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A Comparison of the Quantitative Effects of Seed Moisture Content and Temperature on the Accumulation of Chromosome Damage and Loss of Seed Viability in Lettuce

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ABSTRACT

The rate of accumulation of cells containing chromosome aberrations in lettuce (*Lactuca sativa* L.) seeds is a positive function of temperature and moisture content. It may be described by an equation similar to that for loss of seed viability. The relative effect of temperature on the rates of loss of viability and accumulation of chromosome aberrations is the same. In contrast, the relative effect of moisture on the rate of loss of viability is greater than that for the rate of accumulation of aberrations. Hence considerably more chromosome damage accumulates before death in drier lettuce seeds.

Key words: *Lactuca sativa*, lettuce, seed storage, seed viability, seed longevity, chromosomal aberrations, temperature, moisture content.

There is considerable evidence to show that, as seeds age, they accumulate chromosome damage. Both the rate of loss of viability and the rate of accumulation of aberrant cells (those containing one or more chromosome aberrations) increase with an increase in temperature and seed moisture content. Over the ranges of temperature and moisture content in which these changes were first quantitatively investigated, namely 12–18% moisture content (wet weight basis) and from 20 to 45°C, the effects of the storage environment on both symptoms of ageing are similar enough to ensure that, for a given percentage viability, a predictable percentage of aberrant cells may be found in the surviving seeds. This was first shown in barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.) and faba beans (*Vicia faba* L.) (Abdalla and Roberts, 1968), and later confirmed in barley (Murata, Roos and Tsuchiya, 1979, 1981). Moreover, a comparison of the results for barley between the studies of both groups of workers showed almost precise agreement (Roberts and Ellis, 1984).

In lettuce seeds (*Lactuca sativa* L.) investigated over a similar range of moisture contents, we have come to similar conclusions: following storage at temperatures between 30 and 40 °C with moisture contents of 13·0 and 18·1%, relations between percentage viability and percentage of aberrant cells do not differ significantly amongst different seed storage environments (Rao, Roberts and Ellis 1987). Under these conditions, when viability (normal germination) had dropped to 50%, the percentage of aberrant cells was approx. 3–4% (Rao *et al.*, 1987). This compares with values of 3–4% in barley, 6% in peas and 9% in faba beans (Abdalla and Roberts, 1968). However, in lettuce seeds stored at lower moisture contents, the relationship between percentage viability and accumulation of aberrant cells changes so that, for example, the percentage of aberrant cells present following storage when seed viability had fallen to 50% was about 10% at 9·8% moisture content, 18% at 8·1% moisture content, and 72% at either 5·5 or 3·3% moisture content (Rao *et al.*, 1987).

The distribution of life spans of individual seeds is usually normal when they are stored under constant conditions. Thus, survival curves of seeds of lettuce, as of other orthodox species, tend to be

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cumulative normal distributions of negative slope (Ibrahim and Roberts, 1983). The accumulation of aberrant cells in the surviving seeds also approximates to cumulative normal distributions, in this case of positive slope, so that when percentage viability and percentage aberrations are transformed to probits, a linear relation between the two is found (Rao *et al.*, 1987). Since there is a similarity between the distribution of events in time (i.e. for the accumulation of aberrant cells or non-viable seeds), and since both temperature and moisture content affect the rate of accumulation of both, it seemed possible that similar quantitative approaches might be applicable to both symptoms of ageing.

In the case of loss in seed germination it has been shown that the improved viability equation developed for barley (Ellis and Roberts, 1980*a*, *b*) fits all species of orthodox seeds to which it has so far been applied (Ellis, 1988), including lettuce (Kraak and Vos, 1987). The equation

$$v = K_i - p/10^{K_E - C_W \log m - C_H t - C_Q t^2}, \qquad (1)$$

in which v is the probit of percentage viability, p is the storage period (d), m is seed moisture content (%, wet weight basis), t is temperature (°C), K_i is the seed lot constant (equivalent to the probit of initial percentage viability), and K_E, C_W, C_H and C_Q are the species constants (i.e. invariant within a species), predicts percentage seed viability after any storage period over a very wide range of temperatures and moisture contents. By analogy, the frequency of cells showing chromosome aberrations might be given by

$$a = K_{ia} + p/10^{K_{EA} - C_{WA} \log m - C_{HA} t - C_{QA} t^2}, \qquad (2)$$

in which a is the probit of the percentage of aberrant cells, K_{ia} is the probit of the percentage of aberrant cells at the beginning of storage, with other conventions similar to eqn (1). The denominators of the right-hand sides of eqns (1) and (2) are the standard deviation of the distribution of seed deaths in time (σ) and the standard deviation of the distribution of cells becoming aberrant in time (σ_a), respectively. In this paper we report the use of eqns (1) and (2) to re-analyse earlier data (Rao *et al.*, 1987) on lettuce seeds (cv. Trocadero Improved) stored in 12 different environments.

