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Full Length Research Paper

Effect of drying conditions and harvest time on soybean seed viability and deterioration under different storage temperature

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Quality attributes of seeds may decline during storage. Quantifying loss of seed germination during storage has given rise to an equation proposed by Ellis and Roberts that, "same rate of seed deterioration will take place among seed lots of a species in identical environments". This study was conducted to determine whether deterioration will take place at the same rate, and also to evaluate the factors that affect rate of deterioration. Storage experiments were performed in three constant temperatures of 15, 20 and 25°C and seed moisture contents of 12% using 32 soybean seed lots (cv. Williams) obtained from different harvest date and dried in various combinations of drying. The GENMOD procedure of SAS was used for analysis of standard germination of seed lots, and stepwise regression was used to simulate effects of pretreatment conditions plus storage temperature on seed viability during storage. Data analysis with separate and parallel lines showed that equation of Ellis and Roberts is not applicable for these seeds. Trend of germination reduction were plotted, slops of curves were analyzed and correlated with variables. Harvest moisture, its interaction with storage temperature and second order of harvest moisture had the most effects on rate of deterioration. The moisture content had adverse effect on this rate and interactions of storage temperatures with drying temperature intensify that in higher temperature. Also, high drying temperatures, air velocity, and deep loading exacerbate this rate. Trends of electrical conductivity (Ec) increment that can be related to lipid peroxidation, chemical change or membrane disturb confirmed the results.

Key words: Seed deterioration, seed quality, soybean seed and seed viability.

INTRODUCTION

Seed quality is a multiple criterion that encompasses several important seed attributes: genetic and chemical composition, physical and physiological condition, germination and vigor, size, appearance and presence of seed borne pathogens, crop and varietal purity, weed and crop contaminants and moisture contents. During storage, seed quality can remain at its initial level or decline to a level that may make the seed unacceptable for planting purpose. This is related to many factors: environmental conditions during seed production, pests, diseases, seed oil content, seed moisture content, mechanical damages of seed in processing, storage longevity, packaging, pesticides, air temperature and relative air humidity in storage, biochemical injury of seed tissue, etc. (Simic et al., 2004, 2007). Seed viability denotes the degree to which a seed is alive, metabolically active and possesses enzymes necessary for catalyzing metabolic reaction needed for germination and seedling growth after storage (Basra et al., 2002). Longevity of seed in storage is influenced by the initial quality of stored seed as well as storage conditions. Irrespective of initial seed quality, unfavorable storage conditions, particularly air

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temperature and air relative humidity, contribute to acceleration of seed deterioration in storage. The kinetics of seed survival during storage varies with water content and temperature. Effects of water content and temperature are interdependent (b and Roos, 1993). Hence, it is difficult to assess the effective storage period because the storability of the seed is a function of initial seed quality and the storage conditions (Heatherly and Elmore, 2004).

On the other hand, orthodox seeds can be dried to low moisture contents without damaging the embryo. The longevity of these seed will increase with decrease in moisture content during storage over a wide range of conditions. Contradictory to this view, drying at high temperature leads to significant reduction of moisture content and therefore loss of viability in pea and soybean seeds (Beena and Jayaram, 2010). Also, other studies showed that there was a limit to the water content, beyond which lower water content did not further increase seed longevity (Ellis et al., 1989), and could even have an adverse effect on seed viability and seed vigor (Vertucci and Roos, 1993). Drying temperatures higher than 47°C will decrease vigor indices of soybean seeds. The other drying parameters like air velocity and depth of loading also affect seed quality and indicate interaction with drying temperature and harvest moisture (Abbasi et al., 2009). Thomas (1980) reported that suitable combination for soybean safe storing was moisture content below 12%, temperature less than 10°C and 70% relative humidity. Storage behaviors of these seeds in higher temperatures are not well known and will be discussed in this paper. Moist seeds and uncontrolled storage temperature will affect seed quality with unpredictable results (Ellis and Robert, 1980). Prediction of soybean seed deterioration would be extremely beneficial to seed producers, since seed is frequently stored from one year to the next. Such prediction is dependent on the quantitative relationship between the rate of seed deterioration, seed quality, and storage conditions. During storage, all seeds undergo deterioration, with the rate dependent on storage temperature, seed moisture, and crop species. Attempts to quantify the loss of seed germination during storage have given rise to several prediction equations including the equation proposed by Ellis and Roberts (1980). Temperature, moisture and initial quality are important factors in Ellis and Roberts' equation to predict seed longevity; however, it relies on complex experiments of seed aging (Andreoli, 2004):

$$\nu = ki - \rho/10^{\,k_E - C_w \mathrm{Log}m - C_H t - C_Q t^2}$$

In this equation, ν is the probit of germination (%), ρ is the period of storage (days), m is the moisture content (%, fresh weight basis) and t is temperature (°C). *K*i is the initial seed quality constant of a seed lot (on the probit scale) and K_E, C_W, C_H, and C_Q are constants having common values for all seed lots of a species. These constants quantify the deterioration response with respect to seed moisture content and temperature, and determine the slope of the seed-survival curve. The equation has been applied to many species to predict seed viability or germination during storage and analyzing seed deterioration data. This viability equation is actually a composite of two separate equations. The first describes the seed survival curve in terms of the viability (ν, probit percentage viability) expected after a given storage period (p, days):

$$\nu = ki - \rho/\sigma$$

The assumption is that quality differences between seed lots do not affect δ (standard deviation of the frequency distribution of seed survival times), but are accounted for by *K*i. In contrast, the storage environment has no effect on *K*i, and only affects δ. So, the mentioned equation can be written as:

