Plant Physiol. (1990) 94, 1019–1023
0032-0889/90/94/1019/05/$01.00/0

Received for publication May 3, 1990
Accepted June 21, 1990

Theoretical Basis of Protocols for Seed Storage

Christina W. Vertucci* and Eric E. Roos

U.S. Department of Agriculture, Agricultural Research Service, National Seed Storage Laboratory, Fort Collins, Colorado 80523

ABSTRACT

The protocols presently established for optimum seed storage do not account for the chemical composition of different seed species, the physiological status of the seed, and the physical status of water within the seed. The physiological status of seeds from five species with varying chemical compositions was determined by measurements of rates of oxygen uptake and seed deterioration. The physical status of water was determined by water sorption characteristics. For each species studied, there was a specific moisture content for the onset of respiration, chemical reactions, and accelerated aging rates. The moisture contents at which these physiological levels were observed varied among the species and correlated with the lipid content of the seed. However, the changes in physiological activities and the physical status of water occurred at specific relative humidities: 91% for the onset of respiration, 27% for the increased rates of thermal-chemical reactions, and 19% for optimum longevity. Based on these observations, we propose that equilibrating seeds between 19 and 27% relative humidity provides the optimum moisture level for maintaining seed longevity during long-term storage.

Maintaining the genetic diversity of plants has become a global concern. The International Board for Plant Genetic Resources (IBPGR) was established in 1974 to promote and coordinate an international network of genetic resource centers (11). Much of the germplasm is stored as seeds in numerous facilities around the world. One such facility is the U.S. National Seed Storage Laboratory (NSSL) in Fort Collins, CO. The responsibility of NSSL is to store seeds for indefinite periods of time, maintaining the highest possible viability. In achieving that goal, determination of the best conditions for seed storage is critical.

While scientists have known for many years that dry, cold conditions will increase shelf life of biological material, the optimum moisture level and temperature are poorly understood. Since studies of the optimal environment for storage are intrinsically difficult because they take years, scientists have relied on experiments of seed aging under less optimal conditions and then extrapolated beyond their data. Based on these empirical data, equations have been derived to predict the rate of seed deterioration under various conditions (6, 21). The assumption of these equations has been that the effect of water content on the rate of seed deterioration is a logarithmic relationship and thus, the drier the tissue is, the longer it will maintain viability (5–7).

The viability equations are the basis of the storage recommendations of the IBPGR (3). Based on limited empirical information, calculations from the viability equations, and practical considerations, the IBPGR recommends that seeds be stored at moisture contents between 3 and 7%, depending on the seed (10). For seeds with poor storage characteristics, further drying may be recommended (3, 5, 7). Unfortunately, these recommendations do not aid the gene bank operator in determining the optimal moisture content for storage of a particular seed species, cultivar, or lot.

A more theoretical approach to the study of the effect of water content on seed deterioration would alleviate some of the intrinsic difficulties of a strictly empirical approach. Protocols developed from physical principles will obviate the need to determine optimal conditions for each species, varying cultivars within a species, and specific tissues within an individual seed.

Intuitively, we know that dehydration increases shelf life of seeds because metabolism is diminished. Pathogen growth, depletion of food reserves, and concentration of deleterious by-products of metabolism can be inhibited in the dry state. One of the reasons that drying slows reactions can be understood by considering the Arrhenius equation:

$$k_2 = Z \exp(-E_a/RT) \quad (1)$$

where k_2 is proportional to the rate of a reaction, Z is a coefficient related to the rate of molecular collisions, E_a is the activation energy necessary for the reaction, R is ideal gas constant, and T is temperature. The frequency of molecular encounters (Z) is a function of the flux of molecules, describable by Fick's First Law of Diffusion. Thus,

$$Z \approx 4\ \pi r D L \quad (2)$$

where r is the radius of a molecule and L is the concentration, and D is the diffusion coefficient. By the Stokes-Einstein relation,

