African Journal of Biotechnology Vol. 11(45), pp. 10310-10316, 5 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB11.3505
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Soybean seed viability and changes of fatty acids content as affected by seed aging

Mladen Tatić[1]*, Svetlana Balešević-Tubić[1], Vuk Đorđević[1], Zorica Nikolić[1], Vojin Đukić[1], Milka Vujaković[2] and Gorica Cvijanovic[3]

[1]Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Serbia.
[2]Agricultural Extension Service "Agricultural Station", 21000 Novi Sad, Serbia.
[3]Faculty of Biofarming, Marsala Tita 39, 24300, Backa Topola, Serbia.

Accepted 28 December, 2011

The characteristics of soybean seed chemical composition are related to specific processes occurring in seed during storage. These changes lead to seed aging during storage and affect seed vigour and content of fatty acids. In order to reveal severity of their influence, the following vigour tests were applied: Standard laboratory germination test, cold test, Hiltner test as well as accelerated aging test for three and five days. Six soybean varieties submitted to natural aging process for six and 12 months were tested under conventional storage and controlled storage conditions. Different periods of seed storage, as well as storage conditions adversely affected the seed vigour and fatty acids content, especially linoleic acid. The most reliable results were obtained by cold test and the symptoms observed during accelerated aging test can be used to characterize the degree of aging and storability of soybean seed.

Key words: Aging, fatty acids, lipid peroxidation, soybean seed, vigour.

INTRODUCTION

Changes occurring in seed during aging are very significant with regard to quality and longevity of seed. Characteristics of oily plants seed composition are related to specific processes occurring in seed during storage (Ghasemnezhad et al., 2007). Seed longevity is one of the components of seed quality (Milošević and Malešević, 2004; Baiyeri and Mbah, 2006). The speed at which the seed aging process takes place depends on the seed's ability to resist degradation changes as well as its protection mechanisms, which are species-specific (Balešević-Tubić, 2001).

Many polyunsaturated fatty acids found in seed are particularly sensitive to peroxidative degradation. The results of these processes are not only the lipid degradation, but a series of reactions some of which form toxic products. Fatty acid composition is the most important factor which determines oils susceptibility to oxidation (Morello et al., 2004). In most of the plant species having seed rich in oil, lipids exposed to risk of auto-oxidation include oleic (18:1), linoleic (18:2) and linolenic (18:3) fatty acids chain. Degree of unsaturation has significant influence on degree of degradation (Priestley, 1986). The processes of stored seed deterioration mainly include a decline in the fatty acid content and an increase in the malondialdehyde level (Tian et al., 2008).

Seed viability implies the possibility of forming a new plant both under favourable and adverse climatic conditions. It is believed that high vigorous seed has uniform emergence ability in the field and thus yield stronger plants that provide higher yield (Milošević et al., 1995). Seed vigour is clearly related to many other components of physiological seed quality, such as viability and germination changes in overall seed quality that occur during seed development, maturation, harvesting, conditioning and storage which are all linked to seed vigour levels. Seed vigour testing is used as an indicator of the seed storage potential and proves to be a more reliable indicator than germination test (Balešević-Tubić et al., 2000; Tatić, 2007; Mendes and Moeras, 2009). Cold test (Milošević and Malešević, 2004)

*Corresponding author. E-mail: mladen.tatic@ifvcns.ns.ac.rs. Tel: +381 21 4898116.

provides data on seed viability even in very adverse germination conditions, which gives better insight into seed behaviour during field emergence. Hiltner test imposes a physical stress on the seed, predicting seed emergence capacity under conditions of soil crust formation. Accelerated aging of seed is an excellent method to determine the vigour changes during seed storage (Tian et al., 2008) and shows that the aging seed is characterized by the loss of germination, reduced speed of germination and poor seedling development (Lekić, 2003; Tatić et al., 2009). This would help not only to identify reasons for improving seed storage life but also to provide information that would enable incurporation of trait for better storability in the genetic background of the high yielding varieties (Kapoor et al., 2011). Fabrizius et al. (1999) emphasized the ability to predict the actual germination of soybean seeds during natural aging, by applying the artificial aging test, and one of the important factors are duration of natural aging and degree of damage to the seed.