The first step was to carry out probit analyses on the data showing the reduction with time in seed viability (criterion normal germination, i.e. percentage of seeds producing morphologically normal seedlings) and the increase with time in the proportion of aberrant cells (i.e. those showing one or more chromosome aberrations). These analyses confirmed that, as expected, there was no significant difference ($P > 0.25$) amongst the 12 different storage environments in the values of the constants K_i or K_{ia}, the respective values being 2·80 (s.e. 0·04) and −2·62 (s.e. 0·04). These values are equivalent to an initial normal germination of 99·74% and an initial frequency of chromosome aberrations of 0·44%. They are in reasonable agreement with the observations at zero time of 98% and 1%, respectively, and also with the results of a different analysis which provided estimates of 99·2%, and 0·9%, respectively (Rao *et al.*, 1987).

The estimates of σ and σ_a obtained for each storage environment by probit analysis were then subjected to multiple regression analysis, the independent variables being seed moisture content and temperature. For both symptoms of seed ageing the final term of eqns (1) and (2) (i.e. $C_Q t^2$ and $C_{QA} t^2$) did not explain a significant proportion of the variance ($P > 0.25$). Consequently, these terms were omitted from the models fitted. Their omission was not unexpected as the quadratic term quantifies changes in the temperature coefficient (Q_{10}) which can only be detected with results from a very wide range of temperatures (Ellis and Roberts, 1980*a*, 1981). Table 1 shows the estimates obtained for the remaining constants. The estimates for C_H and C_{HA}, sensitivity to temperature, did not differ significantly ($P > 0.25$) between the two symptoms of ageing and thus a common value (equivalent to a Q_{10} of 3·73) is shown. In contrast, the constants K_E and K_{EA} and the constants C_W and C_{WA} did differ significantly ($P < 0.005$), accumulation of chromosome aberrations being less sensitive to differences in seed moisture content than loss of normal germination.

TABLE 1. *Seed viability and chromosome aberration constants of eqns (1) and (2), respectively, for lettuce (with s.e. in parentheses) determined by re-analysis of data presented by Rao* et al. *(1987) and seed viability constants of eqn (1) reported for lettuce by Kraak and Vos (1987)*

Constant	Values from Rao *et al.* (1987)	Values reported by Kraak and Vos (1987)
K_E	6·851 (0·334)	8·218
C_W	3·837 (0·205)	4·797 (0·163)
C_H	0·0571 (0·0047)	0·0489 (0·0050)
C_Q	ns	0·000365 (0·000056)
K_{EA}	6·221 (0·231)	
C_{WA}	2·880 (0·205)	
C_{HA}	0·0571 (0·0047)	
C_{QA}	ns	

ns = No significant effect of this term within this data set (and omitted from model).

Table 1 also shows, for comparison, the values of the viability constants calculated by Kraak and Vos (1987) for loss of normal germination in lettuce seeds. Within the range of environments studied here, any effect of the differences in constant values shown in Table 1 on predicted longevity are less than they appear at first sight since the lower value of K_E determined here is compensated for in calculations by the similarly lower values of the constants showing the sensitivity of longevity to moisture and temperature. The values of the viability constants reported by Kraak and Vos (1987) are probably more accurate since they were based on more treatments and, moreover, detected a significant difference in Q_{10} at different temperatures. However, it is essential when comparing the constants of eqn (1) for loss in normal germination with eqn (2) for increase in chromosome aberrations to use values determined within the same investigation. Thus, the constants derived from these data were used to calculate planes which show the effects of temperature and moisture content on σ and σ_a, together with the experimental values previously determined by probit analysis for each treatment combination (Fig. 1). In addition to describing the variation in seed longevity or the variation amongst cells in the time taken before an aberrant chromosome can be found, the standard deviation (σ or σ_a) can be used to describe the time taken for viability (germination) to fall from one particular value to another or for the percentage of aberrant cells to increase from one given value to another. This can be done by consulting tables showing the area under a standard normal curve. So, the values shown on the *y*-axis in Fig. 1 can be translated into the time taken for germination to drop, for example, from 99·74 to 96·41 % or from 96·41 to 78·81 %; equally, they could be used to describe the time taken for the proportion of aberrant cells to increase, say, from 0·44 to 5·26 %, or from 5·26 to 26·76 %.

