



## Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean

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### Abstract

Reduced seedling growth is a consequence of seed deterioration. The heterotrophic seedling growth can be considered as the product of three components: (1) initial seed weight, (2) the fraction of seed reserves which are mobilized, and (3) the conversion efficiency of mobilized seed reserves to seedling tissues. It is not clear which of these component (s) is affected by seed deterioration. To study this subject, germination characteristics and seedling growth from deteriorated soybean (*Glycine max*) seeds were studied; seeds were incubated at two different temperatures of 34 °C and 40 °C for varying times. The results indicated that seed deterioration results in decreased percentage and rate of germination and decreased percentage of normal seedlings. Seedling growth and the fraction of seed reserve mobilization indicated a significant decrease with the advance of deterioration. However, the effect of seed deterioration on the conversion efficiency of mobilized reserves to seedling tissues was not significant. Thus efforts to improve seedling germination and growth in plant breeding programs should focus on improvement of fraction of mobilized seed reserves.

**Keywords:** Seed aging; Seedling growth; Seed reserve; Soybean.

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### Introduction

Seed deterioration is a serious problem in developing countries where seeds are stored in places usually without a proper control of humidity and temperature. Temperature and seed moisture content (and/or relative humidity) are the main factors influencing seed deterioration and viability loss in storage (Barton, 1964; James, 1967; Roberts, 1972). Lower temperature and humidity result in delayed seed deteriorative process and aging thereby leads to extended viability period. Seed ageing is generally marked by reduction in vigor (Trawatha et al., 1995; Agrawal and Sinha, 1980; Saxena, 1987; Gupta and Aneja,

2004), viability, rate and capacity of germination (Chhetri et al., 1993; Arefi and Abdi, 2003), increased solute leakage (Kalpana and Rao, 1995; Agrawa, 1990; Basra et al., 2003) and susceptibility to stresses and reduced tolerance for storage under adverse conditions (Duffus and Slaughter, 1980).

High seed vigor, i.e. *rapid, uniform* and *complete* emergence of *vigorous seedling*, leads to high grain yield potential of soybean, especially in double cropping system where the growing season is limited by shortening the days from sowing to complete ground cover. This allows the establishment of optimum canopy structure that minimizes interplant competition and maximizes crop yield. Rapid emergence provides the plants with time and spatial advantages to compete with weeds (Soltani et al., 2001; Soltani et al., 2002).

Knowing that seed ageing and deterioration can affect seed vigor, the present study aims to analyze the influence of seed aging on the different growth components of seedlings. The heterotrophic seedling growth could be quantitatively described as the product of three components (Soltani et al., 2006): (1) initial seed weight (mg per seed), (2) seed depletion ratio ( $\text{mg mg}^{-1}$ ) i.e. the fraction of seed reserve which is mobilized, and (3) the conversion efficiency of mobilized seed reserves to seedling tissues (mg seedling dry weight per mg mobilized seed reserves). Therefore seedling growth can be limited by decreased mobilization of seed reserve and / or the conversion efficiency of mobilized seed reserves. We have found no published data reporting quantitative effects of seed aging on the components of seedling growth in soybean. Being aware of the extent to which each component affects seedling growth, could help agronomists and plant breeders to develop programs for manipulating the critical component (s) in order to improve crop yield.

## Materials and Methods

Soybean seeds (cv. Sahar) were obtained from Gorgan Agricultural Research Station. The experiments were carried out at the Seed Research Laboratory of Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran. To create different aging treatments, seeds were incubated in sealed containers at two different temperatures of 34 °C (Exp. 1) and 40 °C (Exp. 2) for 0, 7, 14, 21, 28, 35 and 42 days.

After aging, a randomized complete block design with 4 replications was used to compare the aging treatments in both experiments. To determine seed dry weight and seed moisture content, 50 seeds from each treatment in four replications were weighed ( $W_1$ ), ground and after drying at 75 °C weighed again ( $W_2$ ) and seed water content (WC) was calculated as  $[(W_1 - W_2) / W_2]$ . Initial seed dry weight (ISDW) in each replication was calculated as  $[W_1 / (1 + WC)]$ .

Treated seeds were germinated between moistened Whatman filter papers, according to ISTA (1983) rules. Thirty seeds per replication were used for each treatment. Seeds were incubated in dark at 20 °C and checked three times per day for up to 10 days. They considered as germinated when their radicle length was approximately 2 mm or more. After ten days, normal and abnormal seedlings and dead seeds were screened. Normal seedlings were counted and were excised from remnant cotyledons. Dry weight of seedlings and remnant cotyledons were obtained after oven drying at 75 °C. Germination rate and germination uniformity were assessed according to Soltani et al. (2001).

