Primed Lettuce Seeds Exhibit Increased Sensitivity to Moisture during Aging

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Keywords: *Lactuca sativa,* seed longevity, viability equation, germination, seed moisture content

Abstract

The Ellis-Roberts seed viability equation is accepted as an accurate predictor of seed longevity over a range of storage temperatures and moisture contents (MC). One application of this model is to identify different combinations of seed MC and temperature that can result in similar seed storage lifetimes. The present study was conducted to determine whether the reduction in storage life of primed seeds is consistent with the predictions of the Ellis-Roberts equation across a range of seed MC and temperatures. Seeds of two lettuce (*Lactuca sativa* L.) varieties (a romaine and a crisp-head type) were primed with polyethylene glycol and along with untreated seeds were adjusted to two different MCs (6% and 9%) and then aged in two different temperature environments (48°C and 38°C, respectively). Control seeds in both storage environments conformed to the Ellis–Roberts equation. Primed seeds aged faster than non-primed seeds but also exhibited different rates of viability loss between the storage environments. Primed seeds stored at 6% MC and 48°C aged slower than those stored at 9% MC and 38°C. These data suggest that priming causes lettuce seed to have a heightened sensitivity to the adverse effects of moisture during storage. To further investigate this observation, the moisture absorption properties of primed and non-primed seeds from both varieties were examined by equilibration over saturated NaCl solutions in sealed containers at 20°C. Primed seeds tended to have higher MC than non-primed seeds, although differences were seldom significant. Thus, an alteration of the RH/MC relationship for primed seeds could not be confirmed as a cause for the differential aging rates between equivalent environments. In conclusion, the predictable effects of MC and temperature on non-primed lettuce seeds as described by the Ellis-Roberts equation may not hold true for primed seeds.

INTRODUCTION

Lettuce seeds are commercially primed to prevent the induction of thermo- or photo-inhibition during the germination process. A secondary advantage of lettuce seed priming is a faster rate of radicle emergence across temperatures when seeds are planted. The combination of these advantages allows for more rapid and uniform field emergence, less variation in plant size, and thus a greater number of cartons per acre at harvest pack-out.

A major disadvantage of priming lettuce seeds is a shorter shelf-life as compared to non-primed seeds. For example, priming protocols that increased germination rates were also shown to decrease longevity under controlled deterioration conditions (10% MC and 40°C) (Tarquis and Bradford, 1992). Primed lettuce seeds stored at 45°C and 50% relative humidity (RH) were reported to exhibit slower and less synchronous germination than the non-primed seeds in as little as 14 days of storage (Hacisalihoglu et al., 1999). Priming has also been shown to shorten seed longevity in many other species (Bruggink et al., 1999). We are not aware of any lettuce priming protocol that can eliminate temperature and/or photo-inhibition without resulting in a more rapid decrease

Proc. IVth IS on Seed, Transplant and Stand Establishment
of Hort. Crops
Ed.: D.I. Leskovar
Acta Hort. 782, ISHS 2008

in longevity.

Because of a shortened longevity, primed seed products from different companies are often tested by potential customers to determine their relative shelf lives. A desirable primed lettuce product is one that can be stored or “carried-over” from one year to the next. If all the primed seeds of a variety are not planted during the current “time slot,” the seeds can be saved and planted during the next year’s time slot. This ability to carry over seed from one year to the next reduces production costs and eases inventory management problems. However, waiting the 6 to 18 months necessary for primed seeds lots to show signs of deterioration under good storage conditions is not feasible for seed dealers and growers. To determine whether primed lettuce products have the potential to store well, aging of seeds may be accelerated by increasing both seed MC and storage temperature. The assumption made is that longevity differences between primed products will remain the same independent of aging conditions. However, this assumption has not been evaluated experimentally. Furthermore, primed lettuce seeds are dried to a low MC (usually < 7%) and then packaged in air-tight containers so that they remain at a low MC during storage. Elevating the primed seed’s MC to a higher level than packaged, so they age faster, may not be indicative of the actual aging process they undergo in sealed containers.

The seed viability equation (Ellis and Roberts, 1980) has been used successfully to describe orthodox seed longevity in relation to seed moisture content (MC) and temperature (T) for many species, including lettuce (Kraak and Vos, 1987):

$$v = K_i - (1/\sigma)\, p$$

where v is the probit of percent viability after a period of p (days) in storage at a given MC (% of fresh weight) and temperature (T, °C), K_i is an index of the initial seed quality of the lot, and σ is defined as the standard deviation of individual seed life spans and varies with storage conditions according to the following equation:

$$\text{Log}\ \sigma = K_E - C_w \log MC - C_H T - C_Q T^2.$$

The constants K_E, C_w, C_H, and C_Q are species constants used to quantify the relative effects of moisture (C_w) and temperature (C_H and C_Q) on longevity. The K_E constant provides a measure of the sensitivity of a species to the effects of temperature and moisture on seed longevity (Roberts, 1986).