$$D = kT/6\pi\eta r \quad (3)$$

where k is the Boltzmann distribution constant, T is temperature, η is the viscosity, and r is the radius of the molecule. Viscosity, the reciprocal of fluidity, is the property of molecules within a fluid to break weak bonds and flow from one site to another. As such, fluidity should follow a Boltzmann distribution:

$$1/\eta \approx \exp(-E_a'/RT) \quad (4)$$

where E_a' is the intermolecular binding energy. By combining equations 1 through 4 and setting $kL = R$, we have

$$k_2 \approx 2\ RT/3 \exp(-E_a'/RT) \exp(-E_a)/RT) \quad (5)$$

Table I. *Moisture Contents (MC) and Relative Humidities (RH) at which Changes in Physiological Activities are Observed for Seed Species with Various Chemical Compositions*

MC data are interpolated from Figures 2 through 4. RHs corresponding to critical moisture contents are determined from the isotherms presented in Figure 1.

Crops	Lipid content	Critical Levels of Moisture for Various Physiological Activities					
		Onset of respiration		Moisture level below which effect of ΔH_{SORP} on reaction rates is negligible		Moisture level at which maximum vigor after storage at 35°C is obtained	
		MC	RH	MC	RH	MC	RH
	%[a]	g/g[b]	%	g/g	%	g/g	%
Sunflower	58	0.17	92	0.034	28	0.031	18
Peanut	45	0.19	92	0.043	25	0.034	16
Lettuce	37	0.21	91	0.055	29	0.047	21
Soybean	20	0.24	91	0.067	28	0.059	20
Pea	2	0.27	90	0.085	28	0.071	19
R^{2c}		0.997		0.992		0.970	

[a] Lipid content expressed as % dry weight. [b] Moisture content as g H_2O/g dry wt. [c] R^2 is correlation coefficient for regression of lipid content *versus* MC.

In a viscous system, such as a dry seed, the rate of chemical reactions can be greatly influenced by the intermolecular binding energy of the solvent, *i.e.* water. The energy for the physical sorption of water is about −57 kJ/mol, compared to about −20 kJ/mol for hydrogen bonding between water molecules; thus, the viscosity of a system where all of the solvent water becomes physically sorbed can be increased by as much as 6 orders of magnitude. Consequently, dehydration increases the viscosity and dramatically reduces reaction rates in aqueous solutions to negligible levels.

In this paper, the principles of increased viscosity in the aqueous domain of seeds are compared with measurements of physiological activity. Evidence on the physical and physiological status of five species of seeds as a function of moisture content suggests the specified moisture content recommended by the IBPGR may be arbitrary and, in some instances incorrect, and that the optimal moisture level for storage can be identified more readily by the relative humidity than the seed moisture content.

MATERIALS AND METHODS

The five species studied represent an array of dicotyledonous seeds with varying chemical compositions: sunflower (*Helianthus annuus,* cv No. 452, Sigco Research, Inc.), peanut (*Arachis hypogea,,* cv Spanish, Burpee Seed Co.), lettuce (*Lactuca sativa,* cv Waldmann's Green, Burpee Seed Co.), soybean (*Glycine max,* cv Williams 82, Dewine Seed Co.), and pea (*Pisum sativum,* cv Alaska Field, Burpee Seed Co.). The lipid content of the seeds were determined by quantitative extractions with a 2:1 chloroform:methanol solution (Table I). Seeds were stored at 5°C prior to use.

The physical status of water in seeds was evaluated using moisture sorption isotherms. Whole seeds were equilibrated at 15 and 25°C over various saturated salt solutions according to methods described earlier (27, 28). The moisture contents of seeds are expressed on a dry weight basis, dry weights being determined after heating material at 95°C for 5 d. The strength with which water is associated to seed macromolecules (ΔH_{sorp}) was determined by van't Hoff analyses of isotherms measured at 15 and 25°C as described previously (27, 28). Values of RHs at given water contents were determined by curvilinear interpolation of the isotherms.