Seedling growth components were estimated as outlined by Soltani et al. (2006). Seed reserve utilization (mg per seed) was calculated as initial seed dry weight minus the dry weight of the cotyledons remnant. Conversion efficiency of mobilized seed reserve into plant tissue was estimated by dividing seedling dry weight to the seed reserves utilization. The ratio of utilized seed reserve to initial seed dry weight was considered as seed reserve depletion percentage.

The electrical conductivity (EC) test, which was only carried out in the Exp. 1 was performed according to the method of Hampton and TeKrony (1995). Four samples of 50 sound looking, unbroken seeds were weighed, and then each sample was placed in 250 ml of distilled water in a 500 ml Beakers. Beakers were then sealed and kept at controlled temperature of 20 °C for 24 h. The electrical conductivity of the seed leachates was then measured using an EC-meter. The electrical conductivity of seed leachates was expressed per gram of seed weight as  $\mu\text{S cm}^{-1} \text{g}^{-1}$  for each sample.

### Results and Discussion

Table 1 represents a summary of the analysis of variance for seed germination characteristics and electrical conductivity test. Seed aging had significant effects on electrical conductivity and seed germination traits, i.e. maximum seed germination, percentage of normal seedlings, and germination rate and uniformity. Figure 1 indicates the effect of seed aging on these traits. The difference between regression lines of maximum seed germination and percentage of normal seedlings in Figure 1 represents the percentage of abnormal seedlings. All the traits have been affected significantly by seed aging as indicated by significant regression. Reduced seed germination following seed aging treatments might have been resulted from the increased solute leakage following imbibition which is usually accompanied with inevitable exit of some necessary materials for germination and normal seedling growth.

Our results are in agreement with those of Ruzrokh et al. (2003), Bradford et al. (1993), Thornton and Powel (1995), Lewis et al. (1998) and Verma et al. (2003). Failure of aged seeds to germinate might be due to lipid peroxidation, mitochondrial dysfunction and less ATP production (Sung, 1996; McDonald, 1999; Basra et al., 2003). Many studies have shown that peroxidative changes in fatty acid composition of membrane lipids lead to massive dysfunction of cellular membranes associated with increased viscosity and permeability of bilayers (Priestley, 1986; Copland and McDonald, 1995). Changes in membrane lipids therefore could account for the increase in solute leakage (Sung, 1996). Lipid peroxidation results in the loss of intact membranes in the mitochondrial cristae thereby reducing ATP production during germination process (McDonald, 1999).

Table 1. *F*-value from analysis of variance for electrical conductivity (EC), germination uniformity (GU), germination rate (GR), maximum germination (MG) and percentage of normal seedling (PNSL) in Exp. 1 (34 °C) and Exp. 2 (40 °C).

	EC	GU	GR	MG	PNSL
Exp. 1	41.01**	0.74 <sup>ns</sup>	9.37**	2.64**	6.62**
Exp. 2	-	8.64**	28.42**	10.47**	18.06**

\*\*Significant at 1% level of probability and <sup>ns</sup> not significant.

