Estimation of seed viability constants for tall wheatgrass, cocksfoot, rye, and sheep fescue to inform gene banking decisions

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Abstract

Stored seeds deteriorate over time and must be regenerated to ensure that the benefits of *ex situ* conservation are realized. Prediction of seed longevity is based on the seed viability equation. This equation has four constants which are species specific. The aim of this project is the estimation of these constants and prediction of regeneration frequency for *Elytrigia elongata, Dactylis glomerata, Festuca ovina, Secale cereal*, *and Secale montanum*. Seeds were equilibrated at 20, 40, and 60% RH and their moisture content was determined after equilibration. Seeds were then sealed in nylon pockets and stored at 30, 35, and 40° C. Germination was tested monthly. Seed viability constants (K_E , C_w , C_H , C_Q) were estimated and regeneration time was calculated for each species. High variation in seed longevity was observed both among the genus and species. *Elytrigia elongata* with K_E=6 and C_w=1.64 had the maximum seed longevity of all species evaluated; seeds stored in an active collection (8% mc, 5° C) would have to be regenerated after 131 years. The minimum seed longevity was obtained for *Festuca ovina* with K_E=4.3 and C_w=0.5; seeds stored in active collections would have to be regenerated after 33 years.

Keywords: seed longevity; regeneration time; seed storage; viability equation constants

Abbreviations:

mc: moisture content; RH: relative humidity

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Introduction

Due to genetic erosion, conservation of plant genetic resources has been significant. Of 2.3 million plant accessions which are conserved in the world, 97% of them are conserved as seed in *ex situ* collections (Plucknett et al., 1987). By conservation of these resources, plant genetic variation associated with beneficial traits will always be available (Gooding et al., 2003).

Seeds of most plants have orthodox behavior and seed deterioration is predictable if storage conditions are defined. Ellis and Roberts (1980) introduced the viability equation for prediction of seed longevity:

$$V = Ki - P \Big/ 10^{K_E - C_w \log m - C_H t - C_Q t^2}$$

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Table 1
List of species and their origin

Seed code	Species	Initial viability	SMC during incubation	Origin
Dactylis glomerata	29859	100	13-22-31	Iran
Festuca ovina	25570	100	12-21-29	Iran
Secale cereal	19199	100	10-21-29	Iran
Secale montanum	28062	100	9-19-28	Iran
Elytrigia elongate	25569	100	9-18-28	Iran

where V = germination (probit) after storage for P days, Ki = initial germination in probit, m = seed moisture content in %, t = temperature in centigrade, P = storage period in days, and K_E, C_w, C_H, C_Q = species specific constants.

The combined effect of moisture and temperature on seed longevity is described by the equation and it can predict viability of each seed lot after a period of storage under defined conditions. This equation has parameters such as storage temperature, storage period, seed moisture content, viability, and four constants (K_E, C_W, C_H and C_Q) which are seed lot specific. This model can be used for any orthodox seeds stored under conditions of -20 to 90° C and 5 to 25 % mc. Planning for managing gene banks and also storing seed under controlled condition will do better by using this equation (Liu et al., 2008). The general recommendation for seed regeneration in any gene bank is when seed viability is reduced to 85% germination (Sackville Hamilton and Chorlton, 1997). Predicting regeneration time is very important to minimize loss of genetic integrity and input of resources.

Time is only one of the several factors relevant to storage. Seed longevity is significantly affected by seed storage conditions. High smc and high storage temperature are major causes of week seed storage.

Dickie et al., (1985) estimated K_E=6.22, C_W=2.67, and C_H=0.0048 for lupin seeds by using probit analysis and fitting data in a viability reduction regression model.

It is known that in many species there is a negative semi logarithmic relationship between seed moisture and its longevity (Ellis et al., 1980a, 1980b, 1981; Kraak and Vos, 1987; Tompsett, 1986). C_W describes the sensitivity of seed longevity to moisture. There is a high difference between C_W of different species (Ellis and Roberts, 1980b; Leon-Lobos and Ellis, 2003).

There was no difference when eight species were compared for relative sensitivity of seed longevity to temperature (Dickie et al., 1990). Although reduction in temperature improves the lifetime but the model predicts that cost-benefit ratio will increase with decreasing temperature. The reason is that, the model unlike the tests also considers temperature till -75° C (Ellis and Roberts, 1980a; 1980b). In such low temperature, relative usefulness comparing to -20° C will be too low and temperatures below -20° C is not recommended (Dickie et al., 1990).

The aim of this study was estimation of seed viability constants for tall wheatgrass, cocksfoot, rye, and sheep fescue which are stored in natural resources gene bank to inform gene banking decisions. By this work the plant genetic resources will be conserved better and the costs of seed banking will decrease.

Material and Methods

Seed samples were taken from the natural resources gene bank of Iran and as listed in Table 1. In order to estimate constants, numerous studies over a wide range of temperatures and moisture contents were conducted (Smith et al., 2003). The current research investigated nine treatment combinations including three temperatures (30, 35, and 40° C) and three moisture contents (obtained from incubation of seeds at 20, 40,and 60% RH).

