AIMS Agriculture and Food, 7(2): 387–402.
DOI: 10.3934/agrfood.2022025
Received: 12 January 2022
Revised: 08 May 2022
Accepted: 01 June 2022
Published: 10 June 2022

http://www.aimspress.com/journal/agriculture

Research article

Effect of storage conditions on seed quality of soybean (*Glycine max* L.) germplasm

Avraam Koskosidis[1,*], Ebrahim M. Khah[1], Ourania I. Pavli[1] and Dimitrios N. Vlachostergios[2]

[1] Laboratory of Plant Breeding, Department of Agriculture Crop Production and Rural Environment, School of Agricultural Science, University of Thessaly, Fytokou Str., 38446 Volos, Greece
[2] Hellenic Agricultural Organization Demeter, Institute of Industrial and Forage Crops, 41335 Larissa, Greece

*** Correspondence:** Email: makisk47@gmail.com; Tel: +306987919575.

Abstract: Soybean is one of the most important oil seed crops. However, soybean seed is structurally weak and inherently short-lived, making the crop vulnerable to long period storage. Thus, it is crucial to study the effect of storage conditions on the quality of soybean seeds (*Glycine max* L.). The genetic material consisted of 10 soybean varieties, whose seeds were stored under both cooling (refrigerator) and room temperature conditions and were subsequently subjected to germination test, electric conductivity test and estimation of free fatty acids percentage. In order to determinethe long-term effects of storage on seed quality, all genotypes were subjected to accelerated ageing at 40°C up to 48 days and viability equation was used to determine the Ki value. Overall findings revealed the significant effect of storage conditions on seed quality and, at the same time, underlined the beneficial effect of storage under cooling conditions, which is expressed as higher germination percentage, reduced electric conductivity and free fatty acids percentage and improved storage longevity. Further, our data provide conclusive evidence for the qualitative superiority of the varieties Adonai, Celina and Neoplanta, especially upon storage under cooling conditions, as they were characterized by higher germination percentage and improved tolerance to storage (storage potential).

Keywords: soybean storage; longevity; germination; electric conductivity; free fatty acids

1. Introduction

Soybean is the world's leading legume [1] among oil seed crops and its cultivation is widespread in the tropical, sub-tropical and temperate region [2,3]. Soybean seeds are structurally weak, inherently short-lived and easily subjected to damage [4]. Seed storage life is influenced by the genotype as well as the storage conditions, mainly referring to temperature and duration [5]. Seed deterioration during storage is largely attributed to lipid peroxidation [6], subsequently affecting seed viability and storage longevity. Among factors influencing seed deterioration, temperature and seed moisture content are most important in terms of loss of viability during storage, ultimately affecting seed vigour and germination potential [7]. Most importantly, deterioration is an irreversible catabolic process that, once occurred, cannot be reversed. However, the seed potential storage life as well as the deterioration rate are greatly subjected to both species and genotype dependency. Soybean is generally viewed as a species of weak seed structure, short-lived and prone to mechanical damage, thus their quality being greatly depended on maturity stage which is related to their longevity during storage [8].

Overall seed longevity during storage is influenced by four major factors i) genotype, ii) seed quality at storage time, iii) seed moisture content and iv) temperature during storage [9]. Several studies related to the physiological quality of soybean seeds during storage for a period of six months under different temperature conditions point to the conclusion that air-conditioned environments positively affect the conservation of seed quality [10,11]. To determine the long-standing storage effects on seed quality, three individual tests were employed: i) seed germination test, ii) electric conductivity test and iii) measurement of the free fatty acids percentage. Such tests collectively provide an excellent means of assessing seed vigour during storage [12], especially in species whose seed longevity is subjected to strong genotypic dependency [13].

Germination is defined as the appearance of the first visible signs of growth or emergence of embryonic root, termed radicle. Germination is affected by several factors, including environmental conditions, mainly temperature and relative humidity [14]. The germination percentage has been widely employed as an indicator of deterioration in various grains during storage. According to Abba and Lovato [15], seed germination is adversely affected by high storage temperature, with the germinated fraction proportionally decreasing as temperature increases. Relative are the findings that popcorn seeds showed a trend of a reducing seedling emergence as exposure period to accelerated ageing increases [16], thus further reinforcing previous conclusions that the prolonged exposition period to accelerated ageing proportionally enhances deterioration rate [17].

Deterioration of grain refers to any degenerative changes occurring after the grain has reached its maximum quality, involving genetic damage, lipid peroxidation, loss of membrane integrity and cellular compartmentalization, selective reduction of capacity and solute leaching [18]. The sequel of cellular, chemical and metabolic alterations is primarily initiated by loss in cellular membrane integrity, thus the deterioration process of grains and seeds is usually associated with poorly structured membranes and damaged cells. Given that the value most affected by genetic factors is the seed's electric conductivity [19], referring to the amount of ions leached into the soaking solution of seeds, it has been proposed that its evaluation provides an accurate means of estimating germination [20]. As such, an increased value of electric conductivity is associated with a proportional decreased seed vigour as a result of loss of cell membrane integrity [17].

Given that free fatty acid content is a factor directly linked to seed vigor and viability, its estimation provides a significant qualitative indicator of seed deterioration during storage [21]. The free fatty acids percentage increases as the storage temperature is rising [22] and vegetable oils are often characterized by high free fatty acid content due to mechanical injury and/or sub-optimum storage conditions of grains or seeds [23]. High free fatty acid content has been associated with excessive losses in refining, while it has been further suggested that its increase contributes to seed deterioration via cell membrane disruption and/or peroxidation-mediated toxicity [24].

Seed ageing is directly associated with the seed moisture content and temperature, thus their manipulation provides a means of technically inducing the seed deterioration process. Under storage conditions, seeds typically lose their viability within a few days or weeks [25]. In this line, it has been evidenced that accelerated ageing causes a remarkable decrease in germination percentage [26–29] while, at the same time, ageing seed is characterized by loss of germination, reduced germination rate and poor seedling development [30,31]. In soybean, it has been confirmed that the accelerated ageing test provides the possibility of predicting the actual seed germination rate during natural ageing [32].