Another use of the Ellis-Roberts equation in predicting lettuce seed aging is that different T and MC values can be inserted to determine how v (probit of percent viability) varies with storage duration (p). Higher MC and T values will always lower v. However, different reciprocal inputs (i.e., increased T along with decreased MC) can be selected that predict the same rate of deterioration.

The purpose of this study was to test whether reciprocal T and MC environments predicted by the viability equation to result in the same longevity for non-primed lettuce seeds would also result in the same relative longevity, albeit a more rapid one, for primed lettuce seeds. Deviations from this expectation may reveal what aspects of longevity are altered by priming. Also, deviations from this expectation may reveal whether elevating the seed MC during accelerated aging tests will provide the same type of aging stress that the seeds undergo during commercial storage.

MATERIALS AND METHODS

Seeds of two lettuce varieties, ‘Big Ben’ and ‘Parris Island Cos’ (hereafter referred to as ‘PIC’) were obtained from Pybas Seed Co. (Salinas, CA). For all primed seed studies, 200 g of seeds from each variety were primed for 24h in an aerated 2 L solution of Carbowax PEG 8000 (336 g L^{-1}) (Dow Chemical, Midland, MI) at 15°C (Bradford, 1986) and allowed to dry overnight on paper towels at room temperature.

To obtain desired MC, seeds were first placed in sealed bottles and allowed to equilibrate for 3 to 5 d at 5°C. The MC for primed and non-primed seeds were adjusted to 6% or 9% (fresh weight basis) by removing water using desiccant bags containing $CaSO_4$ (A.W. Hammond Drierite, Xenia, OH) or by adding water using the following formula:

$$\text{Water added} = [(MC_{desired} - MC_{initial}) \times \text{seed wt}] / [100 - MC_{desired}].$$

All MC measurements were made by oven drying six 1-g replicates for 1h at 130°C.

All seeds were stored at 5°C during MC equilibration with constant mixing to aid in uniformity. Treatments required between two and three weeks of adding or eliminating water to achieve their final MC. After MC adjustment, primed and non-primed seed of each variety x aging environment were divided into 10 sampling dates consisting of 4 replications each. Each replicate contained 2.5 g of seeds in a 5 ml glass bottle enclosed in parafilm. All 4 replicates were sealed together in foil-lined poly-bags and submerged in water baths maintained at 38 or 48°C. After aging and before planting, two 1-g seed samples from each replicate within each sampling date were used for a final MC determination. The mean MC ranges for the 4 seed treatments (variety x seed priming treatment) targeted at 6% and 9% MC were 5.94 – 6.04% and 8.97 – 9.05%, respectively. These mean MCs were calculated for seeds from each of the 8 aging treatments (variety x seed treatment x aging environment) by averaging the mean MC from each sampling date analyzed within a treatment. The number of sampling dates used for each mean aging MC determinations ranged from 6-9 among the 8 treatments. Only sampling dates for which mean MC (averaged across the 4 replicates) were found to be within the desired MC ± 0.1% were used in further data analyses.

To determine percent germination (viability) during aging, 100 seeds from each of the 4 replicates were planted for each sampling date and germinated according to AOSA (1993) guidelines. After 7 days, seeds were counted and classified into normal, abnormal, and non-germinated according AOSA seedling evaluation guidelines (AOSA, 1992). Only seedlings classified as normal were used in the analyses.

MC and temperature values used to define the two aging environments were obtained from the Ellis-Roberts equation (Ellis and Roberts, 1980) using the species constants for lettuce published by Kraak and Vos (1987): $K_E = 6.895$, $C_w = 4.2$, $C_H = 0.0329$, $C_Q = 0.000478$. Storage environments of 6% MC at 48°C and 9% MC at 38°C were predicted to result in identical viability loss time courses. Viability data were transformed to probits and analyzed using CoStat (Cohort Software, www.cohort.com).

To determine seed MC of primed and non-primed lettuce seeds under different RH environments, 10 g of seeds from 4 varieties were placed on accelerated aging trays (Hoffman Manufacturing, Albany OR) over saturated salt solutions of either K_2CO_3 (50 g per 100 ml distilled water) or NaCl (75 g per 100 ml distilled water) in clear polystyrene containers for 2 weeks at 20°C. There were 4 replications per treatment. Seed MCs were determined as described above. Each replicate mean was an average of 4 samples.

