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Quantitative Response of the Longevity of Soybean Seed under Controlled Conditions

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Abstract: The germination potential of soybean seeds (*Glycine max* (L)) declines more rapidly during storage than it does in other other grain crops. This investigation was conducted to determine equation coefficients seed deterioration model for Iran. Seeds of two soybean cultivars were tested using 5 moisture content levels, raging from 7.5% to 18%, in 6 storage temperatures of 25 to 50°C. Seed moisture content level was reached from an initial value (around 7.87) either by adding water, in aluminum-foil packets, or by drying them in desiccators containing silica gel, both at 20°C. Seed sub samples for each moisture content/temperature treatment were sealed in laminated aluminum-foil packets and stored in incubators at those temperatures until complete survival curves were obtained. Seed equilibrium relative humidity was recorded. Estimated viability constants to predict seed longevity were: K_E=5.0833, 6.619; C_W=1.636, 1.762; C_H=0.0076, 0.0365 and C_Q=0.00059, 0.00029 for DPX and Sahar cultivars, respectively. Within a species, estimates of the constants differed (P>0.05). This variant may have been related to Ki or variation among the seed lots in their rates of deterioration at a constant temperature and seed moisture level. These results suggested that variations occurred with the viability constants of a seed deterioration model between the soybean cultivars and that the use of cultivar specific viability constants should be made for predictions of seed germination during storage.

Key words: Soybean · Seed Deterioration model and Longevity Equation

INTRODUCTION

Soybean (*Glycine max* L. *Merr.*) seed lots typically decline in quality faster than seeds of other agronomic crops [1, 2].One of the major constrains to the production of soybean in the tropics is the rapid loss of seed viability and vigor during storage under ambient conditions [3]. The potential longevity of a seed lot is an important component of seed quality in its own right. It is also the basis of several tests for seed vigor that have been developed to rank field emergence potential among seed lots such as accelerated ageing for example [4]. The accelerated-aging (AA) test is one way to estimate seed vigor that has shown to have a high correlation with seed deterioration during storage. However, this correlation for the effect of two key variables affecting seed deterioration, seed moisture and temperature [5]. For this reason seed deterioration prediction models that incorporate the effects of storage temperature and seed moisture have been developed [6]. These models have been revised and expanded to include many crop species, including soybean [7], barley and wheat [4], sugar beet, potato, finger millet, rice and sugarcane [8], Palisade grass [6], Borago [9], cottonseed [2], Sorghum [10] and Pogamia pinnata [11].

Seeds of species that are specific to tropical and temperate zones follow three behavior patterns during storage: orthodox, intermediate and recalcitrant. Orthodox seeds can be conserved long term in low moisture content at subzero storage temperatures [2]. The longevity of orthodox seeds in an air-dried, hermetic storage condition can be estimated with an improved viability equation [12]. This has been applied successfully for many crops and comprises two basic equations. The first is:

$$v = K_i - p/\sigma \quad (1)$$

Where v is probit percentage viability, σ is the standard deviation of the frequency distribution of seed death in time (p, d) and K_i is the intercept (i.e. an estimate of v at

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zero time). This model proposed that as a seed lot, the K_i value will decline, but the subsequent rate of loss of viability ($1/\sigma$) should be constant within a species for a given seed moisture content and temperature [10]. Relation between seed longevity (σ), storage temperature (t, °C) and moisture content (m, % of wt) are described by the equations [12]:

$$\log_{10} \sigma = K - C_w \log_{10} m \quad (2)$$

Equations (1) and (2) can be combined by replacing σ in equation (1) by the antilogarithm of the right-hand side of equation (2). For example at a single temperature, equation (2) can be simplified to (8):

$$\log_{10} \sigma = K_E - C_w \log_{10} m - C_H t - C_Q t^2 \quad (3)$$

Where

$$K_E = K + C_H t - C_Q t^2 \quad (4)$$

Where K_E,C_w,C_H,C_Q and C_2, are constants. The constant K_E, (absolute longevity) denotes the extrapolated value of $\log_{10} \sigma$ at 1% moisture content (because $\log_{10}1=0$) and t= 0°C. C_w, describes the relative effect of moisture content on longevity, being the gradient of the negative logarithmic relation between moisture content (m) and longevity (σ). The combined effect of the constants C_H and C_Q describes the response of seed longevity (σ) to temperature (t).