$$\mathrm{Log}\,\delta = k_E - C_w \mathrm{Log}m - C_H t - C_Q t^2$$

An important prerequisite for the use of equation is validation of the assumption of the same rate of seed deterioration among seed lots within a species when stored in an identical environment. If this assumption is valid, the constants can be determined. Otherwise, the constants will not be universal for all seed lots in a species (Ellis and Roberts, 1980). The reciprocal of δ ($1/\delta$) is the rate of seed deterioration in a given storage environment. Therefore, if plotted, parallel lines should be found among seed lots of a species on the basis of the assumption that all seed lots deteriorate at the same rate irrespective of initial seed quality. In this situation, δ is dependent only on environmental condition. In the present study, the rate of deterioration calculated with probit analysis for nine month storage with parallel (PL) and separate line (SL) firstly for three storage temperature and then, F value was determined accordingly:

$$F = \frac{\dfrac{\text{Scaled Deviance of PL - Scaled Deviance of SL}}{\text{df PL - df SL}}}{\dfrac{\text{Scaled Deviance of SL}}{\text{df SL}}}$$

The significance of F value indicates that the slope of each equation is different and consequently the rate of deterioration is different for soybean seed lots and initial quality or pretreatment of soybean seeds can affect the rate of deterioration. Therefore, simulation of storage

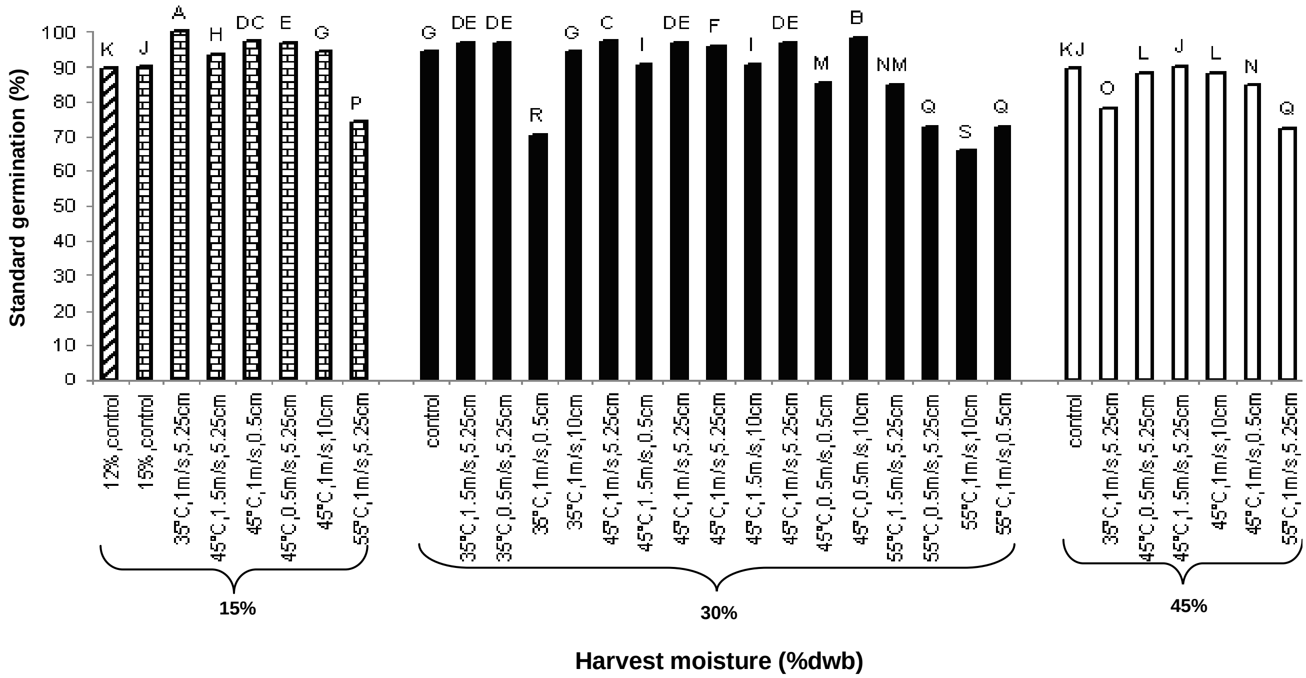

Figure 1. Initial standard germination of soybean seed lots before storage.

temperature combined with various conditions of harvest and drying was used in this experiment in order to create predicting seed qualities equations. Thus, the objectives of this study were: (1) to determine whether soybean seed lots deteriorate at the same rate in identical storage environments; and (2) to evaluate the influence of seed pretreatment conditions and storage temperature on rate of soybean seed deterioration.

MATERIALS AND METHODS

Soybean seeds cv. Williams were sowed at 15th may 2007 in the University of Tehran farm located at 35° 25′ N latitude, 71° 25′ E longitudes and an altitude of 1321 m above sea level. The seeds are hand harvested at three initial moisture contents 45, 30 and 15% dry weight basis (d.b) in 5th, 15th Sep. and 7th Oct., respectively. The seed samples then were sealed in plastic bags and stored in a cold place and equilibration with ambient air was performed for at least 1 h prior to every drying treatment. Drying experiments were carried out using a laboratory dryer consisting of a centrifugal fan that forces heated air from the heating elements to sampling drum. The temperature and air velocity of inlet air were controlled by a digital thermometer (1°C accuracy) and air velocity meter (Lotron model AM 4205) with 0.1 $m.s^{-1}$ accuracy, respectively. Sample weights were determined using an electronic balance with 1 g accuracy for every 15 min until the desired weight corresponding to a moisture content of 12% ± 1 d.b. was reached. The seeds were dried in different drying air temperatures 35, 45 and 55°C, air velocity 0.5, 1 and 1.5 m/s and different loading depth of 0.5, 5 and 10 cm, that is, 32 soybean seed lots using the Box and Behnken (1960) three-level, four-factor fractional factorial design (Abbasi et al., 2009). Williams is a preferred soybean variety in Iran that is cultivated in large areas of soybean production.