There are many circumstances under which it is important to predict the environmental effects on seeds' longevity, ranging from the rapidly occurring loss of viability due to the hot air-drying of wet seeds to slowly occurring deterioration as a result of long-term storage for genetic conservation purposes. Such equations allow for an accurate prediction of the expected percentage of seed viability formed at any given period of medium-term storage and any combination of temperature and moisture content [33,34].

Given that soybean seeds are unusually sensitive to storage conditions [35], this study aimed at investigating the effect of storage conditions and duration on seed quality and longevity of ten commercial soybean varieties, using the accelerated ageing technique.

2. Materials and methods

Soybean germplasm consisted of ten soybean varieties, namely Adonai, Celina, Neoplanta, P21T45, PR91M10, PR92B63, PR92M22, PR92M35, Sphera and Zora. The genotypes were provided by the Institute of Industrial & Forage Crops, Larissa, Greece, while the experiment was conducted in the Laboratory of Plant Breeding, Department of Agriculture Crop Production and Rural Environment, University of Thessaly. A sample of 2 kg per variety was stored either at room temperature (18–22 ℃) or at cooling conditions (2–6 ℃) for a period of 12 months. In order to determine the seed storage behavior, based on the seed viability equation, seed samples from both storage conditions were imported in the accelerated ageing chamber (40 ℃) for a period of 48 days. During accelerated ageing, samples were removed at 3-days-intervals and the long-standing storage effects were determined on the basis of germination, electric conductivity, free fatty acid content and seed viability equation.

2.1. Germination test

A sample of 200 seeds per variety was initially surface-sterilized in a 10% sodium hypochlorite/dH_2O solution, while gently mixing for 5 min, and washed (4x) with sterile dH_2O. Sterilized seeds were subsequently placed in plastic trays and incubated in a germination

chamber (17 ℃). Germination scoring was performed every 2 days and, after counting, germinated seeds were removed. Scoring was continued until there were no seeds left or the remaining seeds were incapable of germination.

2.2. Electric conductivity test

A sample of 50 seeds per variety were placed into a plastic glass filled with 75 mL of dH_2O [38] and covered with plastic membrane to avoid the entrance of dust. Following 24-hours incubation under shady conditions, the electric conductivity value was determined in $\mu Scm^{-1}g^{-1}$, using a conductivity meter. The value of electric conductivity is gradually increasing as the germination percentage decreases [40].

2.3. Free fatty acids

The free fatty acid content was estimated based on the AOCS Official Method Ca 5a-40 [36]. Such measurements were performed in collaboration with Bios Agrosystems.

2.4. Viability equation (Ki)

The Ki value of each genotype was calculated using the formula created by Warren H.J. and Y.W. Wang, National Taiwan University. Based on Ellis and Roberts equation [41], seed survival curve was described as:

$$v = Ki - \left(\frac{1}{\sigma}\right)p \quad (1)$$

where v represents probit percentage viability, 1/σ represents seed survival curve, p represents storage period (days) and Ki represents probit percentage viability at the beginning of the storage.

The slope $\left(1/\sigma\right)$ of the survival curves is not affected by the genotype or the seed quality, instead it is the intercept Ki of the survival curve that is affected by such factors [37]. A higher Ki value is generally related to an enhanced absolute longevity [36].

2.5. Statistical analysis

The experimental layout was completely randomised, with four replications, each consisting of 50 seeds. All analyses were measured in four replicates and expressed as a mean. The differences between mean values were evaluated using Duncan's test at the level of significance $p < 0.05$. Hierarchical Cluster analysis was performed using Ward's method in order to explore the trends and relationships between genotypes. Principal component analysis (PCA) with varimax rotation was performed to explore relationships between traits. All statistical analyses were performed using SPSS statistical software v.20.

3. Results

Traits most affected by storage duration were germination percentage and electric conductivity (Table 1). The storage conditions prior to the accelerated ageing, referring to either room temperature or cooling conditions, was the second most influencing factor in terms of effect on both germination percentage and electric conductivity. On the other hand, the percentage of free fatty acids was mostly affected by the genotype and the interaction between storage conditions and genotype.

Table 1. Total variation for each trait, as explained by each factor.

Factor	Variation (%)		
	Germination	EC	FFA
Storage conditions	10.22	26.81	2.10
Aging days	77.18	42.55	4.64
Storage conditions x Aging days	4.95	4.54	1.37
Genotype	2.21	9.58	65.61
Storage conditions x Genotype	1.40	4.48	16.13
Aging days x Genotype	2.08	5.35	5.37
Storage conditions x Aging days x Genotype	1.93	6.65	4.74

3.1. Germination test

Storage duration in the accelerated aging chamber, as expected, drastically affected germination percentage of seeds that were previously stored in both storage conditions. Seeds that were previously stored under room temperature conditions, showed no significant difference at 6, 9 and 12 aging days (Table 2). Accordingly, no significant differences were noted in seeds that were treated for 18 and 21 aging days, 21 and 24 aging days, 36 and 42 aging days. However, all aging treatments differed significantly from the control (0 aging days). Among varieties, Adonai was least affected compared to all other varieties, as evidenced both by the highest final germination percentage (48 aging days: 10,5 %) and the highest mean germination percentage (57.1%). Seeds that were previously stored under cooling conditions, showed no significant difference at 0 (control), 6 and 9 aging days as well as at 21 and 24 aging days. At the variety level, Celina, Neoplanta and Adonai presented the highest final germination percentage (48 aging days), while Adonai showed also the highest mean germination percentage (76.7%), followed by Celina (74.7%) and Neoplanta (73%). In relation to the previous storage conditions, it was evidenced that a significant decrease in germination percentage was noted at 6 days and 12 days of aging for the seeds stored under room temperature and cooling conditions, respectively. Further, it was shown that the previous storage conditions affected differently the germination potential of all varieties under study. As such, the cooling storage conditions yielded a significantly higher germination percentage in seeds that were aged from 0 up to 24 aging days, and thereafter a drastic decrease in germination percentage was observed. To the contrary, seeds that were stored at room temperature showed a gradual decreasing trend as the aging treatment increased.

