Predicting the Longevity of Finger Millet and Vegetable Amaranth Seeds During Storage Under Controlled Temperature and Moisture Content Conditions

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ABSTRACT

Seeds of finger millet (*Eleusine coracana* (L.) Gaertn. var. KAT/FM-1) and those of Amaranth (*Amaranthus hybridus* (L.) ssp. *cruentus* (L.) Thell.) were stored in hermetically sealed, laminated aluminum foil packets, for up to 252 days at different constant conditions, which included combinations of temperatures ranging from 15° C to 40° C and moisture contents ranging from 5.3% to 17.3% (fresh weight basis). Seeds were sampled from storage for viability determination at different intervals depending on the storage regimens. Viability decline during storage was generally rapid at higher temperature and/or moisture content combinations. The effect of storage conditions on viability was quantified using viability equations. For each of the two species, the estimated periods for viability to fall to 50% (p_{50} value) decreased with an increase in the storage temperature and/or moisture content. Viability constants for each of the two species were obtained and used to predict longevity at various non-extreme hypothetical storage conditions. At comparative storage temperature and moisture content combinations, seeds of the two species differed considerably in longevity. The constants obtained in the present study could be applied in predicting storage life of seeds of the two species, especially under short to medium-term storage conditions.

INTRODUCTION

The primary objective of storing seeds for plant genetic resource conservation is to maintain the genetic integrity of preserved accessions for as long as possible. This is a challenging task due to the inevitable deterioration of seeds in storage, which leads to low vigour, reduced number of viable seeds and genetic drift. The availability of precise guidlines about declines in viability of conserved accessions is therefore important. This would help genebank managers estimate regeneration intervals and scheduling of viability testing on genebank accessions, to increase quality and retain the genetic integrity of the germplasm. On a different time scale, commercial seed companies and farmers are also faced with the task of prolonging the life span of certain seed lots, for future seed production and planting seasons, respectively.

A seed viability equation developed by Ellis and Roberts (1980) can be used as a valuable guide for estimating deterioration of seeds in storage, especially when initial viability levels are high (Mead and Gray, 1999). The equation pre-

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dicts seed longevity over a range of storage temperatures and moisture contents as follows:

$$V = K_i - P / 10^{K_L - C_1 m - C_2 t} \quad [1]$$

This equation [1] relates probit percentage viability V, at any time P, to any combination of moisture content (m) and temperature (t). Once the values of the species constants, K_L, C_1 and C_2, are known for a species, longevity of seeds of that species can be computed for specified conditions of temperature and seed moisture content. The initial viability of individual seed lots is denoted by their K_i value, which is dependent on genotype, the pre-storage environment and their interaction (Ellis and Roberts, 1980). The equation allows accurate predictions to be made of the percentage viability expected after any given period under different combinations of storage temperature and moisture content within medium-term genebank storage. Different investigators (Tompsett, 1984; Dickie et al., 1985; Kraak and Vos, 1987; and Lotito and Quagliotti, 1993) have confirmed the usefulness of the equation in predicting the storage behavior of seeds for many species as long as the initial viability is high. Mead and Gray (1999) have proposed an improved version of the model for cases where initial viability is significantly less than 100%.

Despite the usefulness of the viability prediction equation to genebank managers, only 3% of all plant species have had their viability constants determined (Hong et al., 1996). Most past research has overlooked the majority of the indigenous crops of Africa. Such crops have contributed significantly to the agricultural and nutritional needs in most rural populations of Africa. Finger millet is an important crop for Africa and Asia where it is widely distributed. The grain is used for human consumption and the straw for feeding livestock. Nutritionally, the grains are a rich source of minerals (calcium, phosphorous and iron) and crucial amino acids (tryptophan, cystine and methionine). Amaranth is a fast growing, high yielding and nutritionally important leafy vegetable, grown in many rural parts of Africa. The leaves are a good source of vitamins (A, B and C), minerals (iron and calcium) and nutritionally crucial amino acids (lysine and methionine).

Although concerted efforts have now led to prioritized collection, conservation and utilization of these indigenous crops, little is documented on their seed storage characteristics (National Research Council, 1984; 1996; and Joshi and Rana, 1991). Such knowledge is important to genebank managers for cost-effective maintenance and handling of seed germplasm during storage. The aim of this work was to determine how closely seeds of finger millet and vegetable amaranth follow the Ellis and Roberts (1980) equations and to predict their longevity during storage at different temperature and moisture content regimes.