Where the seed survival curves were described by negative exponentials, however, the fitted time to 50% germination ($P50$) was determined and the term σ replaced by $\log_{10} P_{50}$ [13]:

$$\log_{10} P_{50} = K_L - C_1 m - C_2 t \quad (5)$$

Where K_L, C_1, C_2 and C_2 are constants. Value of K_L obtained as described about K_E. The constant of C_1 and C_2 describes as the response of seed longevity (σ) to seed moisture content (m) and temperature (t).

In this paper we compared seed survival curves of soybean cultivars in various environments in order to: 1) determine viability equation constants; 2) test the hypothesis that the value of Ki, K_L,K, K_e, c_W, C_H, C_Q, C_1 and C_2 varies among cultivars of soybean; 3) test Ki and σ as affected by a seed storage environment.

MATERIALS AND METHODS

Two seed lots of the soybean cultivars (DPX and Sahar) were used. The experiment was designed and carried out in the facilities of the seed science laboratory, Department of Agriculture, at Gorgan University of agriculture science and natural resources, Iran. Seeds were stored at 5°C until the investigation began. Seed quality was measured before seed longevity test. Four replicates of 25 seeds from each cultivar were tested for germination between double layered rolled filter papers at 25±1°C for 7 days. In the accelerated aging (AA) test, 42 g of seed was aged for 72 h at 41°C that Germination, Seedling dry weight and Normal seedling percentage after aging measured with the Standard germination, Seed growth rate tests [14]. In the Electrical conductivity (EC), used 250 mL deionized water in 20°C for 24 h. Conductivity of the leachate was then measured and results were expressed as $\mu S\ cm^{-1}\ g^{-1}$ [13] and then means analyzed as a Completely Randomized Design with four replication.

Seed moisture contents (mc, %) were adjusted from their initial values (around 7.87%) before the experiment to required moisture content by the adding water method [13]. The target weight of the seed samples at this moisture content was calculated as:

$$W_2 = (100-A)*W_1 / (100-B)$$

A= initial seed moisture content (Fresh weight basis), B= target moisture content, W_1 = initial weight of the seed sample (g), W_2= target weight of the seed sample (g).

Drying was done in desiccators containing silica gel at 20°C until mc reached the level of 7.5%. After moisture adjustment to 9, 12, 15 and 18% mc, each of the sub samples was hermetically stored at 3-5°C for 3 days in order for the moisture to equilibrate within and among the seeds. Sand seed moisture contents were then determined on ground samples by the high constant temperature oven method [14]. In each environment, between 13 and 15 sub-samples of 220 seeds of each cultivar were sealed in laminated aluminum foil bags and placed in incubators at the required temperature (25, 30, 35, 40, 45 and 50°C) and stored for 7 days (in high temperatures) and -120 days (in low temperatures). Additional sub samples from all mc levels before storage were taken to detect the relation between seed mc and equilibrium relative humidity, at 20±0.5°C, using appropriate standard salts [12].

Germination tests were done by dividing 200 seeds equally among four replications between moist rolled paper towels (Bowtowel T22) at 25°C. Seed viability in these analysis was normal germination. The observations for germination percentage were subjected to probit percentage germination using SAS software [4]. In this analysis, percentage germination is first transformed to probits. Relation between probit percentage germination and period of storage are then linear is the frequency distribution of seed deaths in time is normal (i.e. equation 1). Where the seed survival curves were fitted to observation by probit analysis, the estimates of σ was provided. Then, the estimate of longevity constants done using the multiple regression analysis, relation between seed longevity and seed storage temperature (t,°C) and moisture content (m,% wet basis). The following, a factorial analysis based on CRD design with three replication Performed for detecting effect of seed storage environment on K_i and $^1/\sigma$. Treatments were three factors: cultivars (DPX and Sahar), temperature (25, 30, 35, 40, 45 and 50°C) and seed content moisture (7.5, 9, 12, 15 and 18 % wet basis). Also analysis variance (t-test) was used to determine if there were significant differences in longevity constants among two cultivars. Seed oil contents were determined by the Sokcele method [16].

RESULTS AND DISCUSSION

Initial Viability and Vigor: Results of analysis variance showed that initial viability (Percentage standard germination) was same (100%) among DPX and Sahar cultivars. Also, the vigor power (Electrical conductivity, Seedling dry weight and Seedling normal percentage) had no significant difference between DPX and Sahar cultivars but in Shahar, values of all vigor trials except EC were higher than DPX and EC in Sahar was lower (Table 1).