Table 2. Germination percentage (%) of seeds that were either stored under room temperature or cooling conditions at different storage duration (aging days).

Genotype	Room temperature											
	Aging Days											
	0	6	9	12	15	18	21	24	36	42	48	Mean (G)
Adonai	81.5 ab	78.0 a	82.0 a	79.0 a	75.5 a	67.5 a	55.5 a	52.5 a	25.5 ab	20.5 a	10.5 a	57.1 A
Celina	64.0 c	62.5 bc	62.0 bc	62.5 abc	58.0 bcd	57.5 ab	56.5 a	52.0 a	23.5 ab	21.5 a	8.5 ab	48.0 C
Neoplanta	74.0 bc	63.5 bc	61.0 bc	60.0 bc	50.0 bcd	44.0 cd	41.0 b	40.5abc	19.0 cd	17.0 a	6.5 b	43.3 D
P21T45	73.5 bc	74.0 ab	69.0 bc	69.0 abc	66.0 ab	50.0 bcd	48.5 a	47.5 ab	23.5 ab	16.5 a	5.0 b	49.3 BC
PR91M10	81.0 ab	56.0 c	56.0 c	55.0 bc	42.0 d	38.5 d	38.0 b	27.5 c	17.0 d	16.0 a	5.0 b	39.3 E
PR92B63	91.5 a	71.0 ab	68.5 bc	68.0 abc	59.0 bc	55.0 abc	55.0 a	53.5 a	26.5 a	20.0 a	5.0 b	52.1 B
PR922M22	75.0 bc	65.5 abc	64.5 bc	54.5 c	52.0 bcd	40.5 d	38.5 b	38.0 bc	18.5 cd	18.0 a	5.5 b	42.8 DE
PR92M35	72.0 bc	73.5 ab	72.5 ab	72.5 ab	63.0 abc	49.5 bcd	49.5 a	45.0 ab	22.0 bc	20.0 a	5.0 b	49.5 BC
Sphera	67.5 bc	68.0 abc	67.5 bc	59.0 bc	46.5 cd	41.0 d	39.0 b	38.5 bc	19.0 cd	16.0 a	5.0 b	42.5 DE
Zora	70.0 bc	66.5 abc	66.0 bc	70.0 abc	52.0 bc	43.5 cd	38.5 b	37.0 bc	17.5 d	16.5 a	4.5 b	43.8 D
Mean (AD)	75.0 A	67.8 B	66.9 D	65.0 D	56.4 C	48.7 D	46.0 D	43.2 E	21.2 F	18.2 F	6.1 G	
Genotype	Cooling conditions											
	Aging Days											
	0	6	9	12	15	18	21	24	36	42	48	Mean (G)
Adonai	100.0 a	98.5 a	98.0 a	96.5 a	95.5 a	93.5 a	92.5 a	89.0 a	37.0 a	33.0 a	10.5 a	76.7 A
Celina	99.0 a	98.5 a	96.0 a	96.0 ab	91.5 a	91.5 a	88.0 ab	86.5 a	37.0 a	26.5 a	11.0 a	74.7 AB
Neoplanta	99.0 a	96.0 a	95.0 a	93.0 ab	91.5 a	91.5 a	83.5 ab	82.0 a	33.0 a	28.0 a	11.0 a	73.0 BC
P21T45	96.5 a	97.0 a	97.0 a	96.0 ab	90.0 a	61.5 c	60.5 c	60.5 c	20.0 c	16.0 b	6.0 b	63.7 F
PR91M10	100.0 a	98.0 a	98.0 a	96.0 ab	84.5 ab	54.5 d	46.0 d	45.0 d	6.5 d	3.5 d	1.0 cd	57.5 G
PR92B63	100.0 a	98.0 a	97.0 a	95.5 ab	76.5 b	50.0 d	49.5 d	41.5 d	0.0 e	0.0 d	0.0 d	55.3 H
PR922M22	100.0 a	99.0 a	97.5 a	95.0 ab	93.5 a	92.0 a	85.0 ab	84.0 a	27.5 b	13.0 bc	3.0 bcd	71.8 C
PR92M35	100.0 a	98.5 a	94.0 a	77.0 c	88.5 ab	88.0 a	86.5 ab	86.0 a	7.5 d	0.0 d	0.0 d	66.0 E
Sphera	78.5 b	98.0 a	93.0 a	89.0 b	88.5 ab	81.0 b	77.5 b	76.5 a	9.0 d	7.0 cd	4.5 bc	63.9 F
Zora	99.0 a	97.0 a	96.5 a	96.5 a	91.5 a	80.0 b	79.5 b	78.5 a	15.5 c	15.0 b	4.0 bc	68.5 D
Mean (AD)	97.2 A	97.9 A	96.2 A	93.1 B	89.2 C	78.3 D	74.9 E	73.0 E	19.3 F	14.2 G	5.1 H	

Note: Different small letters indicate significant differences between the varieties during each aging day according to LSD ($p \leq 0.05$). Different capital letters indicate significant differences between the means of each aging day and each variety according to LSD ($p \leq 0.05$).

The mean germination percentage of seeds previously stored under room temperature and cooling conditions, within each variety tested, are presented in Table 3. Significant differences were noted in varieties Celina, Neoplanta and PR92M22, exhibiting a higher mean germination percentage upon storage at cooling conditions, whereas all other varieties did not differ significantly. Despite the absence of significant differences, overall data point to a general trend of increased mean germination percentage of seeds previously stored under cooling conditions as compared to the respective values of seeds stored at room temperature.

Table 3. Comparison of the mean germination percentage (%), within each genotype, between the two different storage conditions.