MATERIALS AND METHODS

Seed procurement and processing

One sample of approximately 5,000 seeds each for vegetable amaranth and finger millet was obtained locally from production fields at the National Genebank of Kenya in Muguga and Katumani Dryland Research Station in

Machakos, respectively. Each seed sample was placed into a laminated aluminium foil packet and sealed. A sub-sample was randomly drawn from each experimental lot for initial moisture content and viability determinations. Initial viability as determined by a germination test was 97.5% and 97.0% for finger millet and vegetable amaranth, respectively.

Moisture content determination and adjustment

The remaining seeds of each species were subdivided for storage at different moisture contents levels after adjustments. The modified high constant-temperature oven method (ISTA, 1985) was used to determine moisture content, both initially and subsequently during adjustments. Approximately 1.5g of seeds were accurately weighed (to 4 decimal places) in two replicates into clean, pre-weighed dishes and placed into a pre-heated oven at 130 °C for 2h and 1h, respectively for finger millet and amaranth. At the end of each exposure period, seeds were cooled for 30 to 45 min inside a desiccator and re-weighed. Moisture content was expressed on a fresh weight basis.

Cool-air drying and humidification methods were used to adjust seed moisture contents from their initial levels to the targeted storage levels. In the cool-air drying method, sub-samples of seeds in porous cloth bags were placed in a dehumidified room at 15% relative humidity at 20 °C. Occasionally, enough seeds to form a sub-sample for storage at the desired moisture levels were drawn from the bags and placed into laminated aluminium foil packets, where they were sealed and held at 5 °C for 48 h to allow moisture to equilibrate within and among seeds. Moisture content was then determined periodically until the targeted levels were achieved. In the humidification method, sub-samples of seeds in Petri dishes were placed in a desiccator, with the bottom part filled with water instead of silica gel at 20 °C. Using a phsychrometer, the relative humidity inside the desiccator was determined to be above 98%. Enough seeds for eventual storage at targeted moisture levels were drawn occasionally from the desiccator into laminated aluminium foil packets and sealed before the moisture content was allowed to equilibrate. Moisture content was determined periodically to the desired storage level(s). Initial and targeted moisture levels are shown in Table 1.

Seed storage

Approximately 250 seeds at each moisture content were hermetically sealed in laminated aluminium foil packets and transferred for storage into incubators at 15, 25, 35 and 40 °C, depending on the species. A packet was drawn

TABLE 1. Moisture content of amaranth and finger millet seeds before and after adjustment.

Species	Initial moisture content (%)	Adjusted/storage moisture contents (%)			
Finger millet	11.3	5.3	11.3	15.2	16.3
Amaranth	15.8	5.3	11.2	16.4	17.3

from incubators to test germination, at intervals of either 21 or 7 days, depending on storage temperature and moisture content regimen.

Germination test

At the beginning of the study and subsequently at each sampling time in storage, 200 seeds of finger millet in eight replicates of 25 seeds each were arranged on 0.2% aqueous KNO_3 wetted filter papers on a Jacobsen germinator at 25 °C. For amaranth, 200 seeds in four replicates of 50 seeds each were arranged on top of 0.2% aqueous KNO_3 wetted filter papers (size 90 mm, Whatman No. 1). The seeds were then pre-chilled for 7 days at 5 °C before being transferred into an incubator at 35 °C for germination. Both regimens are appropriate for breaking physiological dormancy in the two species as prescribed by ISTA (1985). Normal seedlings (ISTA, 1985) were counted and removed after four days for each species. The final count (normal seedlings) was done on day 10 and 14 for finger millet and amaranth, respectively. Germination was expressed as a percentage of total number of seeds per replicate. Seed survival curves were obtained by plotting percentage normal germination at the final count (viability) against the storage periods for each species.