The aim of this investigation was to determine the degree and acceleration of seed deterioration by submitting soybean seed to natural aging under adverse storage conditions, which was significant from the aspect of seed quality preservation.

MATERIALS AND METHODS

Plant material and experimental design

The seed of six soybean genotypes from different maturity groups (MG): Afrodita and Lasta (MG 0), Balkan and Novosadjanka (MG I), Vojvodjanka and Morava (MG II) were subjected to natural and accelerated aging. Natural aging implies that the seed was stored under conventional storage conditions (uncontrolled conditions) and under controlled storage conditions at 4°C and relative humidity of 80 to 85%. Tests were carried out after 6 and 12 months of storage in four replications. Accelerated aging implies that seed was placed in metal dishes, on metal sieve and into water bath at 42°C, and relative humidity of 100%. The exposure lasted for three or five days in four replications and the seeds were then tested for standard laboratory germinability (Hampton and TeKrony, 1995).

Standard laboratory test

Four replicates x 100 seed of each genotype were tested. Moistened sterilized sand was used as germination medium for soybean. Number of normal seedlings was estimated after 9 days at 25°C and relative humidity of 95%.

Hiltner test

Four replicates x 50 seeds were placed onto moistened sand, and a 3 cm layer of cracked brick (previously sterilized and moistened) was placed upon them. Incubation period under optimal condition lasted for 10 days.

Cold test

Four replicates x 50 seeds were placed onto moistened soil (up to 40% of field capacity) at 5 to 8°C for seven days, and afterwards placed in a germination chamber at 25°C for four days.

Germination (number of normal seedlings) was evaluated for all vigour tests (ISTA, 2004) and length of seedling stem was estimated in 10 normal seedlings. The results of the mentioned treatments were compared with fresh seed germination (measured at the beginning of the experiment and used as the control treatment).

Lipid peroxidation (LP) products

Extraction of malondialdehyde (MDA) was done by using solution of thiobarbituric acid (TBA), trichloroacetic acid (CCl_3COOH) and perchloric acid ($HclO_4$), and concentration was determined spectrophotometrically at 532 nm (Matkovics et al., 1989). Soybean seed (0.5 g) were homogenized in mortar with 4.5 ml extraction solution and incubated in water bath at 90°C for 20 min. After incubation, solutions were cooled to stop the reaction and centrifuged for 10 min at 5500 r/min. MDA concentration (intensity of lipid peroxidation) was expressed as nmol of MDA g^{-1} of fresh mass.

Total oil content was determined using spectroscopic method on NMR–analyzer. The content of oleic (18:1) and linoleic (18:2) fatty acids in seed was determined by esterification using tri-methyl sulfonium hydroxide (TMSH) according to Bute (1983).

Statistical analysis

Intensity of LP, fatty acids and oils represent average of at least three replications of each variety, storage condition and storage time. Pearson coefficient of correlation and linear regression were used to estimate relationships within seed chemical composition during aging process.

RESULTS AND DISCUSION

In order to better recognize the changes in total oil content and tested fatty acids during soybean seed storage, the difference was presented in relation to the initial stage, that is, prior to seed aging treatment (Figure 1). The most pronounced decrease was observed in linoleic acid, particularly after longer conventional storage (7.5%). Oilseed containing large amounts of poly-unsaturated fatty acids would be expected to age most rapidly. The major parameter of the lipid peroxidation model is time. Which is in accordance with the results obtained in different crops by Balešević-Tubić et al. (2004, 2007a) that confirmed that content of linoleic acid decreased from 23 to 6% in sunflower seed after one year of storage under uncontrolled conditions, as well as in maize seed that was in storage for seven years. After a certain storage period, a reduction in the content of linoleic and linolenic acid was observed (Morello et al., 2004) which may lead to conclusion that a combination of high temperature and long storage time can increase the oxidation of oil even in seed (Ghasemnezhad and Honermeier, 2007). In their study, Šimić et al. (2007) indicates that effect of storage longevity on seed oil content is more or less negative and considerably affected by storage conditions.

