103°C for 17 hours. At the end of the prescribed drying period, the foils were placed in a desiccator to cool to ambient temperature. After cooling, the foils were weighed again until constant weight achieved. Seed moisture content was expressed in percentage (%).

Electrical Conductivity of Seed Leachate Measurement

Measurement of electrolytes leakage indicates the strength

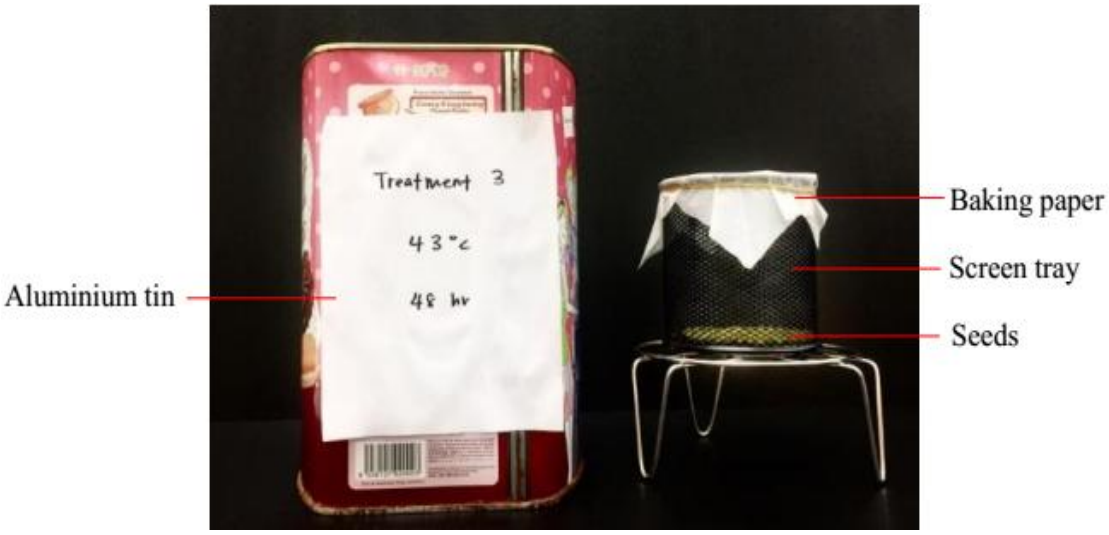

Figure 1: Accelerated aging set up in the study

Figure 2: Rolled towels were set in the upright position in a distilled water filled plastic tray for germination

of the membrane structure of seeds, thus its capacity able to determine the vigour level. Electrical conductivity test was done according to the procedures described by ISTA (2015). Four replications of 15 intact seeds were placed in a 200 ml plastic glass containing 75 ml of deionized water respectively. The seeds were gently stirred using stirring rod to ensure that all seeds were completely immersed and evenly distributed. All glasses were covered and left undisturbed under ambient condition. The seeds were gently stirred before every electrolyte leakage measurement using a conductivity meter (Model Eutech CD650) after 24 hours of soaking. The electrical conductivity was expressed per seed ($\mu S^{-1}cm^{-1}$seed).

Standard Germination Test

Seed germination test was carried out by using the rolled paper towel method (Figure 2). Four replications of 50 aged seeds were used. Non-aged seeds were used as control. A transparent wrapping paper was used as bottom layer. Two layers of moisten paper towel (Scott® Kitchen Towel) were placed on the wrapping paper. Seeds were set centrally lengthwise two centimetres apart from one another on the paper towels. The towels then rolled loosely and set in the upright position in a distilled water filled plastic tray. The level of the distilled water filled in the tray was lower than the last row of seeds in the paper towel (Figure 3). The tray

Figure 3: The level of the distilled water filled in the tray was lower than the last row of seeds in the paper towel