Determination and application of viability constants

Probit analysis (Finney, 1962) was applied to the viability data to provide estimates of K_i, σ (the standard deviation of the distribution of seed deaths in time) and P_{50} (the period for viability to fall to the absolute value of 50% under the respective storage conditions), according to the following linear survival curve equation as developed by Ellis and Roberts (1980):

$$V = K_i - 1/\sigma(P) \qquad [2]$$

Multiple regression of data provided the constants K_L, C_1 and C_2 in the following equation:

$$\text{Log} = K_L - C_1 m - C_2 t \qquad [3]$$

Equation [1] was applied to predict the seed longevity of the two species during storage under different combinations of temperature (20–35 °C) and moisture content (12–17%). The hypothetical storage combinations used in the prediction were within the ranges used in the study to derive viability constants. Ellis and Roberts (1980) have advocated that the modified basic viability equation [1] should only be used to predict longevity within ranges of storage combinations used in determining the constants.

RESULTS

Seed storage

In storage combinations of 15 °C with ≤ 16.3% moisture content (mc), 25 °C with ≤ 11.3 % mc, and 35 °C with 5.3% mc, loss in viability of finger millet seeds after 252d was insufficient to construct complete survival curves. Similarly, for amaranth, viability decline was insufficient after 126d of storage under combinations of 15 °C with ≤ 16.4% mc, and 25 °C and 35 °C with ≤ 11.3% mc.

At each of the remaining four storage combinations for each species, complete survival curves are shown in Figs. 1 and 2. Seed longevity was observed to decrease with increase in the storage temperature and moisture content.

FIGURE 1. Seed survival curves for finger millet stored at various temperatures and moisture contents.

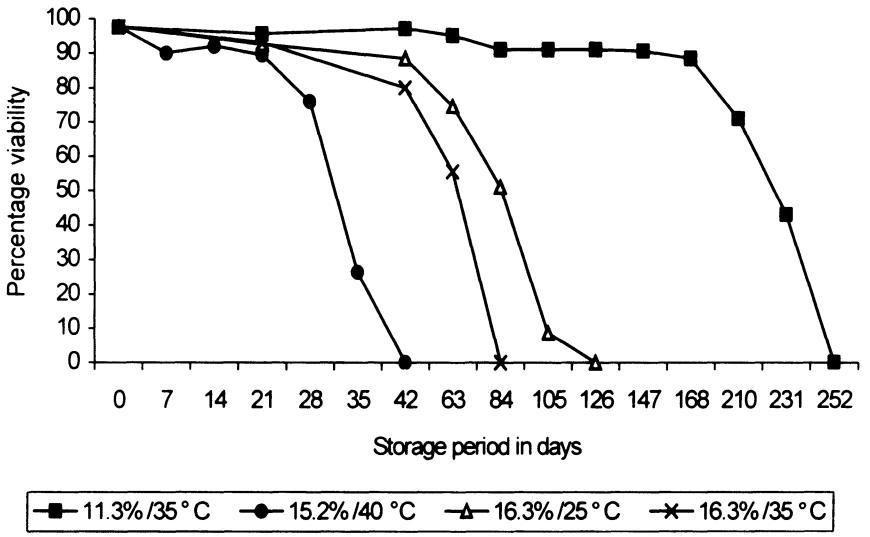
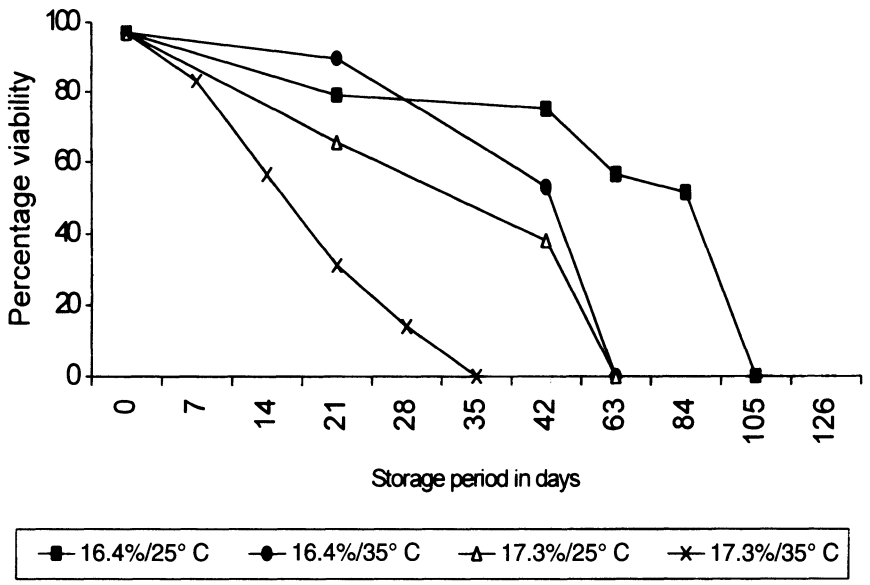


FIGURE 2. Seed survival curves for amaranth stored at different temperatures and moisture contents.



