Risk of cost hike due to failure of maintenance is very high for some of these methods; risk of getting back the conserved material true to type after a time lag in conservation, risk of loss or inability to reinstate the conserved material, risk of high costs involved in the process of conservation have also been ranked as 'low', medium and high and scored based on the desirability of that feature.

The total of the scores indicated that *ex situ* methods were more preferable than the *in situ* methods due to the wider option available for selection. Among the various *ex situ* methods, the field genebank stood first followed by the on farm and the cryopreservation methods getting similar scores. However, they indicate distinct variations. The on farm conservation method provides limited period conservation, cryopreservation offers for infinite time period. The reinstatement of conserved germplasm requires time in on farm while it is almost instantaneous in cryopreservation. The reinstated material is 100% true to type in cryo, which may not be so in on farm method. These features weigh in favour of modern method such as cryopreservation. Further, the other features such as the land and infrastructure availability and the,current priority of a specific method of preservation in terms of global vs local priority also need to be considered while selecting a method. An exercise of this nature would be of great significance in judging the appropriateness of specific methods of conservation. Based on the nature of the product developed, the actual valuation can be done for estimating the cost involved in its development.

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Do Moisture and Temperature Play an Interactive Role on Seed Longevity?

RL Sapra and BB Singh
Division of Genetics, Indian Agricultural Research Institute, New Delhi-110012

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Do moisture and temperature exert independent effects on seed longevity ? A question which seed biologists or genebank managers would like to have an answer. A correspondence published in Seed Science Research (Ellis *et al.* 1991) says "There is no interaction between the effects of temperature and moisture on seed longevity when these relations are quantified by the seed viability equations (*e.g.* Ellis and Roberts, 1980a,b; Ellis *et al.* 1982; Kraak and Vos, 1987), *i.e.* there is no evidence that the relative effect of moisture content on longevity varies with temperature." On the other hand, Vertucci *et al.* (1994) concluded the analysis of their experiment on pea seeds that moisture content and temperature do not exert independent effects on seed longevity.

In light of the abovesaid comments, this paper re-examines and makes an in-depth study of the already published data on *Lupinus polyphyllus* (Dickie *et al.* 1985). It also explains the role of log transformation used in the longevity models in relation to predictive capabilities of the model as well as with respect to the interactive behaviour of the effects of moisture and temperature on seed longevity.

The physical meaning of interaction is that the two factors are said to interact if the effect of one factor changes as the level of other factors changes, and their interaction effect can be measured only if the said factors are tested together in the same experiment. In other words, if we plot the seed longevity (days) against the moisture at various levels of temperature (or against the temperature at various levels of moisture) curves should not be parallel. When data in Table 1 was plotted, the curves at three temperatures levels were found to be non-parallel indicating that the effects of moisture and temperature are non-additive in nature. Whether this non-additivity is significant or not, can be tested through Tukey's non-additivity theorem.

Table 1. Data/results reproduced from Dickie *et al.* (1985) of viability decline curves *of Lupinus polyphyllus* seeds stored at various moisture contents and temperatures

Storage temp.	Mean seed mc	Slope (1/L)	Longevity (L)	Log L
21° C (T_1)	7.91 (M_1)	-.0019	526.32	2.715
	11.73(M_2)	-.0056	178.57	2.254
	14.11(M_3)	-.0059	169.49	2.231
42° C (T_2)	7.92(M_1)	-.0118	84.75	1.926
	11.58(M_2)	-.0789	12.67	1.102
	13.35(M_3)	-.2876	3.48	0.541
62° C (T_3)	8.13(M_1)	-.3027	3.30	0.526
	11.93(M_2)	-.4378	2.28	0.359
	14.32(M_3)	-.5185	1.93	0.285

If data on the longevity is available for more than one replication, then we can analyze the data using the two factor factorial experiment analysis and can test the interaction mean squares against the residual mean squares for accepting or rejecting the null hypothesis of 'no interaction'. However, when one observation per cell is available, we can test the assumption of additivity for the two-way layout using Tukey's theorem which partitions the usual error sum of squares into two components SS_G and SS_{res}. The test assumes the following model with usual anova assumptions:

