Derivation of Constants (K_E , C_W) for the Viability Equation for Pepper Seeds and the Subsequent Test of Its Applicability

Ibrahim Demir¹, B. Begüm Kenanoglu, Kazim Mavi, and Tuba Celikkol

Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, 06110, Turkey

Fiona Hay

Seed Conservation Department, Royal Botanic Gardens Kew, Wakehurst Place, Ardingly, West Sussex RH17 6TN, U.K.

Zeliha Sariyildiz

Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, 06110, Turkey

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Abstract. The effect of moisture on seed longevity during experimental storage was investigated in pepper (*Capsicum annuum*) cultivar Demre. Seeds were stored hermetically at 7.0%, 8.6%, 9.5%, 10.5%, and 12.1% moisture content (MC; percent fresh weight basis) and 35 °C for up to 306 d. Viability (normal germination) was assessed periodically and the seed viability equation moisture constants, K_E and C_W , estimated by regression of $\log \sigma$ (the sd of distribution of seed death in time) with $\log MC$. K_E and C_W values were found to be 7.767 and 4.670, respectively. The newly found moisture constants were combined with the temperature constants that had already been proposed as universal for all orthodox seeds ($C_H = 0.0329$, $C_Q = 0.000478$) and their validity was tested by predicting the viability loss in another pepper cultivar, Carliston. The K_i of the Carliston cultivar was determined by controlled deterioration at 40 °C and 14% MC. The observed viability loss between 30 and 270 d at 25 °C and 10% seed MC was closely related to that predicted by the equation $R^2 = 0.982$ ($P < 0.001$).

Seeds of pepper (*Capsicum annuum* L.) are considered to have a short lifespan in storage (Priestley, 1986). In subtropical regions where pepper seeds are produced, relative humidity is high (greater than 70%) and when associated with high temperatures (30 to 35 °C), the viability of seeds can decline within 6 to 24 months (George, 1985). In previous work, we reported that the germination of the majority of pepper seed lots declined to less than the acceptable commercial level (75%) in Turkey after 8 months, even when they were stored at 5 °C with 8% moisture content (Basak et al., 2006). Commercial producers of pepper seeds may save time and money by allowing the early sale of poor-longevity lots and the holding of better lots for the next growing season. The estimation of viability loss would avoid the use of poorer quality pepper seeds in production. Moreover, prediction of seed lon-

gevity in long-term storage enables effective management of gene bank accessions by setting appropriate regeneration periods.

Three major categories of seed storage behavior have been defined: orthodox, recalcitrant, and intermediate (Ellis et al., 1990; Roberts, 1973). The longevity of orthodox seeds such as pepper can be estimated by the improved viability equations (Ellis and Roberts, 1980):

$$V = K_i - p/\sigma \quad [1]$$

where

$$\log \sigma = K_E - C_W \log m - C_H t - C_Q t^2 \quad [2]$$

v is the probit percentage viability after p days of storage at moisture content, m (% fresh weight basis), and temperature, t (°C); K_i is the initial probit percentage viability of each seed lot before storage. K_E is a species constant, which is equivalent to $\log \sigma$ at 1% moisture content (MC) and 0 °C. C_W indicates the logarithmic response of seed longevity to MC. C_H and C_Q are the linear and quadratic terms, respectively, that describe the effect of storage temperature on longevity (Ellis et al., 1986). This equation has been applied successfully to 53 species (Hong et al., 1996), but so far not to pepper seeds. Use of the equation depends on the determi-

nation of the viability constants (K_E , C_W , C_H , and C_Q), which are species-specific. Ellis and Roberts (1981) and Dickie et al. (1990) found that the relative effect of temperature on air-dry seed longevity in hermetic storage was similar for seeds of many orthodox species. The universal values of 0.0329 and 0.000478 for C_H and C_Q , respectively, were suggested (Dickie et al., 1990). These common C_H and C_Q values were also confirmed for 12 crop species in the recent work of Ellis and Hong (2007).

The objective of this research was to estimate the species-specific constants (K_E and C_W) of the viability equation for pepper seeds and to test the predictive value of the derived equation over time in storage.