Storage experiments were conducted in three constant temperatures of 15, 20 and 25°C and seed moisture contents of 12% (dry weight basis). The initial germination of these seed lots was different and there was variation in vigor levels as determined by accelerated aging test and vigor indices (Figure 1). After seed samples conditioned to desired moisture contents were heat-sealed into plastic waterproof packets and stored in 3 incubators at mentioned temperatures. The length of storage period was 9 months. To monitor survival of the seeds, samples were removed every 45 days and the traits were measured as follows:

Standard germination test

Germination tests were performed with four 50-seed samples from each lot, in rolled papers (sandwich). Germination proceeded in a germinator at 25°C and counts were made for eight days after seeding (ISTA, 1999).

Rate of germination

Rate of germination was calculated as described in following formulae (ISTA, 1996):

$$RG = \frac{\text{No. of germinated seed at first count}}{\text{days of first count}} + \text{-------} + \frac{\text{No. of germinated seed at final count}}{\text{days of final count}}$$

Vigor indices I and II

After final germination day, the lengths of germinated seedlings were measured. The vigor index I was calculated as:

$$VI\ I = \frac{\text{Germination ration (\%)} \times \text{Mean seedling length (cm)}}{100}$$

Also, the weights of germinated seedlings were measured in grams. The vigor index II was calculated as:

$$VI\ II = \frac{\text{Germination ration (\%)} \times \text{Mean seedling weight (cm)}}{100}$$

Accelerated ageing

Accelerated ageing test was performed on 42 g of seeds placed on a wire mesh screen and suspended over 40 ml of distilled water inside plastic boxes (15.0 × 11.0 × 6 cm), held at 41°C and near 100% air relative humidity for 72 h (Hampton and TeKrony, 1995). After the ageing period, seeds were tested for standard germination, and the number of normal, five day-old seedlings was evaluated.

Electrical conductivity test (EC)

Three 50-seed replications were weighed and immersed in 75 ml of deionized water within plastic cups and kept in a germinator at 25°C, during 24 h. After this period, EC was determined in the imbibitions solution with a conductivity meter (Inolab model Listed 8F93), and results expressed as µS $cm^{-1}g^{-1}$ (Hampton and TeKrony, 1995).

A completely randomized design with three replications of each seed lot in each storage temperature was used. The survival data from experiment were used to evaluate the rate of seed deterioration among soybean seed lots and storage temperatures. Seed deterioration rates were calculated for each storage condition using probit analysis of full data sets. The PROBIT procedure (SAS Institute, 1988) provided slopes and intercepts for each probit survival curve where the slope is an estimate of the reciprocal of the standard deviation of the curve, $1/\delta$, (the rate of seed deterioration). After the slopes ($1/\delta$) derived from probit analysis were available for each seed lot and storage temperature, analyses of variance were used to determine if there were significant differences in rate of seed deterioration among seed lots. The GENMOD procedure of SAS Institute (1988) was used. Analyses were performed for parallel and separate lines. The value of F was determined for each temperature. Since the parameter F was significant, therefore, the rates of deterioration of seed lots were different, and we decided to simulate effects of pretreatment conditions plus storage temperature on soybean seed viability using stepwise regression. This regression was performed for rate of deterioration in the time range from 90 (onset of deterioration in most of seed lots) to 225 (getting viability to 0 for some seed lot) days after storage.

RESULTS

Rate of soybean seed deterioration

Analysis of the survival curves from these experiments confirmed that they were generally normally or near-normally distributed for most of seed lots and the others were neglected. Thus, it was appropriate to use probit analysis to determine the standard deviation of seed deaths in time.

If assumed that rate of deterioration is identical for all seeds then the curves of deterioration have to be paralleled and δ must be influenced only with storage coditions. Inorder to test of this hypothesis, data analysis with separate line models and calculation of coefficients for intercept and slope of viability during time were performed for different storage temperatures. For parallel line also, the intercept was calculated and then models were compared, and the values of F were determined (Table 1). Results showed that fitting the parallel line model increased errors more than separate line. Therefore, this intercept cannot be the same for all storage environments and it varies like slopes, hence the curves are not parallel and pretreatment of seed lots, that is, harvest moisture and drying combinations in constant bed dryer, affecting rate of deterioration of soybean seeds. So the equation of Ellis and Roberts is not applicable for these seed lots with various levels of vigor. Therefore, we decided to simulate effects of pretreatment conditions affecting initial quality and rate of seed deterioration, that is, harvest moisture, drying temperature, air velocity, depth of loading and of course storage temperature on the viability and slope of germination decrease curves.