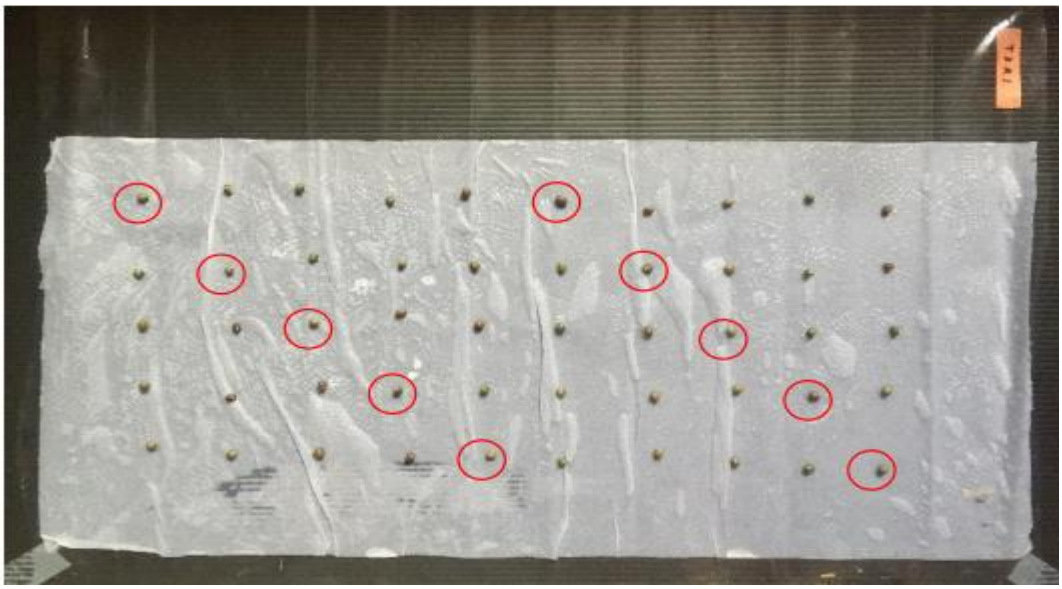

Figure 4: Seedlings in the circled positions were selected for evaluation of radicle and plumule length, radicle fresh and dry weight as well as plumule fresh and dry weight

was then placed in a seed germinator (Seedburo Seed Germinator MPG 3000/C) with alternating temperature regimes of 30°C for 8 hours and 20°C for 16 hours. Germination counts were made and recorded daily until day seven of germination (ISTA, 2015). After seven days, the following attributes were recorded: (i) germination percentage (ISTA 2015); (ii) germination index(AOSA, 2002); (iii) seedling vigour index (Orchard, 1977); (iv) time to reach 50% germination(Farooq et al., 2005); (v) mean radicle length and plumule length (Figure 4); (vii) radicle fresh and dry weight; (viii) plumule fresh and dry weight.

Experimental Design and Statistical Analysis

A completely randomized experimental design with four replicates was used to allocate all samples within each test. Collected data were subjected to analysis of variance (ANOVA) using SAS version 9.4, while the sample means

Table 1. Effect of accelerated aging on seed vigour attributes

Treatment		Seed Moisture Content (%)	Seed Leachate Conductivity (µS/cm/seed)	Germination Percentage (%)	Germination Index	Seedling Vigour Index	T_{50} (day)
Non-aged		6.84±0.38 c[1]	9.95±0.65 c	95.00±3.46 a	39.29±0.96 a	19.68±1.73 a	0.76±0.04 bc
48 h	41°C	11.0890±1.94 b	11.4453±0.85 c	89.5000±4.12 ab	38.0425±0.85 ab	14.1890±1.47 b	0.7150±0.09 bc
	43°C	11.3207±0.87 b	11.4009±1.04 c	85.0000±4.76 b	34.1425±3.01 b	12.1412±1.07 bc	0.9400±0.37 abc
	45°C	12.4899±1.57 b	13.6638±1.67 b	73.0000±5.29 c	28.8325±2.46 c	10.2460±3.09 cd	1.3025±0.38 a
96 h	41°C	21.2830±2.12 a	14.9890±1.54 ab	75.5000±7.19 c	35.6250±3.47 ab	8.9508±2.05 d	0.5650±0.04 c
	43°C	22.4270±2.46 a	16.4472±2.01 a	62.5000±5.26 c	26.4575±3.99 c	4.8369±0.65 e	1.2650±0.44 a
	45°C	23.5680±3.94 a	14.2802±1.50 b	59.5000±9.57 c	25.6250±4.89 c	5.7323±2.26 e	1.0625±0.48 ab
R-Square		0.93	0.80	0.87	0.81	0.91	0.48
[3]C.V. (%)		14.10[***2]	10.39[***]	7.22[***]	8.83[***]	15.85[***]	32.63[***]
Treatment		**Radicle Length(cm)**	**Plumule Length(cm)**	**Radicle Fresh Weight (g)**	**Plumule Fresh Weight (g)**	**Radicle Dry Weight(g)**	**Plumule Dry Weight(g)**
Non-aged		11.09±0.96 a[1]	9.63±0.63 a	1.22±0.19 a	3.87±0.25 a	0.08±0.01 a	0.25±0.02 a
48 h	41°C	8.3150±0.55 b	7.5275±1.11 b	0.9144±0.12 b	3.1200±0.24 b	0.0587±0.01 ab	0.2075±0.02 b
	43°C	7.3600±0.68 bc	6.9275±0.62 bc	0.8155± 0.19 bc	2.7025±0.21 bc	0.06108±0.04 ab	0.1775±0.02 bc
	45°C	6.7475±1.93 bc	7.1200±1.29 bc	0.8209±0.12 bc	2.7750±0.52 b	0.0507±0.01 ab	0.1725±0.03 bc
96 h	41°C	5.7850±1.10 cd	5.9625±0.85 cd	0.7031±0.08 bcd	2.2275±0.35 cd	0.0449±0.01 b	0.1425±0.02 cd
	43°C	3.1150±0.95 e	4.7100±0.67 d	0.5512±0.11 d	1.8600±0.42 d	0.0348±0.02 b	0.1200±0.03 d
	45°C	4.2250±1.48 de	5.2325±0.97 d	0.6118±0.11 cd	2.1650±0.47 cd	0.0381±0.01 b	0.1350±0.04 d
R-Square		0.88	0.84	0.78	0.50	0.84	0.83
[3]C.V (%)		15.98[***2]	12.39[***]	17.12[***]	32.04[*]	12.57[***]	13.21[***]