Material and Methods

Seed material. Pepper seeds (*Capsicum annuum* L.) of cultivar Demre were obtained from the Beta Seed Company (Ankara, Turkey) and stored hermetically at –20 °C before the investigation. Initial seed MC was determined by the low constant temperature method (see subsequently) as 6.9%.

K_E and C_W determinations. Five lots of seeds were humidified in a desiccator over deionized water ($\approx 98\%$ relative humidity) until the target MCs of 7.0%, 8.6%, 9.5%, 10.5%, and 12.1% were reached. During humidification, MCs were checked by frequent weighing and using the following formula:

$$\begin{aligned} \text{Seed weight at desired MC} \\ = \text{initial weight of seed} \\ \times (100 - \text{initial MC}) / \\ (100 - \text{desired MC}) \end{aligned} \quad [3]$$

To ensure even distribution of water within each of the five lots, seeds were sealed inside aluminum foil packets and placed at 5 °C for 3 d. Seed MC (percent fresh weight basis) was determined by the low constant-temperature-oven method prescribed by the International Seed Testing Association (ISTA, 2005) whereby two 4-g samples were dried in a mechanically ventilated oven at 105 °C for 17 h.

Eighteen subsamples of 200 seeds for each MC were sealed in laminated foil packets. All of the 90 subsamples were stored in incubators maintained at 35 ± 1 °C. Samples were removed at intervals varying from 1 to 30 d depending on MC for periods up to 306 d. Two samples for each MC treatment were taken to determine if seed MC changes occurred during storage. Samples were tested for germination by placing seeds between rolled 20 × 20 cm filter (BP) papers (Filtrak, Niederschlag, Germany) moistened with deionized water, rolled towels were then placed in polyethylene bags, and they were incubated at 25 °C in dark. Germination tests were performed by using four replicates of 50 seeds for each MC × storage period. Normal seedling development was used as the germination criterion and counts made after 7 and 14 d (ISTA, 2005).

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¹To whom reprint requests should be addressed; e-mail demir@agri.ankara.edu.tr.

Probit analysis of the germination data for seeds stored at each MC was carried out using GenStat 11 (VSN International Ltd., Hertfordshire, U.K.), thereby fitting Eq. [1]. The effect of the seed storage environment on longevity was quantified using linear regression SPSS (9.05; SPSS Inc., Chicago, IL) of $\log \sigma$ versus $\log MC$ fitting the equation:

$$\log_{10} \sigma = K - C_w \log_{10} m \quad [4]$$

K_E was then calculated using the universal values for C_H and C_Q in the following equation:

$$K_E = K + C_H t + C_Q t^2 \quad [5]$$

Validation of the constants. A separate storage experiment was conducted to test the validity of the constants determined in the previous experiment by comparing the relationship between by the experiment (actual) and predicted (by the equation) germination values.

Seeds of pepper cultivar Carliston, obtained from the Beta Seed Company (Ankara, Turkey), were hermetically stored (aluminum foil packets) at 10.0% MC and 25 °C. Samples of 200 seeds were used to determine germination percent after 30, 45, 60, 75, 90, 105, 120, 140, 200, 230, 240, 250, 260, and 270 d of storage. Seed humidification, storage procedure, and germination tests were conducting as described previously.

The initial quality of this seed lot, K_i , was determined by storing the seeds at 40 °C and 14% MC. Samples of 200 seeds were used for germination testing at intervals of 1 or 2 d up to 42 d of storage. Percentage germination values were converted to probits and linear regression analysis was carried out using SPSS to estimate K_i .

The values determined for K_E and C_w , together with the estimate for K_i , were used to predict the longevity of pepper seeds stored at 25 °C and 10% MC. Linear regression analysis using SPSS was then conducted between predicted and observed values to assess the validity of these species constants for pepper seeds.