Curve of Equilibrium Relative Humidities: The relationship between seed moisture content and equilibrium relative humidities at 25°C are shown in Fig. 1. The third order polynomial curve (r^2=0.96 and 0.98) represents at combination of sorption and adsorption isotherm, depending on whether the value obtained was above or below the initial moisture content value. The isotherm consisted of three regions: 1) at low moisture content, moisture rose steeply at each mal increase in RH (water bonded with sites strongly) 2) at intermediate moisture content, the increase was low (free water at week binding sites) and 3) high moisture content, moisture increased again steeply for each small increase in RH (water bound to other already existent water molecules) [13]. The isotherm inflection point was more in Sahar (8.3%) than in DPX (7.9%). This point, indicated the difference between the first and the second bound water types, so it might account for discontinuity of the logarithmic relationship among seed longevities and the lowest seed moisture contents and from this fact arises the hypothesis that seed longevity increases are linked to moisture content decreases, until the weakly bound water is completely removed [8, 17]. Ellis *et al.* [18] proposed that the critical moisture contents were negatively associated with seed oil content. Within the cultivars, oil content in the DPX cultivar was more than Shahar (23.26% and 20.72% ft.dry weight.), therefore variation in the critical moisture content between the cultivars was expected. Oil content is a major factor to

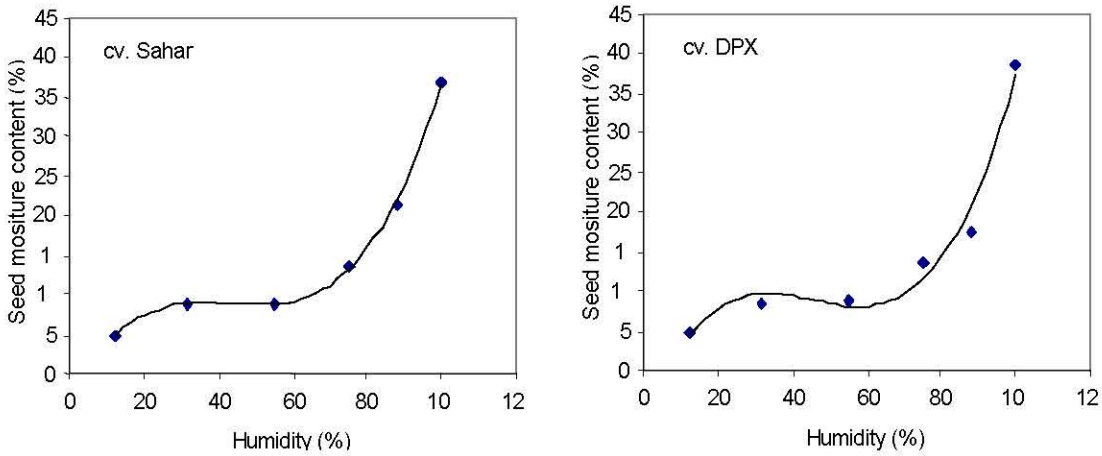

Fig. 1: Relationship of Seed moisture content (%) with Humidity (%) is made by difference salt solutions

Table 1: The results of comparisons mean initial viability and vigor in two soybean cultivars (DPX and Shahar)

Cultivar *	Standard germination (%)	Electrical conductivity (µs/cm.g)	Seedling dry weight (g) (AA)	Normal seedling (%) (AA)
DPX	100	19.38	0.449	78
Shahar	100	18.56	0.507	81

* No Significant differences between all trials among Cultivars.

Table 2: mean square for intercept (Ki) and slope ($1/\sigma$) of the transformed seed survival curves for two cultivar of soybean in different environment.

Seed lot	df	Mean square	Pr>F
Parameters		Ki	$-1/\sigma$
Cultivar (C)	1	115.103**	4.453**
Temperature (T)	5	1.554	48.554**
Moisture (mc)	4	0.4668	66.276**
C×T	5	2.307	0.5860**
C×mc	4	0.6434	0.7527**
T×mc	20	0.3870	12.645**
C×T× mc	20	0.7933	0.4338**
Residual	178	0.2846	0.1221

influence the relation between seed moisture content and equilibrium humidity [18]. Really critical moisture content coincided with the inflection point of the isotherm between seed moisture content and equilibrium relative humidity. It follows that variation in longevity with variation in seed moisture content above the critical value results from variation in the weakly bound fraction of the water held within the seeds, while it appears that removal of the tightly bound water fraction does not influence the rate of seed deterioration.