Probit analysis

Probit analysis estimates for each of the curves for each species are summarized in Table 2. Estimated K_i values at each treatment combination for each species were fairly constant, an indication that the curves extrapolated to equal estimates of the origin. The mean estimated K_i value for finger millet was 7.432 probits (equivalent to 95.3% viability) and 6.680 probits (equivalent to 95.2 % viability) for amaranth, both values are only 2.2% less than the observed initial viability. As temperature and/or moisture content increased, a reduction in seed longevity (P_{50}) was accompanied by a reduction in the seed-to-seed variation in the times at which individual seeds die (σ).

Viability constants

Multiple regression analysis of the curves in relation to equation [3] provided the various viability constants as summarized in Table 3. A comparison

TABLE 2. Summary probit analysis of viability decline in finger millet and amaranth seeds stored at various moisture contents (m.c.) and temperatures.

Species	Temp (°C)	Seed m.c. (%)	K_i	slope (1/σ)	σ	P_{50} (s.e.)*
Finger millet	35	11.3	7.629	-0.012	83.3	216.00(3.499)
	25	16.3	7.308	-0.031	32.3	75.09(1.431)
	35	16.3	7.412	-0.043	23.3	55.92(1.165)
	40	15.2	7.377	-0.082	12.2	29.13(0.5350)
			7.432**			
Amaranth	25	16.4	6.844	-0.029	34.5	63.59(1.435)
	35	16.4	6.556	-0.066	15.2	38.89(0.9378)
	25	17.3	6.601	-0.054	18.5	29.48(0.8798)
	35	17.3	6.719	-0.107	9.3	16.09(0.4314)
			6.680**			

* Standard error

** Average K_i value for each of the two species at four treatment combinations

TABLE 3. Estimates of the viability constants for finger millet and amaranth. Standard errors in parentheses.

Species	Viability constants		
	K_L	C_1	C_2
Finger millet	4.606(1.076)	0.135(0.048)	0.034(0.018)
Amaranth	6.713(0.569)	0.267(0.033)	0.033(0.003)

of the species viability constants for finger millet and amaranth reveals more variability in K_L (the inherent longevity) and C_1 (relative effect of moisture content) values, while the C_2 (relative effect of temperature) values were in close agreement.

Longevity prediction

The estimated viability constants were substituted into the predictive viability equation [1], to calculate the hypothetical times (in days) for seeds of the two species to decline from an initial level of 95% to 85% (regeneration level) at various storage combinations of temperature and moisture content (Tables 4 and 5). In seeds of both species, longevity (P_{85}, the period in days for viability to decline to 85%) decreased with every increasing temperature and/or moisture content. Thus, under the most favorable storage conditions (20 °C and 12% mc), finger millet and amaranth seeds would take approximately 123.1 and 429.8 days, respectively, to reach the regeneration level. On the other hand, under the most deleterious storage conditions (30 °C and 17% mc) the regeneration level is predicted to be reached within approximately 11.2 and 9.2 days for amaranth and finger millet, respectively. It is also appar-

TABLE 4. Predicted time in days for finger millet seed viability to decline from 95% to 85% germination.

Storage temp (°C)	Seed moisture content (%)			
	12	15	16	17
	Days			
20	123.1	48.4	35.5	26.0
27	71.1	28.0	20.5	15.0
30	52.1	20.7	15.1	11.1
35	38.0	15.0	11.0	8.0

TABLE 5. Predicted time in days for amaranth seed viability to decline from 95% to 85% germination.

Storage temp (°C)	Seed moisture content (%)			
	12	15	16	17
	Days			
20	429.8	68.0	36.8	19.9
27	252.5	40.0	21.6	11.9
30	201.0	31.8	17.2	9.2

ent that seeds of amaranth lose viability at a slower rate than those of finger millet under storage regimens with lower moisture contents, while the opposite is true at higher moisture levels (≥ 16%).