Results

Longevity in hermetic storage gradually increased as MC decreased as expected in orthodox seeds. Seeds with 12.1% MC were dead after 53 d, whereas those with 7% MC did not all die until after more than 300 d (Fig. 1). The estimates of the SD of frequency distribution of seed deaths in time (σ , i.e., the time elapsed between e.g., 84% and 50%) ranged between 10.1 d and 102.1 d in corresponding seed MCs, respectively.

The $\log \sigma$ values were regressed against the $\log$ of storage moisture content to estimate the values of the moisture viability constants, K and C_w (Eq. 4) for pepper (Fig. 2). $\log$ seed MC and $\log \sigma$ values were highly correlated (Fig. 2; $R^2 = 0.959$, $P < 0.01$) and showed a negative linear relationship with $K = 6.03$ (SE 0.541) and $C_w = 4.67$

Fig. 1. Survival curves (germination versus storage period) fitted by probit analysis of pepper cultivar Demre seeds stored at five different moisture contents. The curves shown were fitted through the data from the point of rapidly declining viability (i.e., through the data after germination had fallen to $\approx 85\%$).

Fig. 2. The negative logarithmic relation between seed longevity (σ) in hermetic storage and seed moisture contents for pepper cultivar Demre.

(SE 0.555). K_E was then calculated using the universal values for C_H (0.0329) and C_Q (0.000478; Dickie et al., 1990) in Eq. [5]:

$$K_E = 6.030 + (0.0329 \times 35) + (0.000478 \times 35 \times 35) = 7.767$$

Thus, the estimates of the seed viability constants for pepper are:

$$K_E = 7.767, C_w = 4.670, C_H = 0.0329, C_Q = 0.000478.$$

To validate the use of these values for K_E and C_w in the prediction of pepper seed viability, seeds of another pepper cultivar, Carliston, were hermetically stored at 25 °C at 10.0% MC. A typical survival curve from a sequential sampling during storage revealed a slow decline in germination within the first 120 d of storage followed by a much faster reduction (Fig. 3). The initial seed quality

constant (K_i) of this cultivar, Carliston, seed lot was determined by converting the percentage germination values during storage at 40 °C and 14% MC to probits and fitting a linear regression (Fig. 4). The probit intercept value was 2.151 (SE 0.083).

Predictions of the viability of seed lot cultivar Carliston stored at 25 °C and 10.0% MC for the experimental storage periods shown were calculated. Predicted values were obtained by using the estimated K_i for the mentioned seed lot, the values determined for K_E and C_w cultivar Demre, and the universal values for C_H and C_Q . There was a close relationship between the actual and predicted values (Fig. 5; $R^2 = 0.982$, $P < 0.001$, slope = 0.890).

Discussion

In the present work, the viability equation moisture constants (K_E and C_w) for pepper

Fig. 3. Survival curve of a seed lot of cultivar Carliston pepper seeds stored under hermetic conditions at 25 °C with 10.0% seed moisture content.

Fig. 4. Probit values of germination percentages determined pepper cultivar Carliston seeds hermetically stored at 40 °C with 14% moisture content.

Fig. 5. The relationship between actual and predicted germination values for pepper cultivar Carliston seeds stored at 25 °C and 10% moisture content fitted by linear regression (dashed line). The solid line shows the relationship actual = predicted.

cultivar Demre seeds were found to be $K_E = 7.767$ and $C_W = 4.670$. These constants were shown to be valid for the prediction of seed viability for a different pepper cultivar, Carliston, during experimental storage. Validity of moisture constants in a different cultivar than that of constructed one confirms the view that seed moisture constants are applicable to the storage of any cultivar within the same species as indicated by the basic philosophy of the viability equation (Ellis and Roberts, 1981). We did not intend to determine temperature constants for pepper, because universal values for temperature constants were published and proved that they are applicable for all orthodox seeds (Dickie et al., 1990). We used these values to calculate the predicted viability during storage. The highly significant ($P < 0.001$) relationship between predicted and actual germination values (Fig. 5) indicates that the values that we determined for the moisture constants (K_E and C_W) and these universal values for the temperature constants are applicable to pepper seeds.