For equilibrating seeds to specific moisture levels, each seed sample was divided into 15 subsets. These subsets were packed in nylon

Table 2
Seed viability constants and time to 15% loss of viability

Seed Code	Species	Estimated seed viability Constants				Time to loss 15% loss of viability (year)	
		K_E	C_W	C_H	C_Q	5 ° C	-18° C
29859	*Dactylis glomerata*	4.715	0.554	0.03	0.0002	65	280
25570	*Festuca ovina*	4.366	0.50	0.03	0.0002	33	137
19199	*Secale cereal*	6.361	2.059	0.03	0.000201	126	543
28062	*Secale montanum*	4.431	0.472	0.03	0.000201	40	173
25569	*Elytrigia elongate*	6.00	1.642	0.03	0.0002	131	561

mesh and then incubated in sealed containers over solutions of Lithium Chloride (60, 88, and 128 g LiCl per 200 ml deionized water which provided environments of 20, 40, and 60% RH, respectively).

Plastic chambers containing seeds were sealed and placed at 20° C. Seeds were incubated for 21 days according to the protocol of the Millennium Seed Bank Project for moisture equilibrium (Millennium Seed Bank Project, technical sheet 09). After equilibration, seed moisture content was determined according to the ISTA rules (130° C one h). Equilibrated seeds were sealed in moisture proof nylon pockets by a packing machine then were transferred to 30, 35, and 40° C.

Germination was tested for each treatment combination monthly. Pre-chilling treatment was used because of probable seed dormancy. A seed was considered as germinated when its radicle emerged by 2 mm in length (ISTA, 1996).

Data obtained from germination tests was recorded. Percentage germination was converted to probits and the probit was used. First, seed viability equation (described below) was defined for Excel software; then some limitation was considered for the equation (K_E = 6 - 10, C_W = 3 - 6, C_H = 0.03 - 0.06, C_Q = 0.0002 - 0.0005) (Kruse et al., 2005). The model was fitted via Solver option for estimation of the constants with this assumption that sum of square of residues (subtraction of observed viability from predicted one) are zero.

Results

The seed viability constants for *Dactylis glomerata, Festuca ovina*, *Secale cereale*, *Secale montanum,* and *Elytrigia elongata* were estimated (Table 2). Time for viability to fall to 85% was calculated using the viability equation. Assuming initial viability and moisture content are 100% and 8%, respectively, regeneration time for each accession was predicted for base (-18° C) and active (5° C) collections separately. Among them, *Elytrigia elongata* and *Festuca ovina* had the maximum and minimum time for regeneration respectively (Table 2).

Discussion

The results showed that the genus and even species of a genus have different seed longevity. This difference was mainly related to the moisture constants (K_E and C_W). The temperature constants (C_H and C_Q) had a low diversity or were common between genus and species, as other researcher mentioned (Pritchard and Dickie, 2003). For example, C_H and C_Q were reported 0.0329 and 0.000478, respectively for 9 crops and vegetables species (Dickie et al., 1990). Ellis and Roberts (1980a) reported C_H = 0.04 and C_Q = 0.000428 for Barely. However, here C_H and C_Q were estimated to be 0.03 and 0.0002 for all studied species. Kruse, et al. (2005) estimated and reported the seed viability constants for some crops and vegetable in the 7th ISTA Seminar on Statistics. The range of K_E and C_W reported by them were 6.74-10.1 and 3.979-5.896, respectively.

Early research into seed longevity by Harrington resulted in empirical laws which describe the effects of moisture content and temperature on seed longevity. Seed longevity will double with a 1% decrease in seed moisture content (between 0 and 14%) or a 5° C decrease in temperature (between 0 and -40° C) (Harrington, 1972).

All species studied belong to the Poaceae family, but had a wide range of longevity. *Elytrigia elongata* and *Secale cereale* take 131 and 126 years for viability to fall by 15%, whereas for *Festuca ovina* it is only 33 years. It is reported that some other seeds such as *Dracocephalum parviflorum* Nutt. could remain viable for hundreds of years (Conn et al., 2006).

Estimated species constants are very important to gene bank management. Prediction of storage time is helpful for selection of accessions that need regeneration. There are more than 50000 accessions in the natural resources gene bank of Iran; an informed regeneration program will reduce input of resources and minimize genetic erosion.

In addition to initial seed quality and storage conditions, seed longevity is strongly affected by genetic factors. There was a great difference between seed longevity of 92 species which were stored at 13 sites (in uncontrolled conditions) when time to 50% loss of viability was estimated by Priestley et al. (1985).

Several studies leave little doubt that decreasing storage temperature and smc to a definite threshold can increase seed longevity and reduce the frequency of regeneration. However, seed regeneration is eventually inevitable.

High costs and risks of genetic changes induced by repeated regeneration on the one hand and disadvantages of genetic erosion caused by delayed regeneration on the other hand has caused researchers to estimate seed longevity and thus to choose the right time for a regeneration samples. There is no sufficient data about trend of seed deterioration for more than 50000 seed accessions in the natural resources gene bank of Iran. Therefore, it is very important for the regeneration time to be estimated for each accession. By determining this time (using the constants), gene bank manger can design a desirable program that firstly select the accession which exactly needs regeneration, and secondly, will reduce the costs. Finally, such research will result in good conservation of plant genetic resources which are stored ats seed banks.

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