The physiological status of seeds at different moisture contents was determined by measurements of oxygen uptake and rates of seed deterioration. Oxygen uptake was measured manometrically at 25°C in a Gilson Differential Respirometer (27). The moisture contents of seeds were manipulated by storing seeds over various saturated salt solutions (<0.23 g/g[1]), or by adding known quantities of water to preweighed samples (>0.15 g/g). Seeds (at least 1 g of dry material), adjusted to different moisture contents, were loaded into Warburg flasks containing 0.1 mL of 60% (w/w) KOH solution and allowed to equilibrate for 2 h before oxygen uptake measurements were taken. In dry tissues, displacement of manometer fluid was measured every hour for 8 h. In wetter tissues, displacement was measured more frequently. Oxygen uptake rates are expressed as μmol/h/g dry wt of the tissue. Each moisture level was replicated four times.

The effect of moisture level on the rate of seed deterioration was determined by storing seeds at different water contents at 35°C for 2 to 9 months. Moisture contents were maintained by storage over silica gel or saturated salt solutions with RHs between 0 and 45%. After the prescribed storage period, seeds were removed and germinated. To avoid complications from imbibitional injury, seeds were humidified to 0.2 g/g by overnight storage at 100% RH before they were rolled in paper towels and watered. The radicle length of the seedling was measured after incubation at 25°C for 72 (lettuce), 96 (pea, soybean, sunflower), or 120 h (peanut). Seed vigor is expressed as the mean radicle length of 25 seeds × the percentage germination.

[1] Abbreviation: g/g, g H_2O/g dry wt.

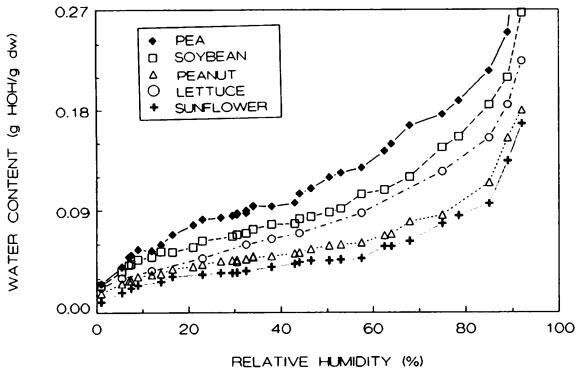

Figure 1. Moisture sorption isotherms of five seed species. Data were collected at 25°C. dw, Dry weight.

RESULTS

Moisture sorption isotherms of whole seeds exhibit the expected reverse sigmoidal shape, which can be divided into three regions: a convex region at RHs below 20%, a linear region at RHs between 20 to 65%, and a concave region at RHs above 75% (isotherms measured at 25°C are given in Fig. 1). The extent of the water sorption varied with the species. Seeds with high lipid contents sorbed less water.

Estimates of water binding enthalpies (ΔH_{sorp}) based on van't Hoff analyses of water sorption isotherms of five species of seeds can be substituted for E_a' in Equation 5. Assuming that the activation energy of a reaction does not change with moisture content (*i.e.* $-E_a$ is constant), the relative effect of decreases in water content (and concomittant increases in viscosity) on the rate of chemical reactions in an aqueous milieu can be predicted as in Figure 2. Since the intermolecular binding energy of water is about −20 kJ/mol, when ΔH_{sorp} is greater than −20 kJ/mol, there should be little further contribution of ΔH_{sorp} to the viscosity term. Consequently, when the rate coefficient in Figure 2 is less than about 3.4×10^{-4} (substitute 20,000 for E_a' in Eq. 4) reactions become much slower. The moisture contents at which reaction rates are predicted to decrease because of the increasing viscosity of the medium correlate with the lipid content of the seed and range from 0.031 to 0.085 g/g for sunflower and pea seeds, respectively (Table I). Desiccating seeds below these moisture levels potentially can limit the rate of chemical reactions by several orders of magnitude.