$L_{ij} = \mu + t_i + m_j + (tm)_{ij} + e_{ij}$

Null hypothesis $H_0: (tm)_{ij} = 0,$

Alternative hypothesis $H_1: (tm)_{ij} \neq 0,$

where L_{ij} is the longevity for the i^{th} temperature and j^{th} moisture, t_i is the effect of i^{th} temperature, m_j is the effect of j^{th} moisture, $(tm)_{ij}$ is the interaction effect of i^{th} temperature and jth moisture and e_{ij} is the error term for ij^{th} cell.

The test for interactions, yielded by the theorem, consists in testing SS_G against SS_{res}. This is done with the statistics,

$(TM\text{-}T\text{-}M)\ SS_G/SS_{res}$,

which has a central F-distribution with 1 and TM-T-M degrees of freedom. T is the number of temperature levels and M is the number of moisture levels in a two way table. We worked out the above said F statistics for the data shown in Table 1. Three levels of temperature are T_1=21°C, T_2=42°C and T_3=62°C; and three levels of moisture are taken as M_1=(7.9-8.2), M_2=(11.5-12.0) and M_3=(13.3-14.4), When the computed F statistics (F=238.6) is compared with the tabulated F value, the hypothesis of 'no interaction' is rejected indicating the presence of non additivity or interactions in the data.

In the above said Tukey's test, both the moisture and temperature have been dealt qualitatively. However, it would be more appropriate to deal them quantitatively and to study the individual effects as well as their joint effects through multiple linear regression analysis approach. For studying these effects, we normally fit a response surface to the data and test the corresponding estimated regression coefficients with the help of Student t-test. The interaction terms in a response surface are products of two or more predictor variables. They are useful when it is believed that the effect of a predictor variable on the response depends on the values of other predictor variables. However, the interaction terms in the regression model repeat information provided by the predictor variables and if the redundancy induced by the interaction terms is too strong, coefficient estimates for the individual predictor variables can be distorted (Gunst and Mason, 1980). We tried to examine the issue of interaction on four scales *viz.*-original, inverse, log and 1.9th root (special scale closer to the square root scale) by fitting response surface to the data and using the stepwise regression analysis. The results of the various fitted models with significant regression coefficients are summarized as follows:

The parameters of the modified viability equations (Ellis and Roberts, 1980a) are estimated statistically in two stages: (i) firstly, estimating the seed longevity L (inverse of the slope of the seed survival curve) for each set of environment (moisture and temperature) using probit analysis between the viability (v) and the period (p) in days, (ii) secondly, after estimating L a linear model is fitted between log L; and log of moisture (m), temperature(t) and quadratic temperature (t^2). The second relationship has been established after the application of a linearizing transformation (log) to the seed longevity (L). Modified viability equations (Ellis and Roberts, 1980a) defining longevity are as follows:

Model I. (Original Scale): $L= K_E-C_w\ m-C_Ht\ -C_I\ m.t-C_Qt^2+e$

$R^2 =0.94499$

Parameter	Estimate	S.E	T	Sig. T
K_E	1792.00	271.41	6.602	0.0027
C_w	87.91	20.12	4.369	0.0120
C_H	46.86	9.427	4.971	0.0076
C_I	-1.48	0.446	3.316	0.0295
C_Q	-0.28	0.096	2.888	0.0447

Model II. (Inverse scale): $1/L= K_E-C_I\ m.t + e$

$R^2 = 0.90072$

Parameter	Estimate	S.E	T	Sig. T
K_E	-0.203267	0.053739	3.782	0.0069
C_I	8.24666E-04	1.0348E-04	7.969	0.0001

Model III. (log scale): $\log L= K_E - C_I \log m.t + e$

$R^2=0.90395$

Parameter	Estimate	S.E	T	Sig. T
K_E	3.368815	0.27303	12.343	0.0000
C_I	-0.04716	0.00581	8.117	0.0001