Genotype	Storage conditions	
	Room temperature	Cooling conditions
Adonai	57.1 a	76.7 a
Celina	48.0 b	74.7 a
Neoplanta	43.3 b	73.0 a
P21T45	49.3 a	63.7 a
PR91M10	39.3 a	57.5 a
PR92B63	52.1 a	55.3 a
PR92M22	42.8 b	71.8 a
PR92M35	49.5 a	66.0 a
Sphera	42.5 a	63.9 a
Zora	43.8 a	68.5 a
Mean	47 B	67.31 A

Note: Different small letters indicate significant differences between the means of each variety in each storage condition according to LSD ($p \leq 0.05$). Different capital letters indicate significant differences between the means of each storage condition according to LSD ($p \leq 0.05$).

3.2. Electric conductivity test

Our data are in accordance with the well-known negative association between germination percentage and electric conductivity, as reflected in the lowest electric conductivity value of the varieties Adonai and Celina after treatment of 48 aging days, which were previously characterized by the highest final germination percentage (48 aging days) in seeds that were stored at room temperature (Table 4). Such findings were further supported by the lowest mean electric conductivity value of Adonai and Celina. The negative association among the aforementioned values was also confirmed in seeds that were stored under cooling conditions. In this case, the varieties Adonai, Celina and Neoplanta, which presented the highest final germination percentages, showed the lowest electric conductivity values after treatment of 48 aging days, while the former two had also the lowest mean electric conductivity values (Table 4). Further, the findings point to a trend of increasing electric conductivity during storage. Seeds that were previously stored under cooling conditions had an electric conductivity value of at least 20 $\mu Scm^{-1}g^{-1}$ lower than those stored at room temperature. Although this difference was preserved until 24 aging days, thereafter declined to the point of neutralization. The observed decreasing difference at 36, 42 and 48 aging days is in agreement with the decreasing difference in the respective values for germination percentage.

Table 4. Values of electric conductivity in seeds that were either stored under room temperature or cooling conditions at different storage duration (aging days).

Genotype	Room temperature										
	Aging days										
	6	9	12	15	18	21	24	36	42	48	Mean (G)
Adonai	157 b	153 e	157 g	155 g	159 e	174 f	183 f	186 d	187 c	188 b	169.9 B
Celina	129 h	127 h	148 i	177 e	185 c	184 e	186 e	186 d	188 c	189 b	169.9 B
Neoplanta	141 f	155 e	165 e	180 d	195 a	193 ab	193 abc	194 ab	194 a	194 a	180.4 AB
P21T45	145 e	153 e	162 f	182 cd	192 b	192 abc	195 a	194 ab	194 a	194 a	180.3 AB
PR91M10	159 b	193 a	194 a	192 a	190 b	192 abc	194 ab	194 ab	193 ab	194 a	189.5 A
PR92B63	180 a	189 b	189 b	191 a	192 b	194 a	191 cd	193 abc	193 ab	193 a	190.5 A
PR922M22	153 c	183 c	186 c	192 a	191 b	192 abc	192 bcd	195 a	194 a	195 a	187.3 AB
PR92M35	119 i	149 f	165 e	168 f	182 d	189 d	190 d	191 c	191 b	193 a	173.7 AB
Sphera	149 d	162 d	174 d	183 c	185 c	190 cd	190 d	192 bc	193 ab	195 a	181.3 AB
Zora	135 g	141 g	153 h	188 b	190 b	191 bcd	191 cd	193 abc	192 ab	195 a	176.9 AB
Mean (AD)	146.7 D	160.5 C	169.3 C	180.8 B	186.1 AB	189.1 AB	190.5 AB	191.8 AB	191.9 AB	193 AB	
Genotype	Cooling conditions										
	Aging days										
	6	9	12	15	18	21	24	36	42	48	Mean (G)
Adonai	92 f	109 f	101 g	104 g	144 e	151 e	153 e	148 e	174 d	175 d	135.1 B
Celina	135 b	134 c	133 c	134 d	137 f	140 g	141 g	142 f	145 f	160 e	140.1 B
Neoplanta	97 e	105 g	108 e	113 e	125 h	145f	148 f	158 d	158 e	175 d	133.2 B
P21T45	142 a	146 b	150 b	177 b	176 b	171 b	183 b	188 a	188 b	190 b	171.1 A
PR91M10	108 d	131 d	185 a	183 a	186 a	182 a	189 a	189 a	191 a	192 ab	173.6 A
PR92B63	76 i	125 e	115 d	150 c	146 d	163 d	170 c	171 c	187 b	194 a	149.7 AB
PR922M22	79 h	79 i	103 f	105 g	131 g	145f	150 f	171 c	183 c	193 a	135.9 B
PR92M35	83 g	95 h	109 e	111 ef	132 g	142 g	172 c	179 b	183 c	187 c	139.3 B
Sphera	117 c	149 a	148 b	152 c	164 c	168 c	172 c	178 b	189 ab	193 a	163 AB
Zora	93 f	94 h	96 h	110 f	124 h	139 h	164 d	179 b	183 c	192 ab	137.4 B
Mean (AD)	102.2 G	118.7 FG	124.8 F	133.9 EF	146.5 DE	154.CD	164.2 BCD	170.3 ABC	178.1 AB	185.1 A	

Note: Different small letters indicate significant differences between the varieties during each aging day according to LSD ($p \leq 0.05$). Different capital letters indicate significant differences between the means of each aging day and each variety according to LSD ($p \leq 0.05$).3.3. Free fatty acids.

As expected, the percentage of free fatty acids (%) was lower in seeds that were stored at cooling conditions as compared to those stored at room temperature throughout the aging period. These findings are indicative of the fact that storage under cooling conditions enhances the longevity of seeds. At the variety level, Adonai variety showed the lowest free fatty acids percentage (Table 5) and, by extension, the lowest deterioration of seeds previously stored at both conditions under study.

Table 5. Free fatty acids (%) of seeds that were either stored under room temperature or cooling conditions at different storage duration (aging days).