These various rates of deterioration occurred with uniform moisture content among seed lots and seed samples. The differences in the rate of deterioration were comparatively large in some cases, which would result in dramatically different estimates of seed longevity. Our finding that the rates of soybean seed deterioration differed significantly among seed lots is in agreement with reports of Tang et al. (1999) for corn, Pieta and Ellis (1992) for wheat, Tarquis and Bradford (1992) for lettuce and Fabrizius et al. (1999) for soybean, but they do not agree with other reports of constant deterioration rates for corn (Ellis and Roberts, 1980) and other species, including barley, wheat, rice, and soybean (Ellis and Roberts, 1980). Thus, the difference in seed longevity among soybean seed lots may result from a difference in seed deterioration rate, initial seed quality, or both. This model does not seem to be applicable to seed lots with low initial quality. Perhaps no model can predict seed longevity of low vigor seed lots because the longevity of these seed lots seems to respond to changes in temperature and seed moisture content differently than medium or high quality seed lots (Tang et al., 2000).

Simulation of seed deterioration rate

Initial standard germination of soybean seed lots before storage was shown in Figure 1. Seed lots were dried at 55°C and monolayer depth had lower germination. High germination percentage was obtained for the seed lot which was harvested at 15% moisture content that dried

Table 1. F values to compare parallel and separate lines at different storage temperature.

Storage temp. (°C)	Model	f	Deviance	Mean deviance	F
15	Separate line	608	233.5	0.38	31.7**
	Parallel line	639	607.29	0.95	
20	Separate line	608	561.24	0.92	40.1**
	Parallel line	639	1707	2.67	
25	Separate line	608	1083.8	1.78	45.2**
	Parallel line	639	3580.8	6.5	

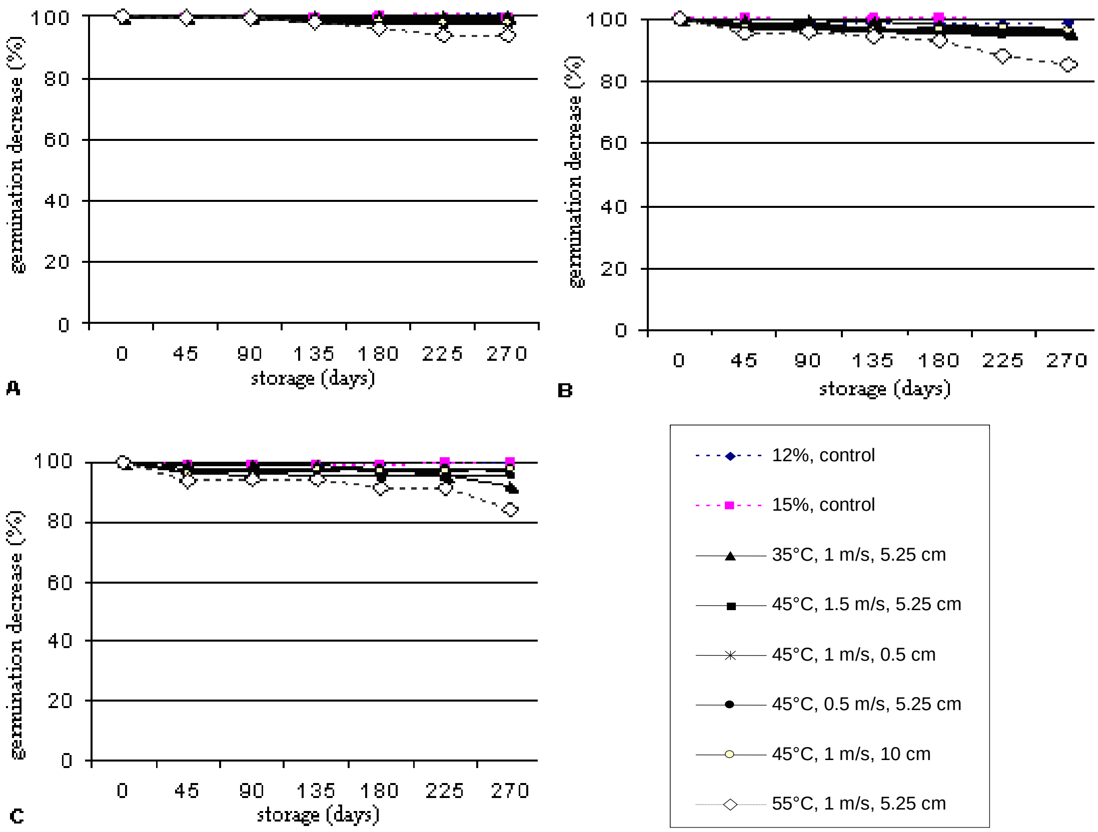

Figure 2. Curves of germination decrease toward initial germination of seed lots for moisture harvest 15% at 15 (A), 20 (B) and 25°C (C).

at 35°C, 1 m/s air velocity and moderate depth. Seed lots which were dried at 55°C had the lowest germination percentage.

Curves of germination decreasing towards initial germination of seed lots were plotted for moisture harvest at 15% (Figure 2), 20% (Figure 3) and 25% (Figure 4). Trend of germination reduction for different seed lots showed that for most of the seed lots, high reduction was observed after 90 days storage falling continuously till 225 days of storage that viability of some lots reaches to zero. For the seed lots harvested at 15% moisture content, only the samples dried at 55°C showed very low reduction than initial viability especially at higher temperatures. The seeds harvested at 30% moisture content indicated more falling especially for storage temperature of 25°C. For example, viability of seed lots dried at 55°C and some seed lots at 45°C, and monolayer depth decreased by half after 180 days of storage. This trend continued to the end of storage period. For 45% moisture content seeds, rate of germination decreased for all seed lots except control (drying at ambient temperature 25°C). Falling trend with time showed that increasing storage temperature resulted in higher rate of deterioration and in most cases, it reach