Rates of oxygen uptake as a function of water content for the five species studied demonstrate that some oxidative activity is observable at nearly all moisture levels (Fig. 3). At moisture contents ranging from 0.17 to 0.27 g/g (Table I), oxygen uptake increases sharply with water content. From studies of respiratory quotients, ATP synthesis, and respiratory inhibitors, we believe that the moisture content at which oxygen begins to be consumed rapidly marks the moisture content at which mitochondrial electron transport is functional (16, 26). The oxidative activity observed at lower moisture contents is probably not a result of mitochondrial electron transport (16, 26, 27), but is indicative of other chemical reactions, for example lipid peroxidation, occurring in seeds. Thus, reducing water content is not totally efficient in preventing physiological and detrimental chemical activity.

Measurements of the effect of moisture content during storage at 35°C on seed vigor demonstrate that there is an optimal moisture content, above and below which seed vigor is depleted more rapidly (Fig. 4). The rate of deterioration varied with seed species. Significant differences ($\alpha < 0.05$) between the driest treatment and the optimum treatment required 2 months for soybean and sunflower and 4 months for peanut, pea, and lettuce. Deterioration was progressive with time and reduction in percentage germination was eventually observed for seeds stored at 1 to 8% or greater than 40% RH within 6 to 9 months (data not shown). Data for each species were fit to a second order polynomial equation ($r^2 = 0.89$, soybean; 0.71, pea; 0.64, lettuce; 0.72, peanut; and 0.87, sunflower) and the optimum moisture content for storage at 35°C was calculated from the first derivative of the equation. The optimum moisture levels for storage ranged from 0.031 to 0.071 g/g for sunflower and pea seeds, respectively (Table I).

DISCUSSION

These experiments were conducted to compare the moisture contents at which changes in the physical and physiological status of seeds are observed for different seed species. The moisture contents at which these changes are observed differ among species, and correlate with the lipid content of the seed (Table I). Seeds with higher lipid contents have lower thresholds of respiration and lower moisture contents for optimum storage. It is evident, then, that values for optimum storage based on moisture content are somewhat arbitrary. They do not account for the chemical composition of the seed which varies among species, among cultivars of the same species, and among tissue types in an individual seed. Since the growing portion and storage structures of the seed are often compositionally different, the recommended 5 ± 2% moisture

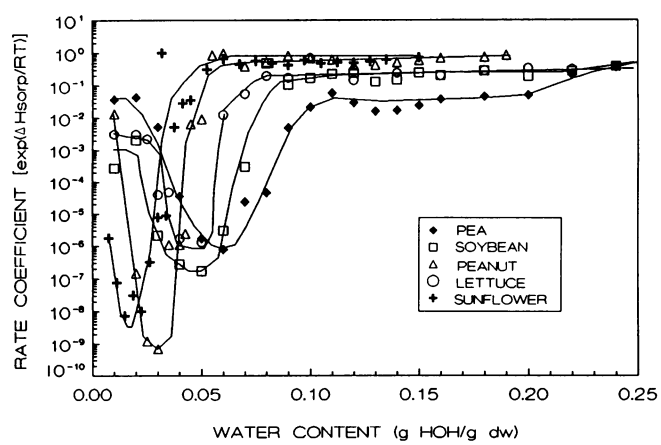

Figure 2. Relationship between moisture content of various seeds and Z, the preexponential rate coefficient in Equation 1. Values are calculated by combining equations 2 through 4 and substituting H_{sorp} values calculated from van't Hoff analyses of isotherms at 15 and 25°C for E_a'. dw, Dry weight.