Genotype	Room temperature					
	Aging days					
	12	24	36	42	48	Mean (G)
Adonai	0,230 d	0,230 e	0,230 e	0,260 f	0,260 f	0,242 E
Celina	0,420 a	0,460 b	0,380 b	0,340 e	0,530 b	0,426 BC
Neoplanta	0,260 c	0,260 d	0,260 d	0,380 d	0,300 e	0,292 DE
P21T45	0,230 d	0,230 e	0,260 d	0,260 f	0,260 f	0,248 E
PR91M10	0,340 b	0,540 a	0,490 a	0,490 b	0,530 b	0,478 AB
PR92B63	0,420 a	0,540 a	0,490 a	0,570 a	0,630 a	0,530 A
PR922M22	0,230 d	0,230 e	0,230 e	0,260 f	0,260 f	0,242 E
PR92M35	0,260 c	0,260 d	0,260 d	0,260 f	0,260 f	0,260 E
Sphera	0,300 b	0,300 c	0,300 c	0,460 c	0,420 c	0,356 CD
Zora	0,260 c	0,260 d	0,340 c	0,340 e	0,380 d	0,316 DE
Mean (AD)	0,295 A	0,331 A	0,324 A	0,366 A	0,379 A	
Genotype	Cooling conditions					
	Aging days					
	12	24	36	42	48	Mean (G)
Adonai	0,230 d	0,230 e	0,230 e	0,230 e	0,230 e	0,230 E
Celina	0,230 d	0,230 e	0,230 e	0,260 d	0,260 d	0,242 DE
Neoplanta	0,230 d	0,230 e	0,230 e	0,230 e	0,260 d	0,236 DE
P21T45	0,230 d	0,260 d	0,260 d	0,300 c	0,300 c	0,270 CD
PR91M10	0,340 b	0,380 b	0,420 b	0,380 b	0,380 b	0,380 B
PR92B63	0,380 a	0,490 a	0,540 a	0,490 a	0,490 a	0,478 A
PR922M22	0,300 c	0,300 c	0,300 c	0,300 c	0,300 c	0,300 C
PR92M35	0,300 c	0,380 b	0,420 b	0,380 b	0,380 b	0,372 B
Sphera	0,300 c	0,300 c	0,300 c	0,300 c	0,300 c	0,300 C
Zora	0,300 c	0,300 c	0,300 c	0,300 c	0,300 c	0,300 C
Mean (AD)	0,284 A	0,310 A	0,323 A	0,317 A	0,320 A	

Note: Different small letters indicate significant differences between the varieties during each aging day according to LSD ($p \leq 0.05$). Different capital letters indicate significant differences between the means of each aging day and each variety according to LSD ($p \leq 0.05$).

3.3. Viability equation (Ki values)

Viability equation (Ki values) indicates the storage potential of each variety for the two storage conditions under study. Storage under cooling conditions had a positive impact on the Ki value, thus

enhancing the storage longevity, of all genotypes. At the variety level, Adonai had the highest Ki value in both storage conditions (Figure 1).

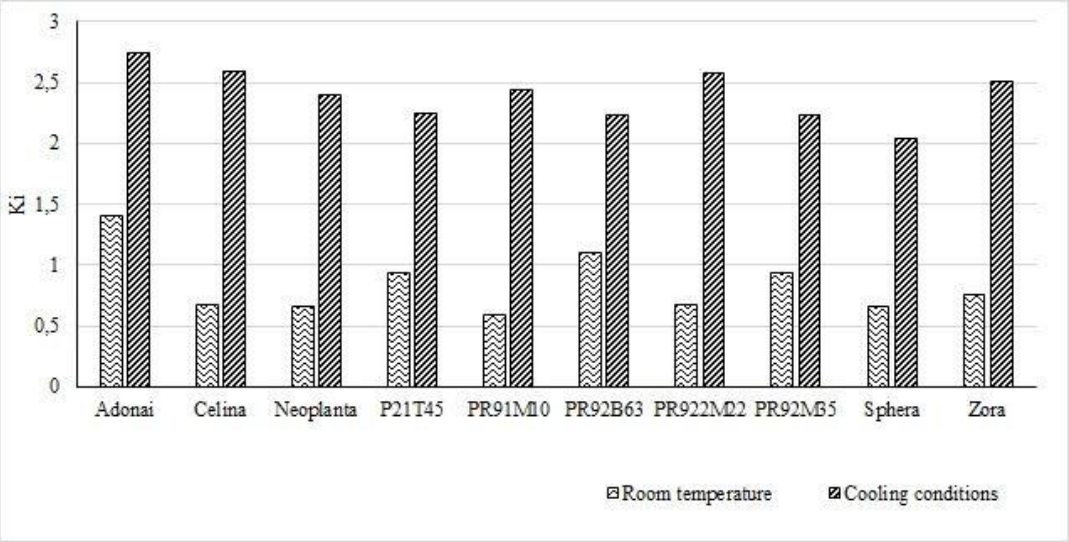
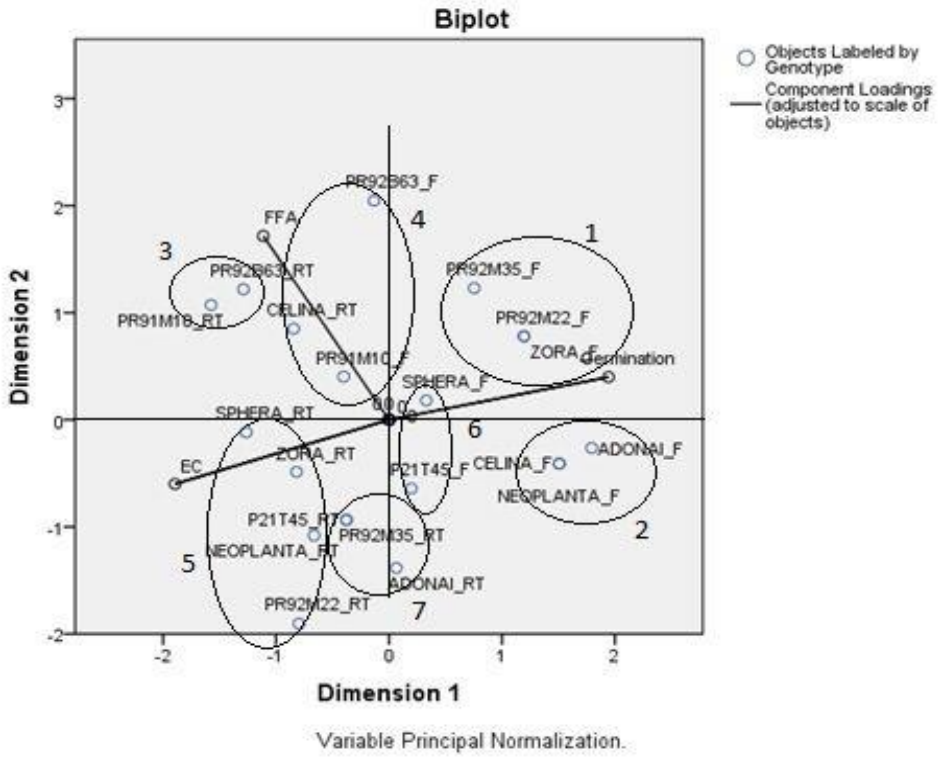