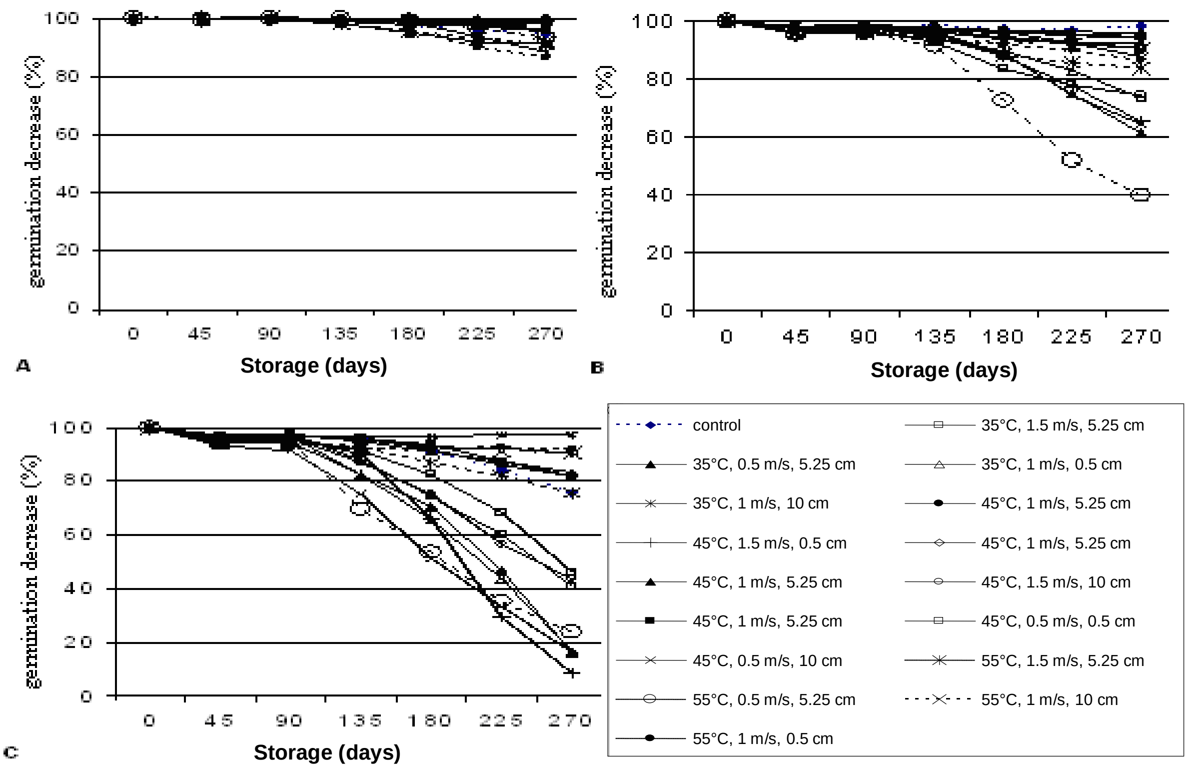

Figure 3. Curves of germination decrease toward initial germination of seed lots for moisture harvest 30% at 15 (A), 20 (B) and 25°C (C).

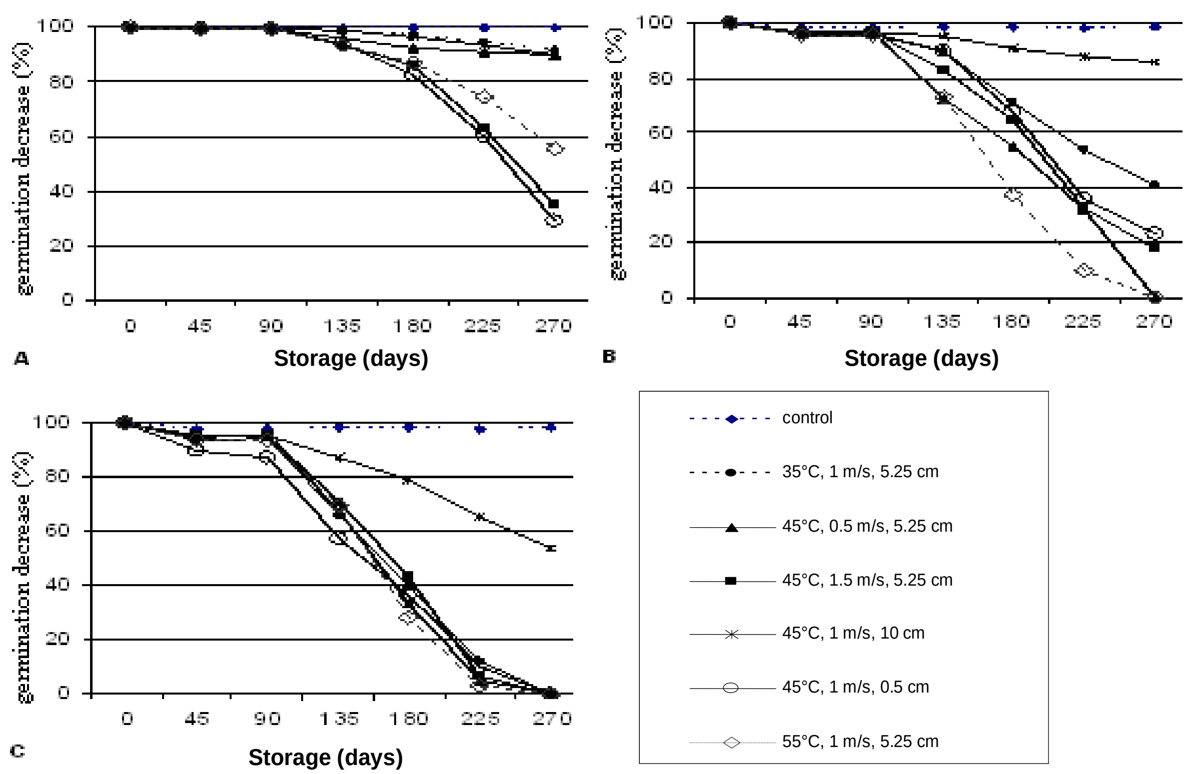

Figure 4. Curves of germination decrease toward initial germination of seed lots for moisture harvest 45% at 15 (A), 20 (B) and 25°C (C).