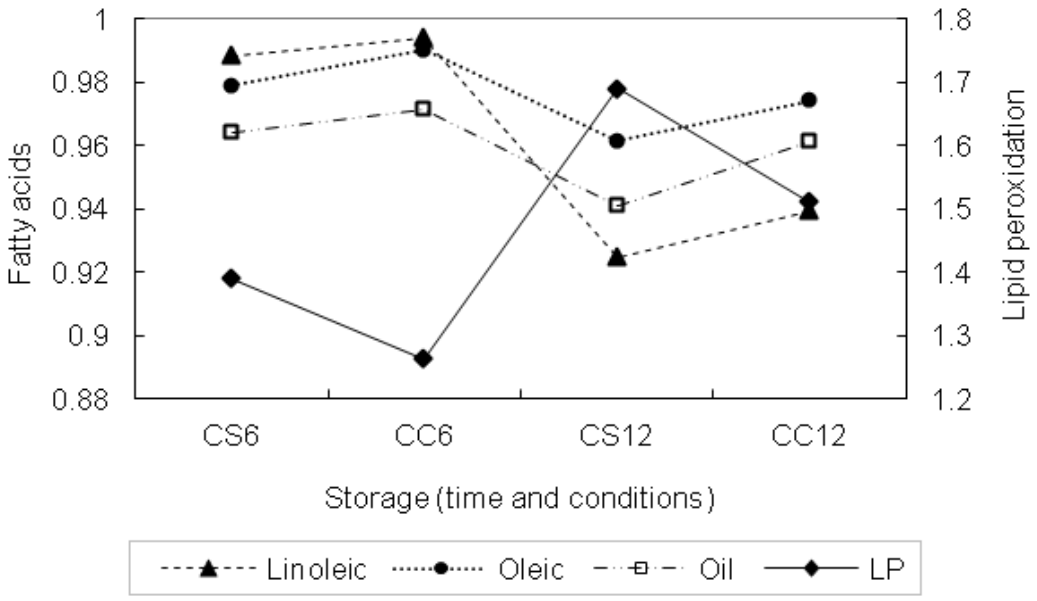

Figure 1. Changes in total oil and fatty acids (oleic and linoleic) content and intensity of lipid peroxidation (LP) depending on the type and duration of soybean seed storage (CC-controlled condition and CS-conventional storage after 6 and 12 month). The values are shown as a percentage/proportion as compared to the initial state-control.

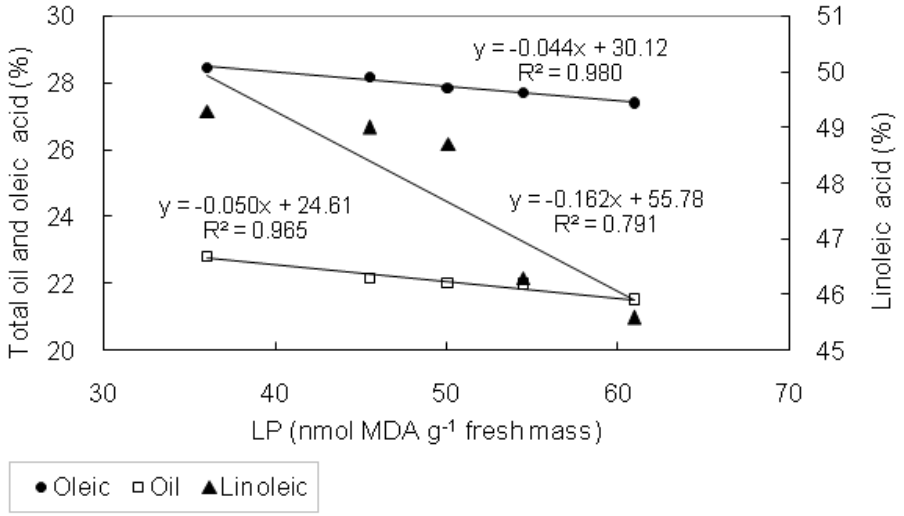

Figure 2. Linear regression analysis for total oil and fatty acids, and lipid peroxidation (LP) during soybean seed storage.

Storage conditions and duration are important factors affecting the degree of biochemical changes in seed. An increase in MDA content in relation to control, that is, the initial state of seed was observed during seed storage, which revealed that LP intensity increased. Obtained results confirmed the possibility of determining the level of LP in seed by determination of MDA content. The greatest intensity of LP with the difference of 70% in relation to the initial state was observed in soybean seed under conventional storage conditions after 12 months. Content of MDA in seed was increased by prolonged storage indicating that lipid peroxidation was more intensive in aged seed (Balešević-Tubić et al., 2005).