Figure 1. Ki values of each genotype in both storage conditions.



Note: RT= storage at room temperature, F= storage under cooling conditions.

Figure 2. Bi-plot analysis for the genotypes whose seeds were previously stored either under room temperature or cooling conditions.

Overall data from germination percentage, electric conductivity and free fatty acids were subjected to cluster analysis, which revealed the classification of genotypes into 7 different clusters. As evidenced, genotypes Adonai, Celina and Neoplanta, which showed the highest mean germination percentage and the lowest mean electric conductivity and free fatty acids percentage after storage at cooling conditions, formed Cluster 2 (Figure 2). To the other end, genotypes PR91M10 and PR92B63, which were previously stored at room temperature and presented the lowest mean germination percentage and the highest mean electric conductivity and free fatty acids percentage, were classified into Cluster 3. Such findings indicate that genotypes belonging to Cluster 2 were the most tolerant to accelerated ageing, whereas those grouped into Cluster 3 were the most sensitive to accelerated ageing and, by extension, to storage. Moreover, overall data underline the significant positive correlation between electric conductivity and free fatty acids percentage as well as their significant negative correlation with germination percentage.

4. Discussion

Soybean belongs to the species whose seed germination and vigour is lower compared to other grain crops, thus often being reduced prior to planting as a result of harsh environmental conditions. The loss in seed vigour is evident by delayed emergence, slow growth and ultimately decline in seed germinability [42]. Under these circumstances, the possibility to robustly predict the relative storability of different seed lots becomes of paramount importance from a scientific, industrial and practical viewpoint. To this direction, the accelerated ageing technique provides an accurate estimation of seed storage longevity, thus enabling rational decision making related to the seed lots which may be retained or should be directly disposed to the market. In this framework, the present study focused on investigating the effect of storage conditions on seed quality and storage longevity in soybean germplasm, consisting of ten commercial varieties.

It is well known that the extent of seed deterioration, caused by seed ageing during storage, strongly depends on storage conditions [43,44] as well as on seed genetic traits [45]. In our study, accelerated ageing adversely affected germination percentage and electric conductivity, with its effects being proportional to the duration of the accelerated ageing, while the free fatty acids percentage was mostly affected by the genotype. Such findings are in accordance with previous reports on loss of viability during accelerated ageing [30,31]. Worth mentioning is the fact that according to de Alencar et al. [49] at 40 ℃ the soybean seeds were classified as out of market after 45 days of accelerated ageing.

In relation to the effect of storage conditions, prior the seed entrance into accelerated ageing chamber, it was revealed that previous storage under cooling conditions was positively associated with a higher germination percentage in all genotypes. The superiority of storage under cooling conditions was further evidenced in seeds exposed to accelerated aging, as they retained a high germination percentage, compared to seeds previously stored at room temperature, thus prolonging storage longevity and delaying seed deterioration. Such findings further reinforce previous reports related to the fact that seed storage at room temperature enhances seed deterioration in soybean, whereas storage under cooling conditions favours seed viability [8,46–48]. Despite the observed differences relating to the previous storage conditions, differences were also noted in the response of genotypes to accelerated aging. As such, Adonai showed a relative superiority as evidenced by a higher germination percentage throughout the observation period as well as a higher mean

germination percentage in seeds that were previously stored under both storage conditions. On the other hand, PR91M10 and PR92B63 exhibited the lowest germination percentage in seeds that were previously stored at room temperature and under cooling conditions, respectively.

In accordance with previous studies, our data point to a negative correlation between electric conductivity and germination percentage, thus indicating that electrolytic leaching is directly linked with germination loss [49]. As expected, electric conductivity increased over time [50], its value being further subjected to dependency on the temperature storage conditions though. Specifically, electric conductivity was consistently maximized in seeds that were previously stored under room temperature conditions, thus confirming its previously reported association with storage temperature [51]. Differences were further observed among genotypes, with Adonai and Celina showing the lowest mean electric conductivity in seeds that were stored at room temperature, while Adonai and Neoplanta presented the lowest values upon seed storage under cooling conditions. In contrast, PR92B63 presented the highest electric conductivity values in seeds that were previously stored under both storage conditions.

Another factor adversely affected by storage duration was free fatty acids percentage, which showed a gradual increase over time. In relation to free fatty acids, previous studies provide evidence that damage of grains or seeds, due to inappropriate storage practices, lead to their increased content in vegetable oils [23], the increase being positively related to storage temperature [46]. Relative in this manner are our findings that the percentage of free fatty acids was higher in seeds stored at room temperature as compared to those stored under cooling conditions. Among genotypes, Adonai and PR92M22 exhibited the lowest free fatty acid content in seeds that were stored at room temperature, whereas in the other group of seeds Adonai showed the lowest respective values. In contrast, PR92B63, as expected based on abovementioned findings, showed the highest free fatty acid content in seeds that were previously stored under both storage conditions.