Table 2. Analysis of variance of model, significance of it and r^2 value.

Source	DF	Sum of squares	Mean square	*F*-value	Pr>*F*	r^2
Model	11	2.50405	0.22764	25.78	<0.0001	0.79
Error	76	0.67098	0.00883			

Table 3. Regression coefficients of polynomial model for slope of germination curves.

Variable	Parameter estimate	Standard error	ss	*F*-value	Pr>*F*
Intercept	0.34106	0.14113	0.05156	5.84	0.0181
x2	-0.05404	0.00881	0.33208	37.61	<0.0001
x5	0.05761	0.01458	0.13793	15.62	0.0002
X_2^2	0.000435	0.00009	0.20402	23.11	<0.0001
X_3^2	0.000121	0.00007	0.02741	3.11	0.0821
X_4^2	0.30134	0.07057	0.16098	18.23	<0.0001
X1x2	0.00142	0.000222	0.35991	40.77	<0.0001
X1x3	-0.00053	0.000159	0.09889	11.2	0.0013
X2x3	0.000386	0.000142	0.06534	7.4	0.0081
X2x5	-0.00109	0.000362	0.08044	9.11	0.0035
x3x4	-0.01004	0.00307	0.09438	10.69	0.0016
x4x5	-0.03243	0.01001	0.09262	10.49	0.0018

to zero after 225 days of storage.

Finally, slope of these curves was analyzed and correlation of these slopes with storage temperature and pretreatment variables were determined with stepwise regression. Slopes were calculated for all seed lots during 90 to 225 days after storage. Then, the effects of storage temperature (x1) and pretreatment variables include drying condition influenced by moisture content (x2), drying temperature (x3), air velocity (x4) and depth of loading in dryer (x5) on this slope were reported as a second order polynomial equation. Table 2 indicates the analysis of variance of the model, significance of it and r^2 value. Regression coefficients also were shown in Table 3. Harvest moisture, its interaction with storage temperature and second order of harvest moisture had the most effects on rate of soybean seed deterioration, and showed higher sum of squares. The moisture content had adverse effect on the rate of deterioration, which means the higher the harvest moisture the more rate of deterioration. Also, interactions of storage temperatures with drying temperature will intensify these slopes in higher temperature. According to other coefficients, high drying temperatures, air velocity and deep loading will exacerbate rate of deterioration, and are not recommended for drying soybean seed needed to be stored.

Electrical conductivity

Data showed that electrical conductivity (Ec) of soybean seed lots were increased during storage period. Curves of Ec increased toward initial germination of seed lots were plotted for moisture harvest at 15% (Figure 5), 20% (Figure 6) and 25% (Figure 7). Trend of Ec increment for different seed lots showed that for most of the seed lots, high increase was observed after 90 days storage continuously to end. For the seed lots harvested at 15% moisture content, the increase was not high even in high storage temperature. The seeds harvested at 30% moisture content indicated more change especially for higher storage temperature. This trend continued to the end of storage period. For 45% moisture content seeds, electrical conductivity increased for all seed lots in any given storage temperature except control (drying at ambient temperature 25°C). Therefore, immature seeds are more susceptible to storage.

DISCUSSION

Damage of seed during storage is inevitable and decrease of quality greatly depends on temperature and relative air humidity in storage, seed moisture content, duration of storage, type of seed, and initial quality of seed (Adebisi et al., 2004; Fabrizius et al., 1999). Initial seed quality is influenced by seed filling conditions, harvest and processing operations of seed. As seeds approach maturity, they characteristically accumulate soluble sugars. These solutes are known to contribute to the development of tolerance to desiccation, as well as to heat denaturation and to longevity itself (Bernal-Lugo and Leopold, 1995). Moreover, it has been proposed that the ratio of sucrose: raffinose in seed tissues is an indicator