[1] Means within the same column followed by different letter are significantly different at p<0.05 according to DMRT.
[2] *, **, *** = Significant difference at p<0.05, p<0.01 and p<0.001, respectively.
[3] C.V. = coefficient variance.

were compared by using Duncan Multiple Range test to the 95% significance level.

RESULTS

Seed Moisture Content Measurement

Seed moisture content after accelerated aging treatment showed a highly significant (p<0.001) elevation trend ranged in between 11.09 to 23.57 percentage points for all the aging combinations. Aging period of 96 hours showed double increment effect on seed moisture level as compared to 48 hours has shown in Table 1. Aging combination of 45°C/96 h marked the highest moisture content with 23.57 %. Results showed that there is no significant difference in between the different aging temperatures within the same aging period, for both 48 and 96 hours. However, significant difference was found in between these two aging periods. All the aged seeds moisture content statistically different with non-aged seeds.

Electrical Conductivity of Seed Leachate Measurement

The electrical conductivity of seed leachate was highly significant (p<0.001) different where it marked an increasing trend for all aging

combinations as compared to the non-aged seeds. Artificial aging for 96 hours obviously marked more electrolyte leakage than 48 hours as shown in Table 1. Seed lots aged at 41°C and 43°C marked a steep elevation when the aging period extended from 48 hours to 96 hours. However, for seed lot aged at 45°C, the electrolyte leakage showed a steep increment at 48 hours and slowed down when aging continued to 96 hours. For 48 hours aging, seed lot 45°C marked the highest leakage of 13.66 $\mu S^{-1}cm^{-1}$seed. While for 96 hours, seed lot 43°C marked the highest leakage of 16.45 $\mu S^{-1}cm^{-1}$seed.

Standard Germination Test: Seedling Evaluations

Germination Percentage

There was a highly significant ($p<0.001$) difference found in the germination percentage of aged seeds. The germination percentage was decreasing as the aging temperature increased across the period as shown in Table 1. Increasing in the aging period gave a more suppressive effect on germination as compared to aging temperature. For seeds aged for 48 hours, the germination percentage decreased to 89.5% for 41°C to 85.0% for 43°C and 73.0% for 45°C regime. For seeds aged for 96 hours, the germination percentage decreased drastically to 75.5% for 41°C to 62.5% for 43°C and 59.5% for 45°C, which all of these were lower than the non-aged seeds of 95.0%.

Germination Index

Accelerated aging highly significant ($p<0.001$) decreased the germination index of mungbean seeds. Increasing aging temperature along the time decreased the germination index remarkably (Table 1). From the findings, all the aged seeds showed lower germination index than non-aged seeds of 39.29. After aging for 48 hours, the germination index decreased to 38.04 for 41°C, 34.14 for 43°C and 28.83 for 45°C regime. Seeds that went through 96 hours aging marked a lower germination index with only 35.63 for 41°C, 26.46 for 43°C and 25.63 for 45°C regime.

Seedling Vigour Index

Seedling vigour index marked a highly significant ($p<0.001$) decline pattern as aging period increasing. Aging period of 96 hours marked a significant reduction in seedling vigour index compared to 48 hours (Table 1). The seed lot vigour index declined to 14.19 for 41°C, 12.14 for 43°C and 10.25 for 45°C regime after 48 hours of aging. Seeds aged for 96 hours resulted in a much lower index with only 8.95 for 41°C, 4.84 for 43°C and 5.73 for 45°C regime. As seed aged, the germination percentage and seedling growth decreased, thus reduced the seedling vigour index.