To further examine the effects of storage conditions on seeds' longstanding viability, the viability equation was calculated. Overall data support the conclusion that storage longevity is significantly affected by storage conditions in all genotypes, with storage under cooling conditions giving rise to higher Ki values, thus indicating its positive effect on storage longevity via delaying seed deterioration. Such negative correlation between seed viability and storage temperature in soybean has been also previously reported [51]. Among genotypes, Adonai consistently presented a higher Ki value, under both storage conditions, providing strong evidence for its suitability for long-duration storage. Further supportive of this conclusion are the clusters defined on the datasets of germination percentage, electric conductivity and free fatty acid content for all genotypes, which place Adonai, along with Celina and Neoplanta, as the best performing cultivars in terms of retaining a relatively high germination potential under cooling seed storage conditions.

5. Conclusions

Collectively, our findings underline that storage under cooling conditions set a ground for retaining a high germination percentage combined with low values of electric conductivity and free fatty acid content. More importantly, the superiority of such storage conditions was evidenced in artificially aged seeds which maintained their seed quality for longer storage period, due to a delay in seed deterioration processes. In relation to the comparative variety performance, Adonai, Celina and

Neolplanta were classified as most superior cultivars, thus providing prospects for maintaining a high seed quality even under long-term storage conditions.

Conflict of interest

All authors declare no conflict of interest in this paper.

References

1. Wesis WA (1983) Oilseed Crop. Tropical Agricultural Series, Longman London.
2. Vlachostergios DN, Noulas C, Baxevanos D, et al. (2021) Response of early maturity soybean cultivars to row spacing in full-season crop and double-crop systems. *Plant Soil Environ* 67: 18–25. https://doi.org/10.17221/433/2020-PSE
3. Akter N, Haque MM, Islam MR, et al. (2014) Seed quality of stored soybean (*Glycine max* L.) as influenced by storage containers and storage periods. *The Agriculturists* 12: 85–95. https://doi.org/10.3329/agric.v12i1.19585
4. Delouche JC, Matthens RK, Dougherty GM, et al. (1973) Storage of seed in sub-tropical and tropical regions. *Seed Sci Technol* 1: 633–692.
5. Villa LG, Roa G (1979) Drying and storage of the industrial soybean and seeds in bulk. Campinas: Fundação Cargill.
6. Kausar M, Mahmood T, Basra SMA, et al. (2009) Invigoration of low vigor sunflower hybrids by seed priming. *Int J Agric Biol* 11: 521–528.
7. McDonald MB (1999) Seed deterioration: physiology and assessment. *Seed Sci Technol* 27: 177–237.
8. Ali IM, Nulit R, Ibrahim MH, et al. (2017) Deterioration of quality soybean seeds (*Glycine max* L.) at harvest stages, seed moisture content and storage temperature in Malaysia. *Int J Biosci* 10: 372–381. https://doi.org/10.12692/ijb/10.5.372-381
9. Gupta PC (1976) Viability of stored soybean seeds in India. *Seed Sci Res* 4: 32–39.
10. Zuchi J, França-Neto JB, Sediyama CS, et al. (2013) Physiological quality of dynamically cooled and stored soybean seeds. *J Seed Sci* 35: 353–360. https://doi.org/10.1590/S2317-15372013000300012
11. Ferreira FC, Vilela FA, Meneghello GE, Soares VN (2017) Cooling of soybean seeds and physiological quality during storage. *J Seed Sci* 39: 385–392. https://doi.org/10.1590/2317-1545v39n4177535
12. 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: 33–40. https://doi.org/10.1134/S1021443708010032
13. Wein HC, Kueneman EA (1981) Soybean seed deterioration in the tropics. *Field Crops Res* 4: 123–132. https://doi.org/10.1016/0378-4290(81)90062-9
14. Al-Yahya SA (2001) Effect of storage conditions on germination in wheat. *J Agron Crop Sci* 186: 273–279. https://doi.org/10.1046/j.1439-037x.2001.00402.x
15. Abba EJ, Lovato A (1999) Effect of seed storage temperature and relative humidity on maize (*Zea mays* L.) seed viability and vigour. *Seed Sci Technol* 27: 101–114.

16. Kavan HC, Catao HCRM, Caixeta F, et al. (2019) Accelerated aging periods and its effects on electric conductivity of popcorn seeds. *Rev Fac Ciênc Agrár* 42: 40–48.
17. Binotti FFS, Haga KI, Cardoso ED, et al. (2008) Efeito do período de envelhecimento acelerado no teste de condutividade elétrica e na qualidade fisiológica de sementes de feijão. *Acta Sci Agron* 30: 247–254. https://doi.org/10.4025/actasciagron.v30i2.1736
18. Santos CMR, Menezes NL, Villela FA (2004) Alterações fisiológicas e bioquímicas em sementes de feijão envelhecidas artificialmente. *Rev Bras Sem* 26: 110–119. https://doi.org/10.1590/S0101-31222004000100017
19. Panobianco M, Vieira RD, Krzyzanowski FC, et al. (1999) Electrical conductivity of soybean seed and correlation with seed coat lignin content. *Seed Sci Technol* 27: 945–949.
20. Hesleihurst MR (1988) Quantifying initial quality and vigour of wheat seeds using regression analysis of conductivity and germination data from aged seeds. *Seed Sci Technol* 16: 75–95.
21. Zadernowski R, Nowak-Polakowska H, Rashed AA (1999) The influence of heat treatament on the activity of lipo and hydrophilic components of oat grain. *J Food Process Preserv* 23: 177–191. https://doi.org/10.1111/j.1745-4549.1999.tb00378.x
22. Bellaloui N, Bruns AH, Gillen AM, et al. (2010) Soybean seed protein, oil, fatty acids, and mineral composition as influenced by soybean-corn rotation. *Agric Sci* 1: 102–109. https://doi.org/10.4236/as.2010.13013
23. O'Brien RD (2004) Fats and Oils Formulating and Processing for Applications. CRC Press, ISBN 0849315999, Boca Raton, United States.
24. Trawatha SE, TeKrony DM, Hidebrand DF (1995) Relationship of soybean seed quality to fatty acid and C_{6-} Aldehyde levels during storage. *Crop Sci* 35: 1415–1422. https://doi.org/10.2135/cropsci1995.0011183X003500050026x
25. Murthy UMN, Kumar PP (2003) Mechanisms of seed ageing under different storage conditions for *Vigna radiate* (L.). Wilczek: lipid peroxidation, sugar hydrolysis, millard reactions and their relationship to glass state transitions. *J Exp Bot* 54: 1057–1067. https://doi.org/10.1093/jxb/erg092
26. Gutierrez G, Gruz F, Moreno J, et al. (1993) Natural and artificial seed ageing in maize germination and DNA synthesis. *Res J Seed Sci* 3: 279–285. https://doi.org/10.1017/S0960258500001896
27. Ruzrokh M, Golozani KG, Javanshir A (2003) Relation between seed vigour with growth and yield in pea (*Cicer arientum* L.). *Nahal o Bazr* 18:156–169.
28. Verma SS, Verma U, Tomer RPS (2003) Studies on seed quality parameters in deteriorating seeds in Brassica (*Brassica compestris*). *Seed Sci Technol* 31: 389–396. https://doi.org/10.15258/sst.2003.31.2.15
29. Rastegar Z, Sedghi M, Khomari S (2011) Effects of accelerated aging on soybean seed germination indexes at laboratory conditions. *Not Sci Biol* 3: 126–129. https://doi.org/10.15835/nsb336075
30. Lekić S (2003) Vigour of seed. Association of breeders and seed researchers of Serbia, Belgrade.
31. Tatić M, Balešević-Tubić S, Vujaković M, et al. (2008) Changes of germination during natural and accelerated aging of soybean seed. In: *Proceedings of The Second PSU-UNS International Conference on BioScience: Food, Agriculture and Environment*, Serbia, 256–259.