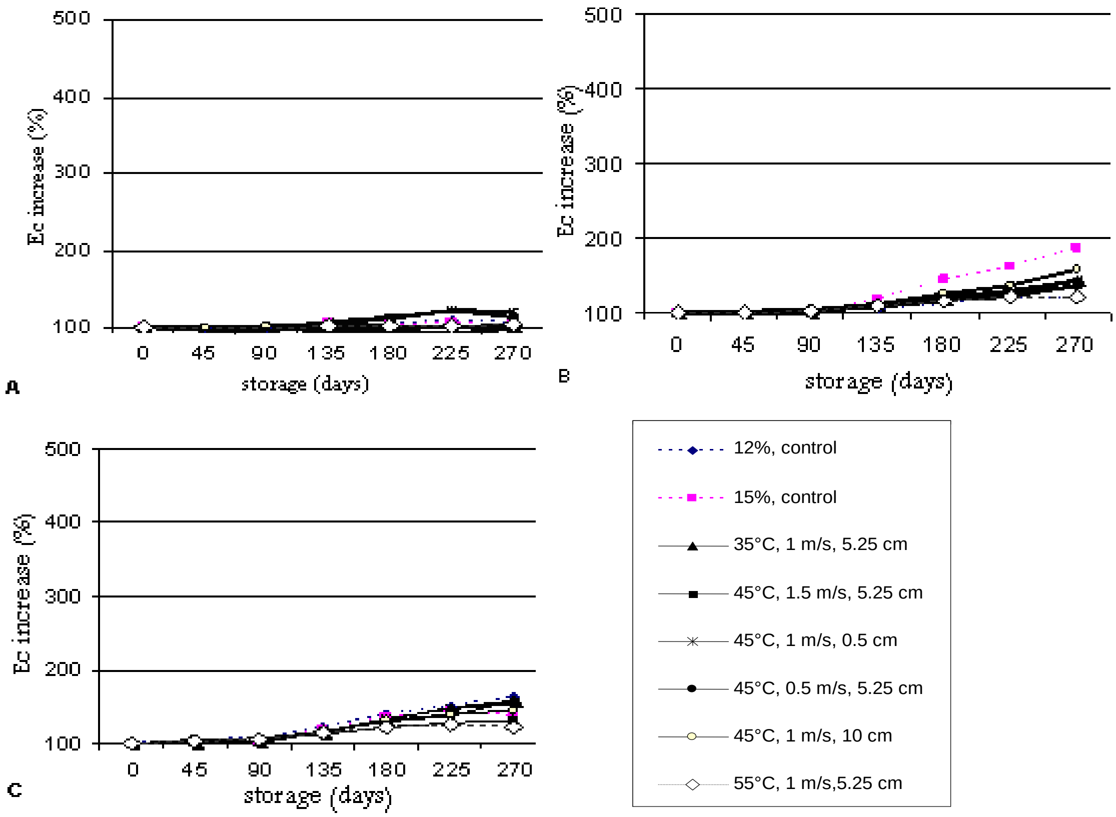

Figure 5. Curves of Ec increment toward initial Ec of seed lots for moisture harvest 15% at 15 (A), 20 (B) and 25°C (C).

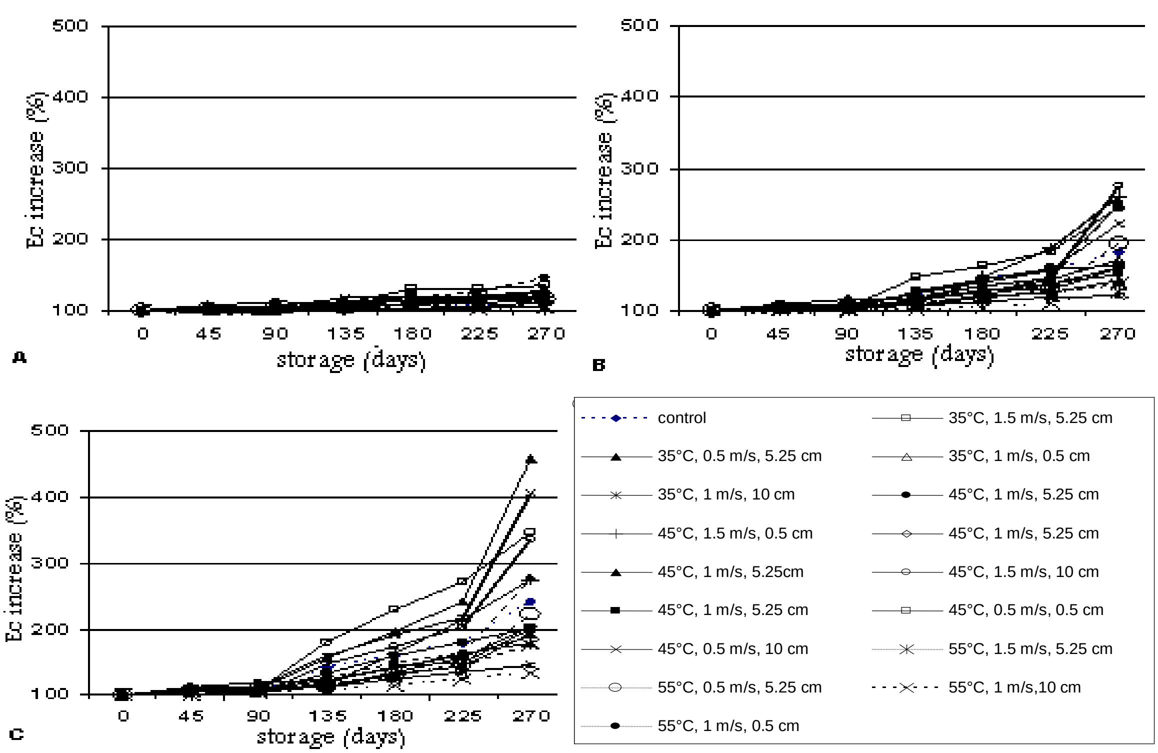

Figure 6. Curves of Ec increment toward initial Ec of seed lots for moisture harvest 30% at 15 (A), 20 (B) and 25°C (C).