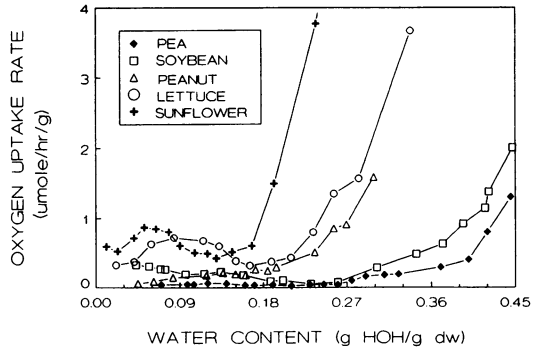

Figure 3. Relationship between moisture content and oxygen uptake for five seed species. Each point represents the mean of four replicates. The standard error of the mean ranged from 0.015 to 0.04 μmol O_2/h/g dry weight (dw).

content may not even pertain to the tissue that is most important in terms of the viability of the seed.

Comparisons between the physiological data (Table I) and isotherms (Fig. 1) suggest that the RHs at which physiological changes are evident are similar among the species tested. The onset of respiration occurs at a RH of 91 ± 1% in all five species. Similarly, the ΔH_{sorp} 'data' show a threshold at a RH of 27 ± 2%. Interpretation of the RH for optimum storage using the sorption curves in Figure 1 is difficult since the longevity data were collected at 35°C and isotherms are for 25°C. Previous studies have shown that water sorption isotherms vary predictably with temperature (28): at a given RH, less water is sorbed at higher temperatures. Thus, the actual RHs for the aging data collected at 35°C will be higher than those interpolated from isotherms collected at 25°C. The optimal RH for seed storage should, therefore, be slightly higher than the 19 ± 2% predicted by our data.

Several studies have shown that the properties of water change discretely with moisture content of protein or biological tissues (2, 16, 24, 26), and also that the physical status of water changes at specific relative humidities (16, 20, 26, 30). It has been suggested that changes in the physical status of water are associated with changes in the physiological activities of seeds (16, 26–30). Thus, it is more relevant to discuss the changes in the physiological status of seeds in thermodynamic terms rather than by moisture content. Such an approach is frequently used in the food science literature (15, 23). Given this premise, we suggest that at a given RH, seeds with varying chemical compositions will be at a similar physiological status and that RH, rather than moisture content, can best define the conditions which optimize seed longevity.

The RH at which seeds show optimal storage performance is not precisely known. The data presented in this paper suggest that the upper limit of the optimum RH should be at the point where the reaction rates are slowed because of the increasing viscosity (about 27%, Table I).

Estimates of the lower limit of the optimal RH for seed storage are more difficult to make. As RH is reduced below 27%, calculations based on changes in viscosity of the aqueous milieu predict a reduction in reaction rates by several orders of magnitude. These estimates of the effects of water content on rates of reactions often do not correspond to measurements of the effects of water content on rates of seed aging. For example, a soybean stored at 15% water and 25°C will lose viability within 4 weeks, but if stored at 5% water it is unlikely that it will remain viable for 75 years (The P50 for soybean longevity is about 3.7 years [20].) Thus, predictions of the optimum RH for storage based on calculations of viscosity and Arrhenius behavior are not supported by empirical evidence. This may be because (a) all of the water in a soybean seed with a water content of 5% is not physically sorbed (and so the estimates of viscosity are inaccurate), (b) some of the aging reactions do not occur in an aqueous domain and so are not governed by the solvent properties of water, or (c) other factors besides the rate of diffusion are involved in the aging kinetics.