Our estimate of K_E for pepper seeds is within the range of values published for other species. In a previous summary of the viability constants for 53 diverse species (Hong et al., 1996), the estimates of K_E ranged from 3.278 for a tree species, *Pinus taeda* (Bonner, 1994), to 10.858 for the pulse, *Vigna radiata* (Ellis et al., 1989). Some of the crop seeds had reasonably high K_E values, i.e., 8.037 in potato, 8.988 in beet root, and 9.993 in maize (Ellis and Hong, 2007). K_E has been found to vary with seed maturity (Hay et al., 1997; Zanakakis et al., 1993) and between ecotypes (Hay et al., 2003), mutants (Lyll et al., 2003) and subspecies within a species (Ellis et al., 1992). Nonetheless, the successful use of the derived K_E for pepper to predict germination of a seed lot from another cultivar within the same species is compatible with the view that K_E is a species constant (Ellis and Roberts, 1980).

Like with K_E , the close fit between our predicted and observed values for viability during storage (Fig. 5) supports the view that C_W is generally a constant specific for a species (Ellis and Roberts, 1980). Estimates of C_W are known to vary among species, largely as a result of differences in seed oil content (Dickie et al., 1990; Ellis et al., 1988; Roberts and Ellis, 1989). Among 53 diverse species (Hong et al., 1996), estimates of C_W varied from 0.983 for the tree *Pinus elliotti* (Bonner, 1994) to 6.305 in sorghum (*Sorghum bicolor*; Kuo et al., 1990). Seed oil content in pepper has been seen to range between 18.2% and 22% (Demir et al., 2008). Our derived C_W value of 4.498 for pepper seeds is close to those for crop seeds that have similar oil contents such as soybean, which has $C_W = 3.979$ and 17% oil content (Ellis et al., 1982). On the contrary, more recent work in tree seeds showed that there were poor correlations ($P > 0.05$) among seed oil content, C_W , and longevity (de Lima and Ellis, 2005). Such insignificant correlation indicates that a generalization regarding differences in seed

longevity among orthodox species in relation to oil content is not possible.

The negative logarithmic relation between seed longevity and MC in hermetic air-dry storage is well described in various crop seeds (Fig. 2) but is subjected to low moisture limit (Ellis et al., 1988, 1989, 1990, 1992). Reducing the seed MC lower than this critical MC does not provide an increase in longevity. In this experiment, the lowest MC used in pepper seeds was 7.0%. This is well above the low critical MC found in some crop seeds, e.g., 4.47% in sugar beet (Ellis et al., 1990), 2.04% in sunflower (Ellis et al., 1988), and 6% in pea (Ellis et al., 1989). Storage at lower MCs than those investigated in this work would have been required determining the low critical MC for pepper seeds.

This work may be of practical significance for the commercial storage of pepper seeds, which are sensitive to adverse conditions and may lose viability within a short time if conditions are suboptimal (Priestley, 1986). The seed viability constants estimated here could provide a sound approach to estimate declines in viability in commercial storage. Although the relationship between predicted and actual values was highly significant (Fig. 5; $R^2 = 0.982$, $P < 0.001$), there is inevitably some modeling error and, in our case, there was some systematic deviation. The fitted regression (Fig. 5) suggested that as viability declined during storage, observed germination became increasingly better than that predicted. This would occur if our estimated K_i was close to the actual value, but σ was being underestimated by our species constants. The actual data indicated that although there was departure over the middle part of the survival curves when viability was declining the most rapidly (between 30% and 80%; Fig. 5) and where the error associated with percentage values would be expected to be greater anyway, observed and predicted values were close at high and low percentage values. Usberti et al. (2006) reported very similar R^2 like in our case in the relationship between predicted and actual seed germination in cotton. If we could have determined moisture constants on more than one seed lot as was done in some

other crop seeds (Ellis and Hong, 2007) and/or a range of temperatures (Hay et al., 2003), the equality between observed and predicted values may have been higher. We intend to go on to control the present constants and their validity with other pepper cultivars and for a larger number of seed lots. The equation could also be used to determine gene bank regeneration intervals after investigations into the effects of lower moisture and temperatures than covered by the present work.

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