Model IV. (Special scale): $L^{1/1.9}= K_E-C_w\log m-\ C_Ht -C_I \log m.t -C_Qt^2 + e$

$R^2 = 0.98391$

Parameter	Estimate	S.E	T	Sig. T
K_E	126.9939	14.96438	8.486	0.0011
C_w	80.07609	13.81944	5.794	0.0044
C_H	2.68035	0.389262	6.886	0.0023
C_I	-1.202638	0.308854	3.894	0.0176
C_Q	-0.012116	0.002714	4.465	0.0111

$$v = K_i-(1/L)p \tag{1}$$

$$K_E - C_w\log m- C_Ht - C_Qt^2 \tag{2}$$

$$L = 10$$

$$v = K_i - p/L \tag{3}$$

where K_E, C_w, C_H and C_Q are the viability constants and K_i is the initial viability; v is the viability on a probit scale and L is the longevity *i.e.* number of days required to loose a unit probit of viability. The longevity coefficients of model (2) are estimated using the multiple linear regression analysis to the following model:

$$\log L= K_E-C_w\log m- C_Ht - C_Qt^2 + e, \tag{4}$$

where e is an error term which is assumed to be independently, identically and normally distributed with constant variance (σ^2) *i.e.*

$$e \sim \text{i.i.d } N(0, \sigma^2). \tag{5}$$

Though, a log linearization transformation has been used practically in all the viability models helping in converting a non linear model to a linear one and thereby making the effects additive in model (4). The required linearization transformation depends upon the parameter λ. A useful family of transformation on the (necessarily positive) response variable Y is given by the power transformation (Box and Cox, 1964)

$$W= (Y^\lambda -1)/\lambda \qquad \text{for } \lambda \neq 0 \tag{6}$$

$$= \log Y \qquad \text{for } \lambda = 0$$

This continuous family depends on a single parameter λ. We will now use the data (Table-1) to estimate this parameter as well as the regression parameter β in the model to be fitted

$$W = X\beta + \varepsilon, \tag{7}$$

where W is a transformed response and ε is an error term. Here W, Y, β and ε are in the matrix notation. There are two main ways to estimate λ as suggested by Box and Cox (1964). However, we have used the maximum likelihood method for estimating the parameter λ. The corresponding values of likelihood function $L_{max}(\lambda)$ for several values of λ in the selected range (-1, 1) were obtained. The values of $L_{max}(\lambda)$ were plotted against. λ . The value of λ which maximizes $L_{max}(\lambda)$ was approximately equal to zero, suggesting again a log transformation for achieving the required additivity. Thus, after the application of the desired transformation, the seed longevity (L) can be linearly related to moisture and temperature in the following form:

$$\log L = f(m, t, \theta) + e, \tag{8}$$

where θ is a parameter vector to be estimated.

When we take log(m) and t as the only explanatory variables and regress them on log(L) we get a model V with the following details:

Model V. (log scale): log $L=K_E-C_w \log m- C_H t + e$
$R^2=0.92975$

Parameter	Estimate	S.E	T	Sig. T
K_E	6.223945	1.026285	6.065	0.0009
C_W	2.765399	0.963299	2.871	0.0284
C_H	0.048609	0.005832	8.335	0.0002

Model V was also obtained by Dickie *et al.* (1985). The quadratic temperature term being insignificant is dropped from the model.

Let us examine critically all the curves, Tukey's theorem and the fitted models in terms of interpretation of effects and their predictive behaviour. Intersecting nature of curves at three levels of temperature clearly indicated the differential response of seed longevity as the level of temperature varies. Temperature and moisture do not exert independent effects is also indicated by the Tukey's theorem which rejects the null hypothesis of "no interaction".