RESULTS AND DISCUSSION

The priming protocol used in this aging study successfully increased the percentage of seeds that could germinate at 30°C in the dark. Primed ‘Big Ben’ and ‘PIC’ seeds germinated 99 and 100% after four days versus 1% and 2% for the non-primed controls, respectively. Priming also decreased the time to 50% radicle emergence at 20°C in the light from 21.5 and 21.9h for the non-primed ‘Big Ben’ and ‘PIC’ seeds, respectively, to 17.5 and 15.0h for primed seeds of the same varieties. Thus, this priming protocol enabled these seeds to germinate under conditions that could impede their stand establishment if they were not primed.

Primed seeds of both varieties deteriorated faster than their respective non-primed controls within both aging environments (Table 1). When averaged over aging environments, non-primed seeds of ‘Big Ben’ and ‘PIC’ decreased to 50% viability in 21 and 29 days, respectively, whereas the primed ‘Big Ben’ and ‘PIC’ seeds decreased to 50% viability in 9 and 8 days, respectively. The faster rate of deterioration for primed than for non-primed seed treatments confirms previously reported results in lettuce (Tarquis and Bradford, 1992).

In order to obtain the best estimates of the intercept, K_i (initial seed quality), and the slope, $1/\sigma$ (rate of deterioration), viability data collected prior to the onset of rapid aging (viability of a sampling date was > 95%) were excluded from the probit analyses

(Tarquis and Bradford, 1992; Kraak and Vos, 1987). Exclusion of this lag phase data (defined as the time between the start of aging and the first significant decline in seed viability) allows for an estimation of K_i that is more indicative of the initial quality of a seed lot than can be observed using the normal germination test (Kraak and Vos, 1987). For both varieties, the K_i's for the primed seed treatments tended to be lower than for the non-primed seed treatments (Table 1), although differences were not significant. The more rapid decline in viability for the primed 'Big Ben' seeds stored at 6% MC + 48°C as compared to either non-primed 'Big Ben' storage treatment could be accounted for by a lower K_i, since the $1/\sigma$ of these storage treatments were not significantly different. Tarquis and Bradford (1992) also reported that PEG priming resulted in lower K_i values for lettuce seeds that were primed than for non-primed seeds. This effect can be attributed largely to a reduction for primed seeds of the length of the lag phase before viability begins to decline during aging. Priming can therefore decrease initial seed quality if initial quality is measured by the seed's ability to maintain shelf life over time. Under field stand establishment conditions, priming increases the initial seed quality of a lot because primed seeds can emerge under conditions that non-primed seeds can not. Thus, the effect of priming on lettuce seed lot quality depends upon the circumstances in which seed quality is being measured.

Larger differences in the rate of seed deterioration ($1/\sigma$ values) were observed between aging environments for primed than for non-primed seeds in both varieties. For example, the $1/\sigma$ value for the primed 'Big Ben' seed treatment was 3 times greater when seeds were aged in the 9% MC + 38°C environment (-1.34 probits day^{-1}) than when seed were aged in the 6% MC + 48°C environment (-0.26 probits day^{-1}). $1/\sigma$ values for the non-primed 'Big Ben' seed treatments were similar between the aging environments (-0.32 and -0.29 probits day^{-1}, respectively). The lack of differences in $1/\sigma$ values for non-primed seeds was predicted by the Ellis-Roberts equation and confirms the equation's validity and the similarity of these aging environments. For primed seeds, the large difference in $1/\sigma$ values between aging environments (i.e., 6% MC +48°C vs. 9% MC + 38°C) indicates that primed seeds respond differentially to environments that produce similar aging rates in non-primed seeds. Deterioration rates of primed seeds are not accurately described by the current constants used in the Ellis-Roberts equation, which were developed using non-primed seeds, and further studies with primed seeds across a broader range of MC and temperatures would be required to determine the changes needed in the equation to account for their aging characteristics.

The more rapid aging of the primed seeds at 9% MC versus 6% MC irrespective of storage temperature suggests that priming increased the sensitivity of seeds to the aging effects of higher MC. For the purposes of this study, the lack of reciprocity between the longevities of primed seeds stored in the two storage environments indicates that accelerating the aging of primed lettuce seed by increasing their MC may well result in an underestimation of their potential shelf life when stored at lower MC, as would be normal practice with hermetically sealed packaging.