Time to Reach 50 % of Germination

The finding showed an interesting significant ($p<0.05$) result in time to reach 50 % of mungbean seed germination. The results revealed that aging condition at 41°C allowed seeds to reach 50% germination faster than untreated seeds for both 48 and 96 hours with only 0.72 days and 0.57 days respectively as compared to the untreated seeds of 0.76 days (Table 1). For 43°C aging regime, the seeds took 0.94 days and 1.27 days to reach 50% germination for 48 and 96 hours respectively. Aging combination of 45°C/48 hours marked the slowest for seeds to attain 50% germination with 1.30 days, while 96 hours aging under the same temperature took 1.06 days.

Radicle Lengthand Plumule Length

Both the radicle and plumule were significantly decreased in length as the aging temperature increasing along the aging period. Aging at 48 hours showed less impact in slowing down the seedling growth compared to 96 hours aging (Table 1). Exposure of seeds to high temperature and relative humidity affects the seedling growth where the radicle length declined more than plumule length. Seed lot aged at 43°C for 96 hours marked the shortest radicle (3.12cm) and plumule (4.71cm) length among all aged lots.

Radicle Fresh Weightand Radicle Dry Weight

The finding revealed that accelerated aging significantly influenced the radicle fresh weight and dry weight in mungbean seeds. All the aged seeds were recorded with lighter radicle than non-aged seeds of 1.22 g for fresh weight and 0.08 g for dry weight (Table 1). For 48 hours aging, radicle fresh weightdecreased to 0.91 g for 41°C, 0.82 g for both 43°C and 45°C regime. When the aging extended to 96 hours, radicle fresh weight continued to drop to 0.70 g for 41°C, 0.55 g for 43°C and 0.61 g for 45°C regime.

Plumule Fresh Weight and Dry Weight

The analysis also revealed that accelerated aging significantly affected the plumule fresh and dry weight in mungbean seeds. Non-aged seeds recorded the highest plumule fresh weight of 3.87 gand plumule dry weight of 0.25 g (Table 1). As seeds aged, the plumule fresh and dry weight decreased. Aged lot of 41°C resulted in better plumule growth than 43°C and 45°C regime aged lots.

DISCUSSION

Physiological Changes in Mungbean Seeds after Accelerated Aging

In artificial aging, seeds are subjected to high temperature (41 to 45°C) and high relative humidity (up to 100%) conditions to allow rapid seed aging. Hence, seed deterioration occurs much more rapid in artificial aging compared to natural aging. Artificial aging is useful to lower the seed viability within a short period for the experimental

purpose (Kaewnaree et al., 2011). Present findings revealed that all the aging conditions significantly elevated seed moisture content, delayed days to reach 50% germination, slower seedling growth, decreased seedling vigour index and finally a decline in germination percentage and germination index as compared to non-aged seeds (Table 1). The severity of these changes is related to the degree of seed deterioration. It is believed that these detrimental effects have resulted from several mechanisms including free radical-mediated lipid peroxidation, protein structure degradation, enzyme inactivation, disruption of cellular membranes and genetic integrity destruction (McDonald, 1999).

Exposure of seeds to high temperature and relative humidity during artificial aging cause the reactive oxygen species to accumulate in the bilayer phospholipid membrane. Reactive oxygen species has been widely recognized as the main factor of seed aging causing seed deterioration (Laloi et al., 2004). The species include free radicals like superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$); non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O2$), and so forth (Sharma et al., 2012). Kibinza et al. (2006) reported the loss of viability in sunflower seeds during aging was associated with the decline of antioxidant enzymes activity caused by the accumulation of free radicals hydrogen peroxide and lipid peroxidation. Once the seeds imbibed in the water, enzymatic mechanisms in the seeds initiating the production of reactive oxygen species especially in the mitochondrial respiratory chain of the metabolically active seeds (Bailly, 2004). Excess of ROS oxidised and denatured protein structures in the cells, caused cellular membrane start to disorganize, gradually loss its integrity and selectivity, leading to the rapid increment of water imbibition in the cells and affects embryo viability (Krishnan et al., 2004; Bailly et al., 2008; Kapoor et al., 2011; Peng et al., 2011).