A high correlation was found between the changes of total oil, oleic and linoleic acids content and intensity of LP during soybean seed storage (Figure 2). It may be

Table 1. Prediction of LP intensity in soybean seed based on total oil and fatty acids content using accelerated aging test (AA).

Fatty acids	Correlation							
	Three-day AA				Five-day AA			
	CS6	CC6	CS12	CC12	CS6	CC6	CS12	CC12
Total oil	0.55*	0.61**	0.47*	0.56*	0.56*	0.64**	0.37	0.55*
Oleic (18:1)	0.13	0.60**	-0.01	0.55*	0.35	0.81**	0.26	0.76**
Linoleic (18:2)	-0.11	-0.14	0.37	-0.08	-0.15	-0.18	0.33	-0.13

Significance at level *p<0.05 and **p<0.01; CS, conventional storage (6 and 12 month); CC, controlled storage (6 and 12 month).

Table 2. Correlation of LP intensity and vigour of soybean seed during natural aging.

Applied vigour tests	Type of storage			
	CS6	CC6	CS12	CC12
Standard laboratory	-0.660**	-0.648**	-0.883**	-0.853**
Cold	-0.713**	-0.226	-0.878**	-0.666**
Hiltner	-0.698**	-0.566*	-0.879**	-0.759**

Significance at level *p<0.05 and **p<0.01; CS, conventional storage (6 and 12 month); CC, controlled storage (6 and 12 month).

noted that decline in linoleic acid content was the most pronounced with increased LP intensity, as compared to oleic acid and total oil content. Intensified activities of enzymes that participate in lipid metabolism, caused especially by increased moisture content in seed and higher storage temperature, increased the usage of lipids in the respiration process, leading to significant reduction of oil content in sunflower seed (Beratlife and Iliescu, 1997). Many other authors emphasized the connection between LP intensity, that is, peroxidative degradation of fatty acid and changes of their content in seeds during storage, pointing out the length and conditions of storage, as one of the most important factors that determine the degree of seed damage (McDonald, 1999; Verma et al., 2003).

Extreme aging conditions such as accelerated aging make processes of LP in seed more intensive than natural aging. By application of accelerated aging test, it is possible to predict the degree of damages in aged seed, which is evident from the obtained results (Table 1). The best indicator is the change in oleic acid content obtained by application of a five-day accelerated aging test, which can reveal the intensity of LP in seed during natural aging under controlled storage conditions. The high correlation coefficient was noted for a period of six months (r = 0.81), as well as the period of 12 months (r = 0.76).

Prediction of possible negative consequences during seed storage is very important, especially for seed with higher oil content, in which lipid auto-oxidation during aging leads to changes in fatty acid content, and degradation processes that eventually lead to complete loss of seed viability. Results obtained by application of accelerated aging test represent a good starting point in deciding on the duration of soybean seed storage, with no significant influence on seed quality. Many authors have observed changes in biochemical parameters of artificially aged seed, as well as certain relations to natural aging (Walters et al., 2001; Bailly et al., 2002; Žilić et al., 2006).

Seed vigour is used as a measure of accumulated damage in seed as viability declines. Considering intensity of LP in soybean seed during storage and seed germination obtained by applied vigour tests, a high correlation was observed after 12 months (Table 2). The above mentioned dependence revealed that with increased LP intensity, a significant decline in germination, that is, soybean seed vigour occurred. Increased oil content in stored soybean seed can easily cause damage to the seed and loss of seed vigour. Three months after storage, a strong reduction in seed germination was observed in high oleic sunflower cultivars, especially when stored in high temperature conditions (Ghasemnezhad and Honermeier, 2007). The results of Mohammadi et al. (2011) also indicated that seed deterioration results in decreased percentage and rate of germination and decreased percentage of normal seedlings. Seed aging is generally marked by reduction in vigour (Gupta and Aneya, 2004; Tatić et al., 2009).