Seed Survival Curves: Values of the intercept of the regression lines (Ki) were variable among the cultivars. The differences in Ki among seed lots were significant (Table 2 at P>0.001). Between the two lots of soybean cultivars, DPX had greater initial germination than Sahar and a higher Ki value. Differences in estimates of Ki between the two cultivars were because of the difference in vigor, cultivar DPX had vigor power greater than that of Sahar. The results for these two seed survival curves are in agreement with results of Pieta Filho and Ellis [4], Ellis and Hong [19] and the viability equation, equation (3), proposed by Ellis and Roberts [12]: the seed survival curves can be described by negative cumulative normal distributions and the origin of these curves (Ki) was unaffected by the storage environment of the seeds but was a characteristics of the seed (Table 2).

Effect of Seed Storage Moisture Content and Temperature on Longevity: Multiple regression analyses were carried out to determine response of longevity (σ, d) to moisture content (%, f.wt) and temperature (°C) of the two cultivars in accordance with equation (3). The results showed the expected negative logarithmic relation between longevity and moisture content at each temperature and the negative effect of temperature on longevity (Fig. 2). So, analysis variance showed that the response seed longevity to change in moisture content and temperature was significant (Table.2; P>0.01). As expected, the response seed longevity to change in moisture content at the higher values was significant for each of the two cultivars, i.e. at higher moisture contents longevity declined significantly with increased moisture (Table 3). The appropriate values of the constants of equations (3, 5) derived from these analyses, which described the fitted lines of negative slope at the moisture and temperature contents. Although, the viability model [12] is based on the assumption that all seed lots, regardless of genotype or initial quality, deteriorate at the same rate under identical storage conditions, however differences in σ occurred between the cultivars, suggesting that the cultivars did not deteriorate at the same rate under the storage conditions. Also one assumption of the viability model is that all seed lots regardless of genotype to initial quality had the same rate of deterioration during storage when exposed to the same storage conditions, but this appears to have been invalid for the soybean seed lots [1]. Tang *et al.* [17] also indicated that differences in deterioration rates among seed lots of corn in the same environment were relatively large.

Comparison of regression among two soybean cultivars found significant difference in the estimate of each of Ki, K_L, K, K_E, C_w, C_1 and C_2 but those of C_Q and C_H did not (Pr>0.05) (Table 4). Consequently, common values of C_Q and C_H could be applied within each species to quantify the results. The common estimates K_E of, C_Q and C_H obtained for these soybean cultivars (DPX; Sahar)

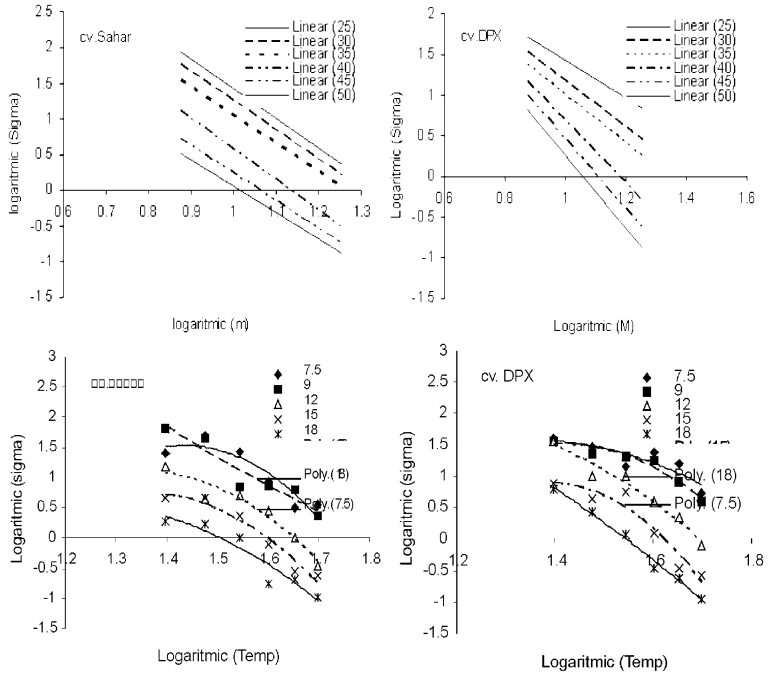

Fig. 2: Relations between seed moisture content (%, f.wt. logarithm scale) and temperature (°C, logarithm scale) with longevity (σ, days and logarithm scale) for two cultivar of soybean be stored.