Since the theoretical considerations based on water sorption isotherms are not reliable predictors of aging kinetics, we must rely on empirical observations to determine (a) whether there is a lower limit to the RH at which seeds store best, and (b) what that lower limit may be. At RHs between 20 and 92% (about 0.06 and 0.25 g/g for soybean seeds), rates of oxygen uptake by soybean and pea correlate with rates of seed deterioration predicted by the longevity equations of Roberts and co-workers (27). However, at RHs below about 20%, oxygen uptake does not decline with further decreases in moisture level (Fig. 3) and is disproportionately high compared to seed longevity predicted by the viability equations (27). Reasons for this observation are unknown, but it is suggested that oxidative reactions do not decline with further decreases of water content because (a) viscosity is not observed to increase further (Fig. 2), or (b) the reactions occur in a nonaqueous milieu. The latter hypothesis is consistent with the idea that lipid peroxidation is largely responsible for deterioration of dry seeds (20).

Actual measurements of seed deterioration under very dry conditions have shown that the viability equations fail (6, 8) and that either very low moisture contents have no beneficial

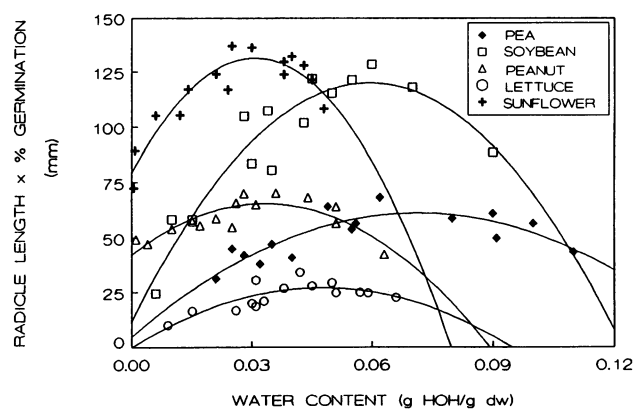

Figure 4. Effect of moisture content during storage at 35°C on seed vigor. Data is presented for soybean and sunflower stored for 2 months and peanut, pea, and lettuce stored for 4 months. Vigor is expressed as the germination index: the mean radicle length × the percentage germination. The average standard error of the mean radicle length of 25 seeds was 6.3 (soybean), 3.6 (pea), 2.3 (lettuce), 8.4 (sunflower), and 4.1 (peanut). The curves are drawn from fitted second order polynomial equation. dw, Dry weight.

effects (8, 18, 19), or that they have detrimental effects (1, 9, 12, 18, 19, 29). Most of these experiments assayed viability as percent germination. In this study, a sensitive test of seed health, seedling growth, was used, and all five seed lots tested showed reduced viability within 4 months when stored below a critical moisture level of about 19% RH (Fig. 4; Table I).

Clearly, the idea that the drier the seed is, the better it stores is not generally applicable and we contend that drying below a specific water content can be deleterious to seed viability. This view can be supported by three lines of arguments: (a) The germination tests presented in previous studies (8) are not sensitive enough to measure decline in seed vigor. (b) In previous studies (8), the storage temperature was 65°C. It is well documented that dehydration can prevent thermal denaturation. Thus, such a rigorous temperature treatment can obscure aging phenomena in very dry tissue since these tissues are also preferentially protected from denaturation. (c) Numerous studies demonstrate that water which is tightly associated with macromolecular surfaces has protective effects and its removal may cause deterioration of macromolecules with time (4, 13–15, 17, 23, 25, 29).

From these and previous studies, we suggest that there is an optimum moisture level for seed storage. If the moisture level is expressed in terms of water content, the optimum value varies among seeds; however, if the moisture level is expressed in terms of RH, it is almost constant among species. The optimum moisture level is between the RH, where reactions become thermodynamically less feasible because diffusion is slower, and the level below which seeds deteriorate more rapidly. This moisture level corresponds to RHs between 19 and 27% and corresponds well to the water activity range of 0.2 to 0.3 suggested as optimal for stability of dehydrated foods (15). Recently, co-workers (8) have suggested that 10% RH is optimal for storage. Based on problems associated with interpreting aging experiments at 65°C and isotherms at 20°C, we believe that 10% RH is an underestimate.