The effect of interaction can be seen on all the scales (Models I to IV). However, the interaction has been more sensitively expressed through model II and III as compared to I and IV. Surprisingly, in these two models (II & III), regression parameters corresponding to temperature as well as moisture are missing. Should we conclude from these models that the seed longevity or the rate of dying of seeds is not at all affected by the temperature or moisture, and it is the interactive effect which is primarily responsible for deterioration of seeds? The reason for this is quite simple. The interaction term (log m.t or m.t) in these models is highly correlated with the response variable (log L or 1/L) and thus, explains the maximum variation in the response variable. Due to this reason, the other main effect terms become almost redundant and do not find significant place in these models. Out of all these five models, model IV explains the maximum variation (98.4%) in the longevity and has been iteratively obtained and appears to approach the true deterministic model. However, this particular model IV fitted on a special scale and other models I, II and III suffer from a severe drawback of simplicity of interpretation of effects, and conclusions drawn from these models are very complicated and messy. These models may behave well under interpolated conditions, but under extrapolated conditions may give even negative estimates of longevity. The only best alternative choice here seems to be model V which does not contain interaction term and has a simplicity in the expression of results. It also behaves well under the extrapolated conditions. We should here clearly differentiate the construction of regression models for prediction purposes from the formulation of theoretical models. Model IV is approaching towards a theoretical model (true relationship), whereas model V has been formulated for the prediction purposes. It is worth noting here that in the model V the log transformation has eliminated the need for second order terms (quadratic and interaction) in the regression equation. Thus, if we are interested in whether there is interaction between temperature and moisture we should not simply use this additive effects model (V) for making conclusions about the presence or absence of interaction as this model has been selected with minimum sample interactions. We should look for a properly formulated model or a model on a reasonable scale on which the effect of interaction could be tested.

Box and Cox (1964) maximum likelihood method when applied to the data suggested a log linearizing transformation to achieve the additivity of effects. While discussing the issue of interaction and transformation we refer to the paper on 'An analysis of transformations' by Box and Cox (1964). In analysis of variance and multiple regression problems we are concerned not merely with finding a transformation which will justify anova assumptions but rather to find, where possible, a metric in terms of which our findings may be succinctly expressed. Thus, we look for a scale on which the effects are additive, *i.e.* to see whether interactions are removable by a transformation. Of course, only a particular type of interaction is removable. Having chosen a suitable candidate from a parameteric family of transformations, we should make the detailed estimation and interpretation of effects on this transformed scale. In longevity models a log scale has been chosen to estimate and interpret moisture and temperature effects. Model III and model V both have been fitted on log transformed data. Though, model III explains a slightly less variation (2.5%) when compared to model V yet, it estimates the interaction parameter with more sensitivity and has only one explanatory variable *i.e.* the interaction component. Longevity parameters (K_E and C_W) estimated through model V have high standard errors and thus, have poor reliability. However, we will prefer selecting and using the model V as interaction(s) are missing in the said model which may cause unnecessary nuisance in the prediction of longevity.

The possible reason, perhaps, for the difference of opinions made in respect of the interactive behaviour of moisture and temperature on seed longevity is the analysis of the data with altogether different objectives. Vertucci *et al.* (1994) analyzed peas experimental data on the original scale and concluded that the temperature and moisture do not exert independent effects, whereas Ellis *et al.* (1991) tried to prove the absence of interaction on the basis of longevity models - models developed with minimum sample interactions.

Logarithmic transformation has been used practically in all the modified viability models to estimate, express, and to predict the effects of moisture and temperature on the seed longevity. We believe this transformation has performed the task of removing the interactions in the additive type of the models suggested by Ellis and Roberts (1980a) and hence there is a need to re-examine these interactions on valid scales through properly formulated models. The purpose of log transformation used in the viability models is to avoid the undesirable interactions to appear in the model which otherwise would have led to messy conclusions. The objective of transformation should not be mistaken here as 'only a mathematical relationship'. It has its own statistical significance. In addition to the reduction in interactions, transformations also reduce non-normality, but most frequently reduce inequality of variance in the data (Scheffe, 1949).

The longevity models should not be used for drawing inferences about the presence or absence of interactions between moisture and temperature as they have been developed for simplicity and for better expression of the effects by avoiding undesired interactions to appear in these models. Thus, we disagree with the statement given by Ellis *et al.* (1991) in reference to the interactive behaviour of moisture and temperature on seed longevity.

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