Table 3: Estimates of the slope ($1/\sigma$) of the transformed seed survival curves for two cultivar of soybean in different storage environments

		Temperature(°C)					
Cultivar	Content moisture (%)	25	30	35	40	45	50
DPX	7.5	0.027a	0.033a	0.035a	0.042a	0.066a	0.184a
	9	0.028a	0.044a	0.049a	0.057a	0.126a	0.254a
	12	0.030a	0.100b	0.103a	0.262a	0.47a	1.314)b
	15	0.127b	0.234c	0.26a	0.806b	2.88b	3.78c
	18	0.169b	0.365d	0.854b	3.23c	4.76c	10.012d
Shahar	7.5	0.018a	0.02a	0.036a	0.119a	0.322a	0.287a
	9	0.015a	0.022a	0.042a	0.142a	0.397a	0.272a
	12	0.065b	0.23b	0.196b	0.348b	0.975b	2.93b
	15	0.225c	0.253b	0.436c	0.248c	3.500c	4.296c
	18	0.516d	0.589c	0.985d	5.78d	4.95d	9.432d

Table 4: Estimates of the viability constants for two soybean cultivars

Cultivar	K_E	K_L	C_1	C_2	C_W	C_{Ht}	C_Q
DPX							
Constants	5.8901	4.340	0.1653	0.0527	1.653	0.0078	0.00059
SD	0.6449	0.1491	0.0885	0.0031	0.088	0.00341	0.000447
Shahar							
Constants	6.6197	4.4916	0.1476	0.0584	1.7626	0.0365	0.000293
SD	0.4459	0.1029	0.00485	0.0021	0.0597	0.0232	0.00030

Table 5: Compare values of equations constants of survival curves among two soybean cultivars

Seed lot	df	Tvalue	Pr>t
K_E	1	17.14	0.037
K_L	1	58.48	0.010
C_1	1	17.68	0.036
C_2	1	19.49	0.032
C_W	1	31.33	0.020
C_{Ht}	1	1.54	0.36
C_Q	1	2.93	0.20

at temperatures from 25 °C to 50 °C were 5.8901(s.e=0.6449), 6.6197 (s.e=0.4459) and 0.00059(s.e= 0.000447), 0.000292(s.e=0.00003) and 0.0078(s. e=0.00341), 0.0365 (0.0278) respectively. The values of C_H and K_E similar to those (C_H=0.00022 and K_E=7.748) were pre determined [7]. But the values of C_w did not (Table 5). Kundu [11] reported that extension of storage period to higher temperature may increase the applicability of the C_Q as well as the fitness of the equation. Similarly Ellis *et al.* [17] indicated that the estimate of 5.39 for C_w for pea varied rather than earlier estimates Ellis and Roberts [7] and the estimate of 5.25 for C_w for lettuce was slightly greater than 4.8 as determined by Kraak and Vos and substantially greater than 3.84 reported by Rao, Roberts and Ellis [18]. Comparison viability constants of this study with viability constants obtained by Ellis *et al.* [7] showed no significant difference was evident between the values of us (cv. DPX) with Ellis *et al.* [7] but K_E varied significantly in Sahar cultivar (P>0.05.). Estimates of K_E and C_w are known to vary among species, largely due to differences in seed composition [20, 19]. Although research by Pieta-filho and Ellis [4] and Ellis and Hong [19] indicated invariant estimates of each of K_E and C_w within species, the results from this research showed that those constants varied among cultivars of soybean [19, 4]. It means that difference between the two soybean cultivars was because of Slope ($-1/\sigma$) in Sahar more than DPX.

The estimate of C_w that is considered as the specific value for each species also falls under the same ranges observed in other species. As the oil content of the seeds differed among the species so did the affect of value of C_w [21]. However Bonner [22] indicated that there was no relation between the value of C_w and oil content of seed in a study on several species. Accordingly, inter specific variation in the several of C_w is ascribed to differences in seed composition [18]. Differences in longevity constants among the two cultivars are in agreement with results of Tang *et al.* [17]: since the assumption of the same rate of seed deterioration for all seed lots stored an identical environments used to support equation (3) was not valid for hybrid corn seed, it was not possible to develop valid estimates of the viability constants K_E, C_w, C_H, C_Q of equation (3), as has been done for many other crop species.

CONCLUSION

- This research suggests that constant rate deterioration and same constants of longevity equation among seed lots or cultivars may be invalid and we cannot develop and use the common constants of seed deterioration model for all of soybean cultivars. This may limit its use for predicting deterioration of soybean seeds.
- A reliable equation is available to predict soybean longevity thus seed producers can accurately predict germination prior to field planting in Iran. (Longevity constants were only found to be useful for cultivates of Sahar and DPX).

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