The cause for the lack of reciprocity in primed seed viability loss rates between the aging environments is not known at this time. PEG priming has been reported to alter the water sorption properties of embryonic tissue of mung bean seeds (*Vigna radiata* L. Wilczek) by increasing the number of weak water-binding sites (Sun et al., 1997). Primed seeds were observed to have slightly higher MCs, as compared to non-primed seeds at the same water activity (RH/100). An increase in the number of weak water-sorption binding sites could partially explain the faster decline in viability for primed lettuce seeds stored at 9% MC as compared to the 6% treatment. However, the large $1/\sigma$ differences between aging environments for the primed lettuce seed that we report may not solely be due to a change in water sorption properties. We measured the MC of primed and non-primed seeds of 'Big Ben' and 'PIC' as well as two other varieties after equilibration over saturated salt solutions (Table 2). The primed lettuce seeds of all four varieties exhibited slightly higher MC than the non-primed seeds after two weeks of equilibration over the saturated NaCl solution, but differences were non-significant except for the 'Big Ben' seeds stored at 9% MC. Little or no difference in MC was observed between the primed versus the non-primed seeds of ‘Big Ben’ and ‘PIC’ equilibrated at 6% MC. Thus, more rapid aging of primed lettuce seeds does not appear to be due to altered seed moisture absorption properties.

In a later study, Sun et al. (2003) showed that priming also caused an increase in biological surfaces of embryonic tissue in mung bean. A greater surface area for biological reactions to occur could cause primed seed to exhibit a heightened predisposition to the aging effects of moisture. This heightened predisposition to moisture could cause primed lettuce seeds to age faster at 9% than at 6% MC, irrespective of temperature, while no difference would be noticed in the non-primed seed. This possibility remains to be tested in lettuce.

These results illustrate the need for caution when increasing the MC of primed lettuce seeds for the purpose of accelerating their aging. Commercial quantities of primed lettuce seeds are normally packaged to prevent the migration of moisture between the seed and the environment. Increasing the MC of primed lettuce seeds above that at which they were packaged may not provide an accurate prediction of their potential shelf-life, especially when comparing different primed seed products. Different primings may increase the seeds’ sensitivity to moisture to different extents such that the results from an accelerated aging test may not mimic the natural aging that these products undergo at low MC. Thus, the results of accelerated aging tests at elevated MC may not accurately reflect the quality of different priming protocols for achieving improved stand establishment under stressful conditions.

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Tables

Table 1. The effects of priming on longevity (days to 50% viability), initial storage quality (K_i), and rate of deterioration ($1/\sigma$) for 'Big Ben' and 'PIC' lettuce seeds stored in two environments (6% MC + 48°C and 9% MC + 38°C). K_i (y-intercept) and $1/\sigma$ (slope) values were generated by linear regression. The coefficient of determination (R^2) of the regression is also provided.

Variety	Seed Treatment	Storage environment	Days to 50% viability ± 95% CL	K_i ± 95 % CL	$1/\sigma$ ± 95 % CL	R^2
Big Ben	Non-primed	6% MC + 48°C	21.9 ± 1.4	11.2 ± 1.0	-0.29 ± 0.04	0.99**
		9% MC + 38°C	20.4 ± 1.4	11.6 ± 7.1	-0.32 ± 0.28	0.82*
	Primed	6% MC + 48°C	14.4 ± 0.3	8.5 ± 1.1	-0.26 ± 0.08	0.95**
		9% MC + 38°C	3.2 ± 0.6	9.2 ± 1.6	-1.34 ± 0.36	0.98**
PIC	Non-primed	6% MC + 48°C	27.3 ± 1.1	14.3 ± 2.4	-0.34 ± 0.08	0.98**
		9% MC + 38°C	30.8 ± 1.3	12.1 ± 3.8	-0.24 ± 0.11	0.89**
	Primed	6% MC + 48°C	11.9 ± 0.4	10.9 ± 4.4	-0.49 ± 0.34	0.87**
		9% MC + 38°C	4.1 ± 0.5	8.8 ± 2.6	-1.19 ± 0.54	.090**

CL- Confidence limits

*, ** Significant at the 0.05 and 0.01 level of probability, respectively.

Table 2. Moisture content (MC) of primed and non-primed lettuce seeds from 4 varieties after 2 weeks of equilibration over saturated salt solutions at 20°C.

Variety	Seed Treatment	Seed MC over sat. K_2CO_3 solution %	Seed MC over sat. NaCl solution %
Big Ben	Non-primed	6.44	9.49
	Primed	6.44	9.85
	Significance	NS	*
PIC	Non-primed	6.45	9.96
	Primed	6.49	10.14
	Significance	NS	NS
Big Star	Non-primed	-	9.60
	Primed	-	9.96
	Significance		NS
Tango	Non-primed	-	9.58
	Primed	-	9.79
	Significance		NS

*Significant at 0.05 probability level according to 2-tailed student's "t" test.