High vigour seeds are expected to tolerate high temperature and humidity, while retaining their capability to produce normal seedlings. Applied vigour tests

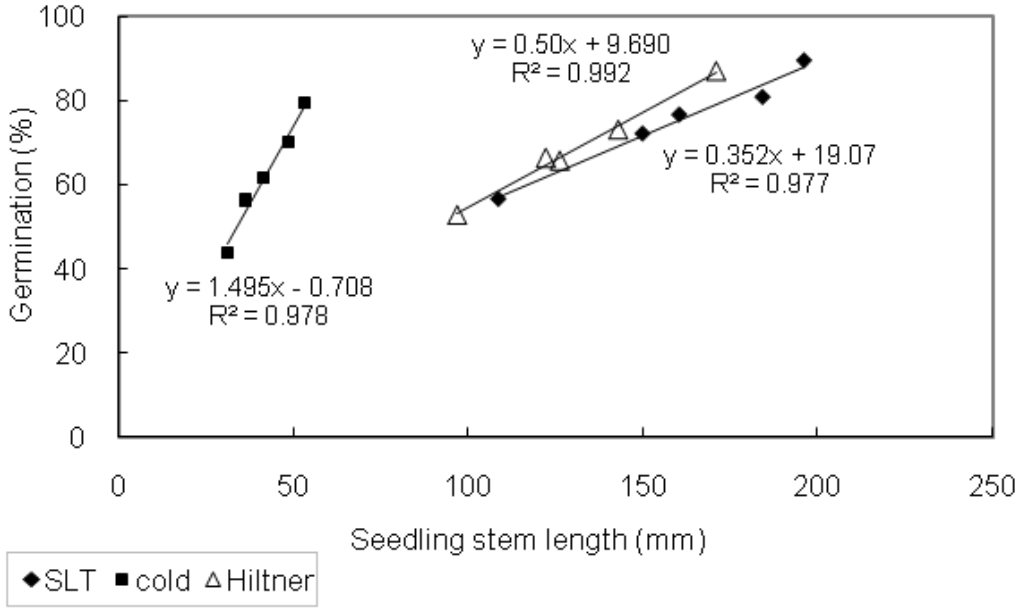

Figure 3. Dependence of soybean seedling stem length and germination during storage with applied vigour tests (standard laboratory test-SLT, cold and Hiltner tests).

revealed that soybean seed was damaged during storage, which adversely affected the seedling growth under unfavourable germination conditions. High regression coefficient (ranging from R^2 = 0.977 to R^2 = 0.992) in all three applied vigour tests, revealed high dependence of germination and seedling stem growth (Figure 3). When germination was lower, the seedling stem growth was weaker, and in aged soybean seed, the most pronounced decline in viability was observed when cold test was applied. Balešević-Tubić et al. (2007b) claimed that during natural aging of sunflower seed, a significant decline in seedling growth was observed, especially when cold test was applied. The smallest increase in seedling growth was observed in seed stored under uncontrolled conditions for 12 months. When standard laboratory test was applied, even damaged seed was capable of forming normal seedlings due to optimal moisture and temperature prevailing during testing. Cold test was more reliable in assessing the viability of aged seed and reaction of seed under field conditions, which was in accordance with the results obtained by other authors studying the effects of seed aging on vigour (Carvalho and Nakagawa, 2000; Mendes and Moraes, 2009; Vieira et al., 2010).

Initial plant growth is very significant for its further development and survival under unfavourable environmental conditions. More rapid formation of assimilation surface and root system early in plant life, gives certain advantage to the plants in the subsequent stages of growth and development. Due to the above mentioned reasons, the seedling growth is considered a very important indicator of seed vigour.

Model for estimation of seed germination during storage can be based on accelerated aging test, and can be helpful in making a decision on duration of soybean seed storage. In this study, the three-day accelerated aging test proved as one of the most reliable for the prediction of possible seed changes under controlled conditions after 6 and 12 months of storage. Using changes occurring during seedling growth, it is possible to predict changes in soybean seed germination under controlled storage conditions by application of the three-day accelerated aging test. The cold test proved to be the most reliable due to the obtained highest regression coefficient R^2 = 0.578 after six months of storage (Figure 4), while standard laboratory test (R^2 = 0.440) could be used with somewhat lower reliability. However, with the prolonged period of storage, that is, after 12 months (Figure 5), the standard laboratory test became less precise, and cold and Hiltner tests were more reliable. This case also proved that under less favourable conditions and prolonged storage period, the standard laboratory tests were not an accurate indicator of vigour decline, and that more vigour tests must be used in order to get a clearer picture of damages to the seed caused by aging.