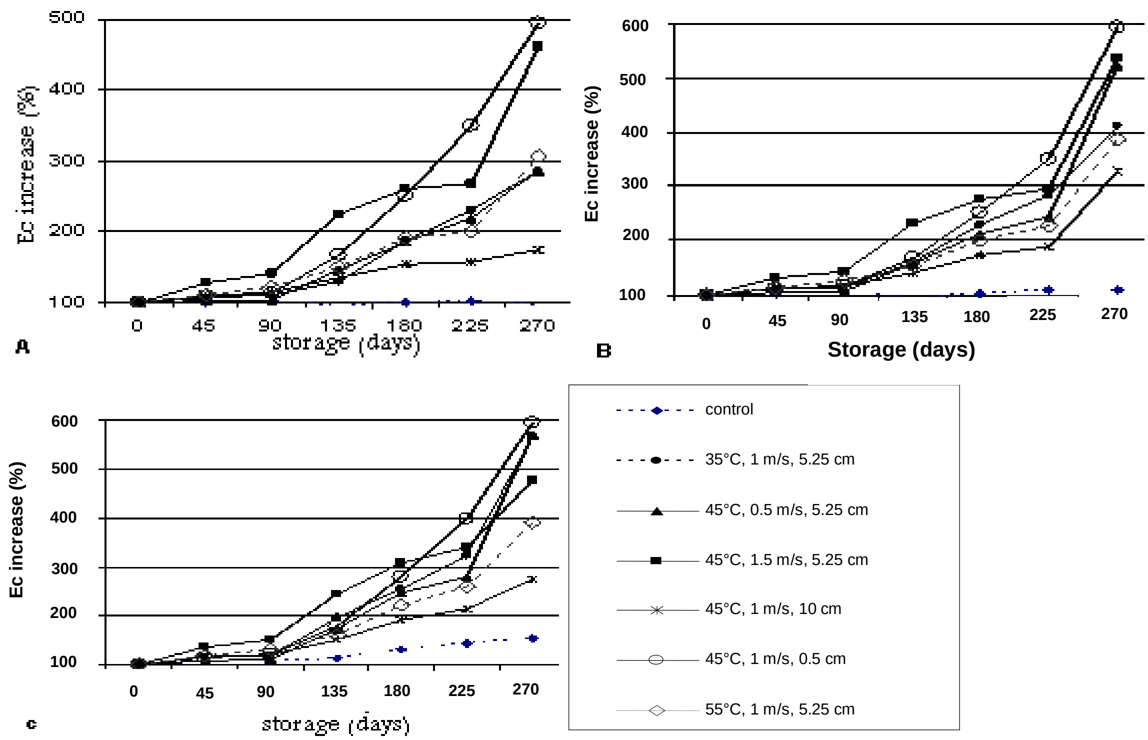

Figure 7. Curves of Ec increment toward initial Ec of seed lots for moisture harvest 45% at 15 (A), 20 (B) and 25°C (C).

of seed storage stability and storage category (Bernal-Lugo and Leopold, 1998). Obendorf et al. (1998) showed that germinability was improved by slow drying. During slow drying, immature embryos accumulate stachyose. An increase in the speed of germination and seedling growth accompanies with the change in sugars during maturation and storability of seeds has been related to the mass ratio of these oligosaccharides (Blackman et al., 1992). It has been proposed that sucrose and raffinose play a role in the storability of seeds. The content of raffinose as a mass fraction of total sugars and the magnitude of the glassy state showed positive correlations with storage stability. In this study, the seeds with harvest moisture more than 30% may not completed their growth and some solutes are not present. Therefore, they are vulnerable to storage. It is suggested that in soybean seeds, dry storage stability is a reflection of the total soluble sugar mixture, which could be more stable in fully matured seed (that is, harvest moisture of 15%). Also, late embryogenesis abundant (LEA) proteins, together with oligosaccharides and possibly small heat shock proteins (sHSPs) participate in glass formation and stabilization (Kioko et al., 2006). LEA proteins have interesting functional properties related to their presumed role as cellular stabilizers under stress conditions and storage, and amount of these proteins will be more in fully matured seed (Hundertmark and Hincha, 2008). Hence, the seeds that are harvested at 15% moisture content may accumulate these proteins more than the other harvest dates.

During storage phospholipids content of cellular membrane in general and that of mitochondria inner membrane in particular is decreased. This event causes disorganization of membrane, hence loss of membrane integrity or loss of selective permeability. This could be indicated by leakage. On the other hand, the increase of membrane permeability and activation of hydrolytic enzymes disrupt the cell structure and compartments. The increase leakage associated with aging might be the result of a more permeable membrane or of a larger pool of electrolytes. It can be the reason for higher Ec value for seed that are harvested early and stored in higher temperature.

Seed germinability decreased during storage, with a greater fall as storage temperature increased. Seed aging processes are controlled by temperature and moisture conditions, and may be associated with various chemical reactions and metabolic alterations in seeds. Electrical conductance of seed leachates also increased with ageing. Sung and Chiub (1995) indicate that storage of soybean seed at 5 and 25°C, aging inhibited seed germination and enhanced lipid peroxidation, but with more rapid seed deterioration and a greater extent of lipid peroxidation at higher temperature.

Saha and Sultana (2008) showed that seed germination and field emergence percentage decreased and electrical conductivity of seed leachate increased with increasing seed age in soybean varieties. Under tropical conditions, it has been established that soybean seed viability and vigor rapidly reduced during storage at ambient temperatures (Nkang and Umoh, 1996).

Conclusion

Seed deteriorates during periods of prolonged storage, but the speed of deterioration varies greatly among species and of course seed lots. Finally, deterioration of soybean seed occurs for any reason but it is not favorable for seed producers, and we have to manage harvest, drying storage temperature and moisture content to save acceptable quality for oilseed crops. According to this study, soybean seeds, especially those harvested early and passed through some processes like drying at high temperature, needs to be storage at temperature lower than 20°C; seed moisture content and relative humidity have to be controlled too.

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