It was believed that membrane degradation is the first sign of seeds aging. Phospholipid bilayers and protein structures are the main components in the cellular membrane. Most of the membrane phospholipids are polyunsaturated fatty acids which are more susceptible to attack by free radicals to produce peroxide than monounsaturated fatty acids (Priestley et al., 1980, Basra et al., 2003). Peroxide generated tends to transform into aldehyde to combine with enzymes. The enzymes have then been inactivated and dysfunctional, thus gave deteriorative damages to seeds. Free radicals released as by-product at the end of peroxidation eventually attack other subcellular structures in seeds, including organelle membranes, proteins, and DNA (Liu et al., 1993). Degradation of the mitochondrial membrane due to artificial aging also reducing the essential energy supply, thus suppressed the seedling growth (Gidrol et al., 1998). Peroxidation of membrane lipids consequently damaging the membrane structure, increasing membrane viscosity and enhancing bilayer permeability (Priestley, 1986), thus lead to a decline in seed quality and vigour.

All seeds undergo natural aging process after physiological maturity stage which leads to deterioration in seed quality with vary rate among various plant species (Merritt et al., 2003). Aged seeds produce weak seedlings with low adaptation to the adverse environment due to their lower vigour level (Aiazzi et al., 1997). When vigorous seeds are under warm moist conditions, their embryos enlarged rapidly to develop primary roots. For epigeal germination species such as mungbean, cotyledons served as an important organ for food storage and assimilation. Seedling development is affected by the ability of the cotyledons to expand. Accelerated aging caused the incomplete or faulty essential enzyme synthesis, essential enzymes inactivation and declined biochemical activities in seeds (Kapoor et al., 2010). Seed cotyledons then unable to provide an essential usable food supply for normal seedling growth, thus interrupt the early stage of germination and seedling development (Iqbal et al. 2002). This reduction in seedling growth and vigour might be associated with the weak hypocotyls and primary roots due to the accelerated aging (Basra et al., 2003).

Biochemical Changes in Mungbean Seeds after Accelerated Aging

Present results demonstrated that electrolyte leakage was significantly increased by accelerated aging with a fluctuation trend among aging combinations over 24 hours soaking (Table 1). Fick and Hibbard (1925) first proposed the use of electrical conductivity measurements as a means of determining seed viability in Timothy and red clover. Solute leakage from seeds reflect the state of cell membranes and its ability of solute retention during imbibition (Espanany et al., 2015). During the conductivity test, measurement of electrolyte leakage indirectly indicate the differences in seed deterioration and vigour levels (Peksen, 2007). These differences are often associated with the differences in cellular membrane integrity, biochemical changes, physical disruption and ability of the seeds to reorganize and repair the damage (Brouwer and Mulder, 1982; Powell, 1988; Duczmal and Minicka, 1989).

Damage of cellular membrane is a continuous process during early stage of seed deterioration. As seeds age, degradation of phospholipids caused the membrane structure disorganized and more permeable, resulting in more ions leakage from the seeds into soak water (Ouyang et al., 2002). Impaired membrane activity and increase in dead tissue on the cotyledons are also often associated with an increases in leakage, which might not reflected in germination failure in the laboratory (Matthews and Rogerson, 1976). Conductivity test able to detect cellular membrane disorganization during early seed deterioration, while the loss of germination ability can only be detected at the end of the deterioration process. Several studies have been made to study the conductivity test as vigour test in in soybean (Parrish and Leopold, 1978; Mattoni et al., 2015), groundnut (Pearce and Samad, 1980), cowpea (Peksen et al., 2004), corn (Fessel et al., 2006), horse gram (Sharma

et al., 2011) and radish (Mavi et al., 2016).

Several literatures had documented the similar results. Abass and Shaheed (2012) revealed that mungbean seeds aged at 45°C with 100% relative humidity caused more electrolytes leaked from the aged seeds. Hussein et al. (2012) a linear increase in the electrolyte leakage of maize seeds as the aging period increased. However, contradict result was reported by Gidrol et al. (1998) where no significant difference was found in the loss of legume seed viability and electrolyte leakage during accelerated aging. This was supported by Hussein and Yasser (2011) in sunflower seeds.

CONCLUSION

Accelerated aging were found to have significantly deleterious effects on mungbean seed quality. The laboratory experiments demonstrated that high vigour seeds outperform low vigour seeds in almost all traits evaluated. The extent of deterioration corresponded linearly with temperature and duration of aging. The higher the temperature and the longer the aging duration, the more severe was the damage.

DECLARATION OF INTEREST

The authors reported no conflicts of interest for the content and writing of this article.

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Conflict of interests

The authors declare that they have no conflicting interests.

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