The decreases in both root and shoot lengths and seed germination by accelerated aging may be a result of progressive loss of seed viability and can be used as an excellent method for determination of vigour changes during storage (Mosavi et al., 2011). Based on their results, other authors (Filho et al., 2001; Balešević-Tubić et al., 2009; Kapoor et al., 2011) also concluded that accelerated aging test could be used to predict the

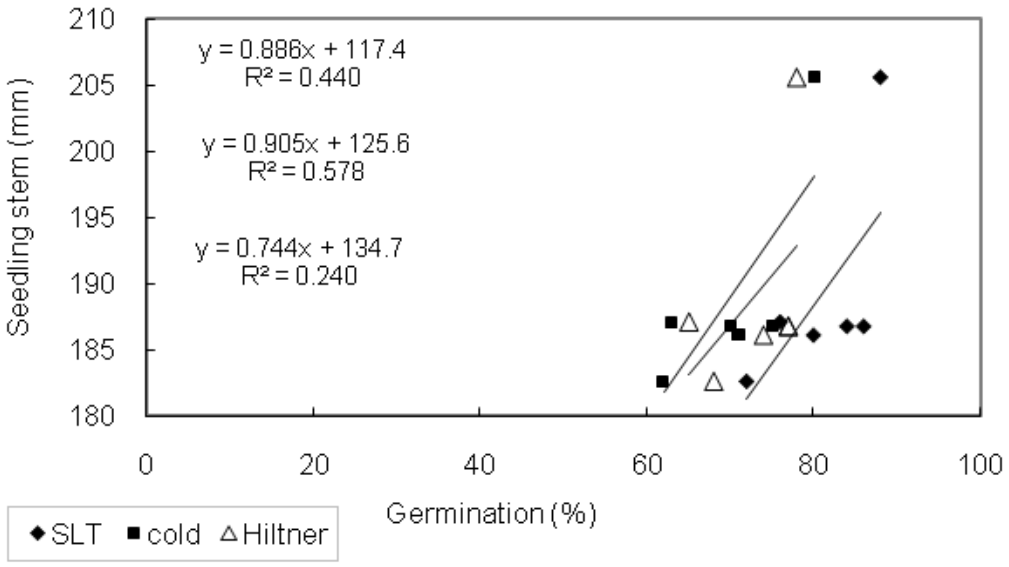

Figure 4. Dependence of soybean seedling stem length after application of the three-day accelerated aging test, and seed germination (standard laboratory test-SLT, cold and Hiltner tests) after 6 months of storage under controlled conditions (the order of equations for the regression line is equal to the order shown in the chart legend).

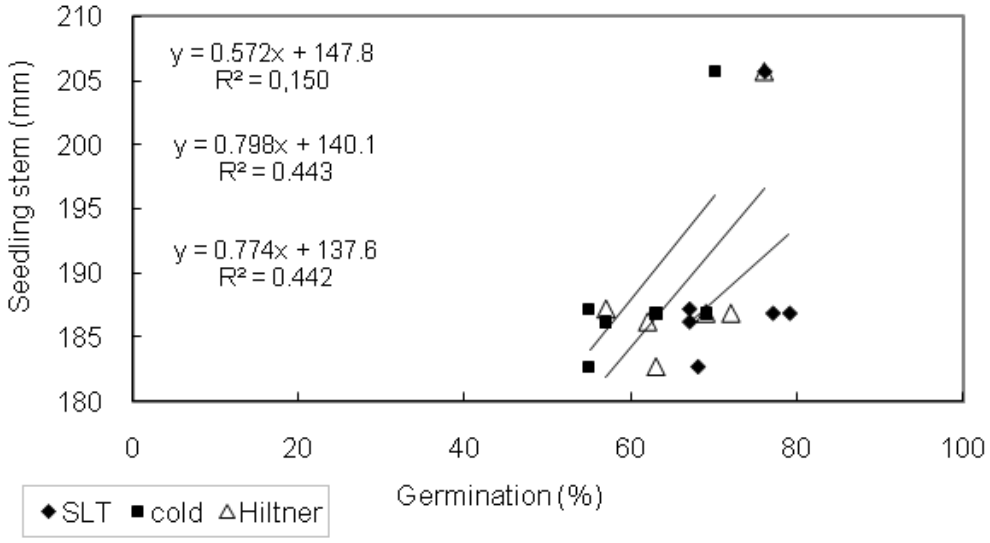

Figure 5. Dependence of soybean seedling stem length after application of the three-day accelerated aging test, and seed germination (standard laboratory test-SLT, cold and Hiltner tests) after 12 months of storage under controlled conditions (the order of equations for the regression lines is equal to the order shown in the chart legend).

degree of seed damage during natural aging and loss of seed vigour under laboratory conditions.