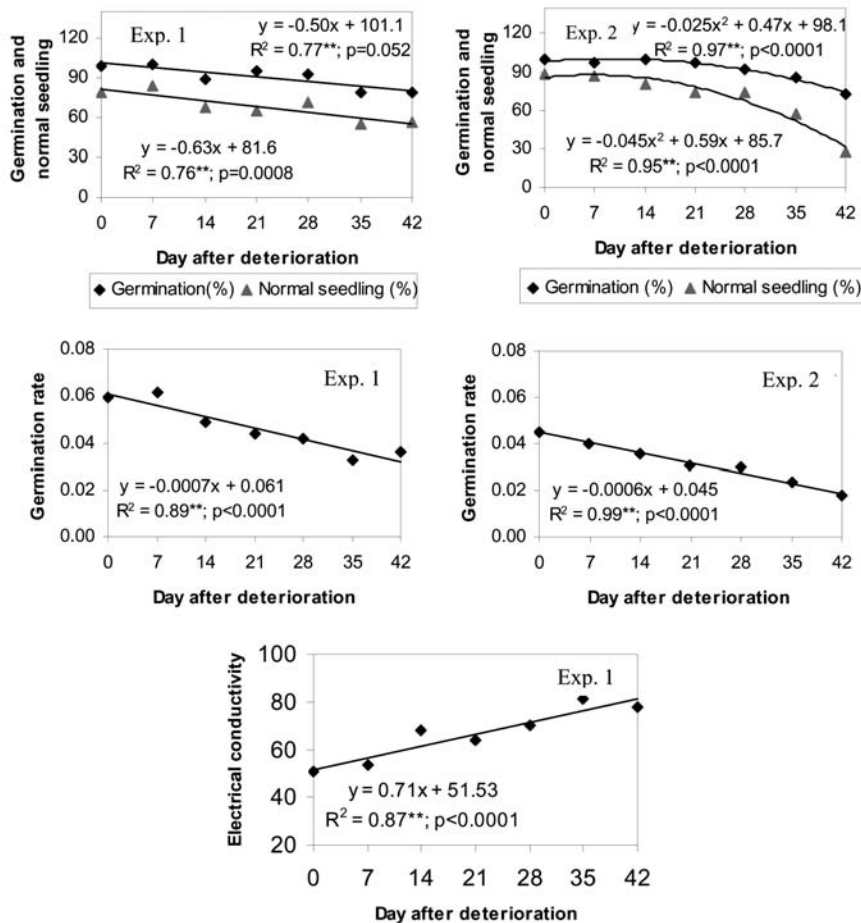


Figure 1. Maximum germination, percentage of normal seedling and germination rate in Exp. 1 (34 °C) and Exp. 2 (40 °C), and electrical conductivity in Exp. 1 (34 °C), as influence by seed aging.

Table 2 represents a summary of the analysis of variance for seedling growth and its components as affected by seed aging treatments. The effects of seed aging on seedling dry weight and seed reserves depletion percentage were significant in both experiments, but not on the conversion efficiency of mobilized reserves. Figure 2 indicates that seedling dry weight and seed reserve depletion percentage decreased significantly (and quadratically) as seed aging progressed. Studying the effect of drought and salinity stresses on seedling growth in wheat, Soltani et al. (2006) reported that the increase of osmotic pressure leads to reduction of seed reserve mobilization, but no significant differences were found between stressed and non stressed plants with respect to the conversion efficiency of mobilized reserves. The decrease in seed reserve mobilization rate was the cause of decreased seedling growth. They had reported similar results for chickpea seedling growth as influenced by salinity and seed size (Soltani et al., 2002).

Table 2. F-value from analysis of variance for seedling dry weight (SLDW), seed reserve depletion percentage (SRD) and seed reserve utilization efficiency (SRUE) in Exp. 1 (34 °C) and Exp. 2 (40 °C).

	SLDW	SRD	SRUE
Exp. 1	4.86**	3.70**	0.51 <sup>ns</sup>
Exp. 2	25.33**	16.39**	1.81 <sup>ns</sup>

\*\*Significant at 1% level of probability and <sup>ns</sup> not significant.

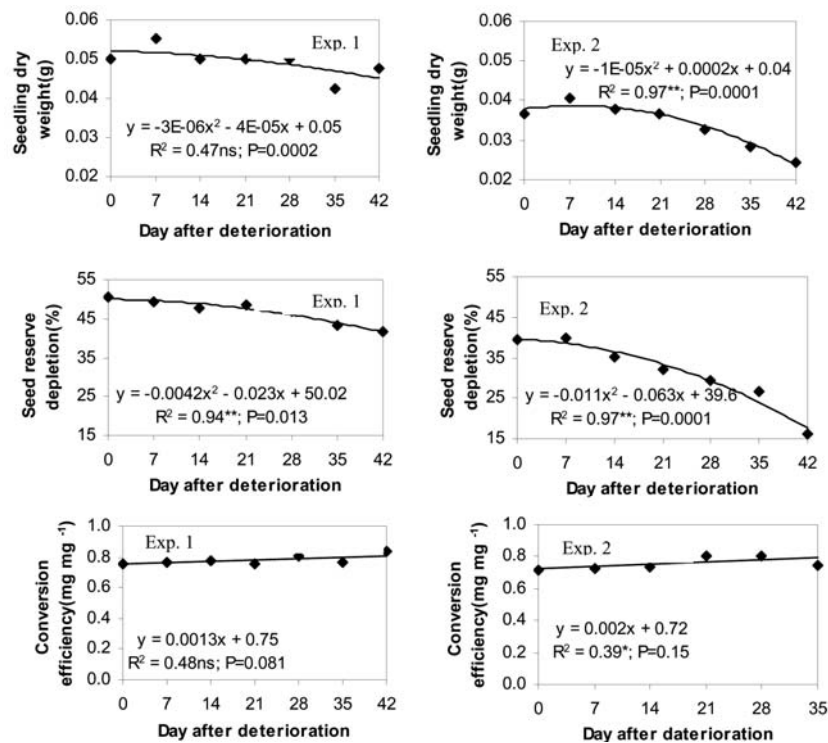


Figure 2. Effect of seed aging on seedling dry weight, seed reserve depletion and seed reserves conversion efficiency in Exp. 1 (34 °C) and Exp. 2 (40 °C).

Overall, the results obtained in this study show that seed aging results in reduced seedling growth and this is a consequence of decline in weight of mobilized seed reserve (seed reserve depletion percentage), not seed reserve utilization efficiency. Seed aging has no effect on the conversion efficiency of mobilized reserves. Therefore, sensitive component of seedling growth is the weight of mobilized (utilized) seed reserve and plant breeding efforts or physiological remedies (say chemical application) should be focused on improvement of seed reserve mobilization.

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