32. Fabrizius E, TeKrony D, Egli DB, et al. (1999) Evaluation of a viability model for predicting soybean seed germination during warehouse storage. *Crop Sci* 39: 194–201. https://doi.org/10.2135/cropsci1999.0011183X003900010030x
33. Ellis RH, Osei-Bonsu K, Roberts EH (1982) The Influence of Genotype, Temperature and Moisture on Seed Longevity in Chickpea, Cowpea and Soya bean. *Ann Bot* 50: 69–82. https://doi.org/10.1093/oxfordjournals.aob.a086347
34. Khah EM, Ellis RH, Roberts, EH (1986) Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat, Department of Agriculture, University of Reading. https://doi.org/10.1017/S0021859600087232
35. Ferreira FC, Vilela FA, Meneghello GE, Soares VN (2017). Cooling of soybean seeds and physiological quality during storage. *J Seed Sci* 39: 385–392. https://doi.org/10.1590/2317-1545v39n4177535
36. Zanakis GN, Ellis RH, Summerfield RJ (1993) Response of seed longevity to moisture content in 3 genotypes of soybean (*Glycine max* L.). *Exp Agric* 29: 449–459. https://doi.org/10.1017/S0014479700021165
37. Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. *Ann Bot* 45: 13–30. https://doi.org/10.1093/oxfordjournals.aob.a085797
38. Vieira RD, Penariol AL, Perecin D, et al. (2002). Electrical conductivity and initial water content of soybean seeds. *Braz J Agric Res* 37: 1333–1338. https://doi.org/10.1590/S0100-204X2002000900018
39. Firestone D (1989) Official Methods and Recommended Practices of the American Oil Chemists' Society. 4th edn., Ameri-can Oil Chemists' Society, Champaign, Ca 5a-40.
40. Lin SS (1990) Changes in leakage, germination and vigor of bean seeds aged under high relative humidity and high temperature. *Braz J Plant Physiol* 2: 1–6.
41. Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. *Ann Bot* 45: 13–30. https://doi.org/10.1093/oxfordjournals.aob.a085797
42. Woodstock LW, Taylorson RB (1981) Ethanol and acetaldehyde in imbibing soybean seeds in relation to deterioration. *J Plant Physiol* 67: 424–428. https://doi.org/10.1104/pp.67.3.424
43. Elias SG, Copeland LO (1994) The effect of storage conditions on canola (*Brassica napus* L.) seed quality. *J Seed Technol* 18: 21–22.
44. Balešević-Tubić S, Malenčić Đ, Tatić M, et al. (2005). Influence of aging process on biochemical changes in sunflower seed. *Helia* 28: 107–114. https://doi.org/10.2298/HEL0542107B
45. Malenčić Đ, Popović M, Miladinović J (2003) Stress tolerance parameters in different genotypes of soybean. *Biol Plant* 46: 141–143. https://doi.org/10.1023/A:1022384600538
46. de Alencar ER, Faroni LRD'A (2011) Storage of soybeans and its effects on quality of soybean sub-products. In: Krezhova D (Ed.), *Recent Trends for Enhancing the Diversity and Quality of Soybean Products*, InTech, 47–66. https://doi.org/10.5772/18022
47. Balešević-Tubić S, Tatić M, Đorđević V, et al. (2010) Seed viability of oil crops depending on storage conditions. *Helia* 33: 153–160. https://doi.org/10.2298/HEL1052153B
48. Kandil AA, Sharief AE, Sheteiwy MS (2013) Effect of seed storage periods, conditions and materials on germination of some soybean seed cultivars. *Am J Exp Agric* 3: 1020–1043. https://doi.org/10.9734/AJEA/2013/3590

49. de Alencar ER, Faroni LRD'A, de Lacerda Filho AF, et al. (2006) Influence of different storage conditions on soybean grain quality. International Working Conference on Stored Product Protection 15–18 Oct 2006, Sao Paulo (Brazil).
50. Singh J, Paroha S, Mishra RP (2016) Effect of storage on germination and viability of soybean (*Glycine max* L.) and Niger (*Guizotia abyssinica*) seeds. *Int J Curr Microbiol Appl Sci* 5: 484–491. https://doi.org/10.20546/ijcmas.2016.507.053
51. Vijay D, Dadlani M (2009) Molecular marker analysis of differentially aged seeds of soybean and sunflower. *Plant Mol Biol* 22: 282–291. https://doi.org/10.1007/s11105-008-0085-9

AIMS Press