The results of the present study indicate that seed storage conditions (temperature and relative humidity) are highly important factors that affect seed aging and seed viability along with the duration of storage. Content of MDA in soybean seed was increased by prolonged storage, which shows that lipid peroxidation was more intensive in aged seed. Some changes also occurred in fatty acids content, especially in linoleic acid. Natural aging led to decreased seed germination, and more intensive decrease was observed under conventional

storage conditions than under controlled conditions. Cold test was the most reliable indicator of changes occurring in aged soybean seed. Effects of accelerated aging tests on changes in soybean seeds have proved that degree of seed damage during natural aging and the ability of seed to be stored can be predicted in the laboratory.

REFERENCES

Baiyeri KP, Mbah BN (2006). Surface sterilization and duration of seed storage influenced emergence and seedling quality of African breadfruit (*Treculia africana* Decne). Afr. J. Biotechnol. 5(15): 1393-1396.

Bailly C, Bogatek-Leszczynska R, Come D, Corbineau F (2002). Changes in activities of antioxidant enzymes and lypoxigenase during growth of sunflower seedlings from seeds of different vigour. Seed Sci. Res. 12: 47-55

Balešević-Tubić S (2001). The influence og aging process on seed viability and biochemical changes in sunflower seed. PhD Thesis – University of Novi Sad, Novi Sad.

Balešević-Tubić S, Milošević M, Vujaković M, Zlokolica M, Nikolić Z (2000). Seed ageing. Field Veg. Crop Res. 33: 207-212.

Balešević-Tubić S, Tatić M, Miladinović J, Malenčić Đ. (2004). Lipid peroxidation and activity of superoxide dismutase associated with natural aging of oil maize seed. Abstracts. 27th ISTA Congress Seed Symposium. Budapest, Hungary.

Balešević-Tubić S, Malenčić Đ, Tatić M, Miladinović J (2005). Influence of aging process on biochemical changes in sunflower seed. Helia, 28(42): 107-114.

Balešević-Tubić S, Tatić M, Miladinović J, Pucarević M (2007a). Changes of fatty acids content and vigour of sunflower seed during natural aging. Helia, 30: 61-67.

Balešević-Tubić S, Tatić M, Hrustić M, Miladinović J, Maksimović L (2007b). The influence of aging process on germination and seedling growth of sunflower seed. Proceedings of the First Joint PSU-UNS International Conference on BioSci: Food, Agri. the Environment, Thailand, pp. 198-202.

Balešević-Tubić S, Nikolić Z, Vujaković M (2009). Soybean Seed – the starting point of all food and feed. Proceedings The Second Joint PSU–UNS International Conference on BioSci. Food, Agric. Environment, Novi Sad, Serbia, pp. 23-28.

Beratlife C, Iliescu H (1997). Highlights of proper sunflower seed storage. Helia, 20(26): 121-137.

Bute J (1983). Raped derivatizacion of acids for GC by pre-column transesterification of glycerides. J. Chromatography, 261: 142-145.

Carvalho NM, Nakagawa J (2000). Seeds: science, technology and production. FUNEP, Jaboticabal, SP, Brazil.

Fabrizius E, TeKrony D, Egli DB, Rucker M (1999). Evaluation of a viability model for predicting soybean seed germination during warehouse storage. Crop Sci. 39: 194-201.

Filho JM, November ADC, Chamma HMCP (2001). Accelerated ageing and controlled deterioration seed vigour tests for soybean. Sci. Afr. 58: 421-426.

Ghasemnezhad A, Honermeier B (2007). Influence of storage conditions on quality and viability of high and low oleic sunflower seeds. Int. J. Plant Prod. 3(4): 41-50.