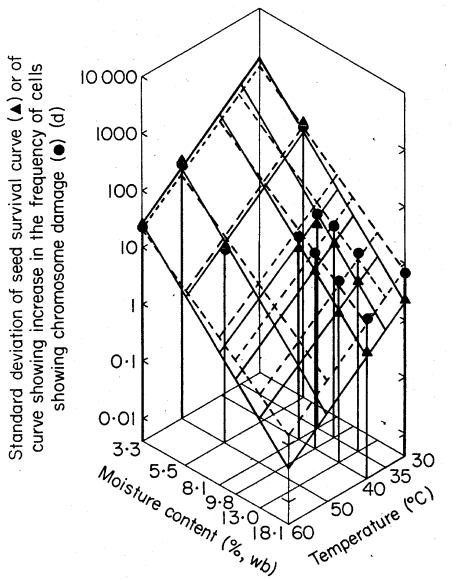

FIG. 1. The effect of seed moisture content and temperature during storage on the standard deviations of the distributions of seed deaths (criterion normal germination) in time (▲) and of cells becoming aberrant (those containing one or more chromosome aberrations during first mitoses) in time (●) for one seed lot of lettuce (cv. Trocadero Improved) which initially showed 98 % normal germination and 1·0 % aberrant cells. The fitted planes shown are those defined by eqn (1) for loss in germination (solid lines) and eqn (2) for accumulation of chromosome aberrations (broken lines) and the values of the constants given in Table 1.

It is clear from Fig. 1 that both temperature and moisture content affect both the loss of seed viability and the accumulation of chromosome aberrations in a similar way. However, as indicated by the different values for the appropriate constants in eqns (1) and (2) (Table 1), a change in moisture content has a relatively greater effect on the rate of loss of germination than it does on the accumulation of chromosome aberrations. Because of this, although loss of seed viability and the accumulation of chromosome aberrations are both slow at low moisture contents, the percentage of aberrant cells can ultimately reach high values before individual seeds finally die. Whereas at high moisture contents, although the accumulation of chromosome aberrations is rapid, loss of seed viability is even more rapid so that the seeds die before a large proportion of aberrant cells has had time to accumulate. Note also that there is no evidence in Fig. 1 of discontinuities in relations between loss in viability and moisture or between increase in chromosome aberration and moisture within the moisture content range investigated.

Since, in lettuce at least, it is now known that the effects of the seed storage environment on both symptoms of ageing (normal germination and chromosome aberrations) differ quantitatively, we have also used the data available here to reconsider the earlier suggestion that the quantitative effects of seed storage environment on normal germination and total germination (defined as radicle emergence) are the same (Ellis and Roberts, 1980*c*, 1981). This was done by subjecting estimates of σ derived by probit analysis for each criterion to multiple regression analysis. As with normal germination, the term $C_Q t^2$ of eqn (1) did not significantly ($P > 0{\cdot}25$) improve the goodness of fit for total germination and it was, accordingly, deleted from the model. The estimates of K_E, C_W,

and C_H determined did not differ significantly ($P > 0{\cdot}25$) between the two criteria and had the common values 6·972 (s.e. 0·305), 3·872 (s.e. 0·175), and 0·0575 (s.e. 0·0044), respectively, i.e. very similar to those provided for normal germination alone (Table 1).

We conclude, therefore, that the quantitative effects of seed storage environment on normal germination and total germination are the same (differences observed being entirely due to differences in K_i). However, although loss of seed viability and the accumulation of chromosome damage are affected by the environment in a similar manner, there are important quantitative differences. Moreover, these are sufficiently large to suggest that loss of viability is not a result of chromosome damage. Since there is generally a correlation between visible chromosome aberrations and with the incidence of heritable point mutations (e.g. Abdalla and Roberts, 1969; Dourado and Roberts, 1984), these results emphasize that under the dry conditions (5 ± 1 % moisture content) recommended for long-term seed storage for genetic conservation (IBPGR, 1985) it is important also to follow the advice for regenerating seed stocks to produce fresh seeds before viability has fallen very far, i.e. not less than 85% in most species (IBPGR, 1985). Because of the practical implications, it would seem advisable to investigate whether the difference in the quantitative effect of seed moisture content on the accumulation of chromosome aberrations and loss of viability in lettuce seeds also occurs in other species, since data obtained at low moisture contents are generally lacking.

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