DISCUSSION

Roberts and Ellis (1984) have emphasized that important considerations on the relationship between seed storage and viability decline should include time, temperature and moisture content. This is in view of the fact that deterioration in any seed lot during storage is a function of these three factors. The insufficient viability decline observed at some storage treatments in the present study could thus be attributed to the short exposure period. Consequently, any deterioration that occurred under these conditions within the respective storage periods was not severe enough to be manifested as a decline in viability.

The present work has confirmed that different storage regimens do not affect the value of K_i for any given seed lot (Ellis and Roberts, 1980; Hong et al., 1996). Thus, the estimated values of K_i for each of the two species are in close agreement under each storage regimen. Since linear survival curves are described by two variables (equation 2), it inevitably means that different conditions of storage will only affect the value of σ, the seed-to-seed variation in longevity (Roberts and Ellis, 1984). This was confirmed by the observed decrease in the standard deviation of the distribution of seed deaths with time (σ) as temperature and/or moisture content increased. Roberts and Ellis (1984) have also stated that in a more deleterious storage environment, the longevity of the longest lived seed within a seed lot will be reduced in units of time much more than the short-lived seeds. This would lead to a reduction in seed-to-seed variation and hence steeper probit curves. In this study, a decrease in the mean longevity period (P_{50} value) was accompanied by steeper survival curves due to a reduction in seed-to-seed variation in the times at which individual seeds die. It implies, therefore, that viability decline in seeds of the two species is effectively described by the basic viability equations of Ellis and Roberts (1980).

A comparison of the species viability constants for equation [3] between the species in the present work shows similar values for C_2 (0.034 for finger millet compared to 0.033 for amaranth), suggesting that their seed longevity may have a similar quantitative response to storage temperature. Similar observations have been made between different seed species by other investigators (Dickie et al., 1985; 1990; Bonner 1994). Results in the present study, therefore, support a proposal by Dickie et al. (1990) that the temperature coefficients do not vary significantly among species and that a common constant may be sufficient to quantify the relationship between seed longevity and temperature. In contrast, C_1 values, which denote the relative effect of moisture content on longevity, vary considerably between finger millet (0.135) and amaranth (0.267). The value of the moisture content coefficient has been observed to differ markedly among orthodox species (Dickie et al., 1985; Ellis et al., 1988; Hong et al., 1996), largely due to differences in the lipid content

of the seeds (Roberts and Ellis, 1989; Walters, 1998). Finger millet seeds are reported to have a lipid composition below 1.5% (National Research Council, 1996), while seeds of amaranth contain up to 7.9% lipid content (National Research Council, 1984; Maundu et al., 1999). Predicted faster viability decline in amaranth than in finger millet seeds at relatively higher moisture levels in comparison to lower levels, could be explained by differences in the respective moisture content coeficients.

At the same time, differences in the predicted absolute longevity of the two species at comparable storage combinations could be explained by variations in seed chemical composition (Cromarty et al., 1985; Walters, 1998). Such longevity differences have been observed in seeds of various species during storage at comparable temperature and moisture content conditions. It has been suggested that seed chemical composition differences affect longevity by influencing the water binding and activity in seeds, and hence the nature and rate of deteriorative reactions (Walters, 1998).

The species constants obtained in our study can be used to give estimates of the expected longevity of seeds for the two species during storage within short- to medium-term storage conditions. In particular, our results also suggest that seed collectors should dry amaranth seeds during prolonged collection missions to avoid probable high rates of deterioration as predicted above 16% moisture content. In order to meet the standards of genebank active collections, seeds of these two species should be dried below 12% moisture content to store for at least two years before regeneration. Predictive results also reveal that normal storage by farmers for over a year is possible for seeds of amaranth without major viability loss, at temperatures ≤ 20 °C in combination with ≤ 12% moisture content.

Conclusion

Results in the present work suggest that the viability equation of Ellis and Roberts (1980) can be applied to these two species. The viability constants obtained in our study can be used as a guide to genebank managers and other seed curators for the expected viability levels of the two species during storage. Application should, however, be limited to active collection (short- to medium-term) storage conditions, given that only short periods and a rather narrow range of temperatures and moisture contents were used to obtain the constants.

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