Ghasemnezhad A, Cergel S, Honermeier B (2007). The impact of storage time and storage temperature on the quality of the oil of evening primrose (*Oenothera biennis* L.). J. Med. Spice Plants, 12: 175-180.

Gupta A, Aneja KR (2004). Seed deterioration in soybean varieties during storage-physiological attributes. Seed Res. 32: 26-32.

Hampton JG, TeKrony DM (1995). Handbook of Vigour Test Methods. 3rd Edition, Published by The International Seed Testing Association, Zurich, Switzerland.

ISTA (2004). International Rules for Seed Testing. International Seed Testing Association, Switzerland.

Kapoor N, Arya A, Siddiqui AM, Kumar H, Amir A (2011). Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). Am. J. Plant Physiol. 6(1): 28-35.

Lekić S (2003). Vigour of seed. Association of breeders and seed researchers of Serbia, Belgrade.

Matkovics B, Gašić O, Varga SzI, Štajner D, Kraljević-Balalić M (1989). The antioxidant enzyme activities in wheat seeds and their F_1 hybrids. Cereal Res. Commun. 17: 113-119.

McDonald MB (1999). Seed deterioration: physiology, repair and assessment. Seed Sci. Technol. 27: 177-237.

Mendes CR, De Moraes DM (2009). Respiratory activity for the differentiation of vigor on soybean seed lots. Revista Brasileira de Sementes, 31(2): 171-176.

Milošević M, Rajnpreht J, Graovac M, Zlokolica M (1995). Effect of aging on the basis of indicators of seed viability of major field crops. Agronomic Knowledge, 3: 2-5.

Milošević M, Malešević M (2004). Seed production. Institute of Field and Vegetable Crops, Novi Sad., pp. 110-114.

Mohammadi H, Soltani A, Sadeghipour HR, Zeinali E (2011). Effect of seed aging on subsequent seed reserve utilization and seedling growth in soybean. Internat. J. Plant Prod. 5(1): 65-70.

Morello JR, Motilva MJ, Tovar MJ, Romero MP (2004). Changes in commercial virgin olive oil (CV Arbequina) during storage with special emphasis on the phenolic fraction. J. Food Chem. 85: 357-364.

Mosavi NSM, Gholami H, Kord GH, Sadeghi M, Sedighi E (2011). Free fatty acid and electrical conductivity changes in cotton seed (*Gossypium hirsutum*). Int. J. Agric. Sci. 1(2): 62-66.

Priestley DA (1986). Seed aging implications for seed storage and persistence in the soil. Comstock Publishing Associates, A division of Cornell University Press Ithaca and London, pp. 137-165.

Šimić B, Sudarić A, Liović I, Rozman V, Ćosić J (2007). Influence of storage condition on seed quality of maize, soybean and sunflover. Agriculturae Conspectus Scientificus, 72(3): 211-213.

Tatić M (2007). Effect of endogenous and exogenous factors on aging process and soybean seed viability. PhD Thesis-University of Novi Sad, Novi Sad.

Tatić M, Balešević-Tubić S, Vujaković M, Nikolić Z (2009). Changes of germination during natural and ascelerated aging of soybean seed. Proceedings the Second PSU-UNS International Conference on BioSci. Food, Agric. Environ. Serbia, pp. 256-259.

Tian X, Song S, Lei Y (2008). Cell Death and Reactive Oxigen Species Metabolism during Accelerated Ageing of Soybean Axes. Russ. J. Plant Physiol. 55(1): 33-40.

Verma SS, Verma U, Tomor RPS (2003). Studies on seed quality parameters in deteriorating seeds in Brassica (*Brassica campestris*). Seed Sci. Technol. 31: 389-396.

Vieira BGTL, Vieria RD, Krzyzanowski FC, Neto JBF (2010). Alternative procedure for the cold test for soybean seeds. Sci. Agric. (Piracicaba, Braz.) 67(5): 540-545.

Žilić S, Milivojević M, Šobajić S, Maksimović M (2006): Effect of multiple alleles on oxidative stability and germination of soybean seeds subsequent to the accelerated ageing test. Genetika, 38(1): 37-48.

Walters C, Pammenter NW, Berjak P, Crane J (2001). Desiccation damage, accelerated ageing and ageing and respiration in desiccation tolerant and sensitive seeds. Seed Sci. Res. 11: 135-148.