CONCLUSIONS

Scientists have known for years that drying seeds can slow deterioration. However, the underlying mechanism of seed deterioration has remained elusive. Because of this, storage protocols which will ensure the maximum longevity of seeds are not known. A better understanding of the relationship between water content and physiological activity will lead to improved practices for long-term storage of seeds and perhaps to insights into the nature of seed aging.

Physiological activity changes with moisture level. The RH at which seeds are equilibrated provides a better measure of the physiological level than does moisture content. Thus, it seems likely that chemical reactions become facilitated at specific water activities. This suggests that there is an interaction between the properties of water in an unimbibed seed and the types of reactions that can occur. To maximize seed longevity, deleterious reactions must be minimized. Our results indicate that this occurs at a RH between 19 and 27%.

LITERATURE CITED

1. **Bartholomew DP, Loomis WE** (1967) Carbon dioxide production by dry grain of *Zea mays*. Plant Physiol **42:** 120–124
2. **Clegg JS** (1978) Hydration-dependent metabolic transitions and cellular water in *Artemia* cysts. *In* JH Crowe, JS Clegg, eds, Dry Biological Systems. Academic Press, New York, pp 117–153
3. **Cromarty AS, Ellis RH, Roberts EH** (1982) *The Design of Seed Storage Facilities for Genetic Conservation.* International Board for Plant Genetic Resources, Rome
4. **Crowe JH, Crowe LM** (1986) Stabilization of membranes in anhydrotic organisms. *In* AC Leopold, ed, Membranes, Metabolism and Dry Organisms. Cornell University Press, Ithaca, New York, pp 188–209
5. **Ellis RH** (1988) The viability equation, seed viability nomographs, and practical advice on seed storage. Seed Sci Technol **16:** 29–50
6. **Ellis RH, Roberts EH** (1980) Improved equations for the prediction of seed longevity. Ann Bot **45:** 13–30
7. **Ellis RH, Hong TD, Roberts EH** (1986) Logarithmic relationship between moisture content and longevity in sesame seeds. Ann Bot **57:** 499–503
8. **Ellis RH, Hong TD, Roberts EH** (1988) A low-moisture-content limit to logarithmic relations between seed moisture content and longevity. Ann Bot **61:** 405–408
9. **Harrington JF** (1973) Biochemical basis of seed longevity. Seed Sci Technol **1:** 453–461
10. *International Board for Plant Genetic Resources Advisory Committee on Seed Storage Report on the Third Meeting* (1985) International Board for Plant Genetic Resources, Rome
11. *Annual Report for 1988* (1989) International Board for Plant Genetic Resources, Rome
12. **Kosar WF, Thompson RC** (1957) Influence of storage humidity on dormancy and longevity of lettuce seed. Proc Am Soc Hortic Sci **70:** 273–276
13. **Kuntz ID, Kauzmann W** (1974) Hydration of proteins and polypeptides. Adv Protein Chem **28:** 239–345
14. **Labrude P, Chaillot B, Vigneron C** (1987) Problems of haemoglobin freeze-drying: Evidence that water removal is the key to iron oxidation. J Pharm Pharmacol **39:** 344–348
15. **Labuza TP** (1980) The effect of water activity on reaction kinetics of food deterioration. Food Technol **34:** 36–59
16. **Leopold AC, Vertucci CW** (1989) Moisture as a regulator of physiological reaction in seeds. *In* PC Stanwood, ed, Seed Moisture. Crop Sci Society of America Special Publication No. 14: 51–68
17. **Mahama A, Silvy A** (1982) Influence de la teneur en eau sur la radiosensibilite des semences d'*Hibiscus cannabinus* I. Role des differents etats de l'eau. Environ Exp Bot **22:** 233–242
18. **Nakamura S** (1975) The most appropriate moisture content of seeds for their long life span. Seed Sci Technol **3:** 747–759
19. **Nutile GE** (1964) Effect of desiccation on viability of seeds. Crop Sci **4:** 325–328
20. **Priestley DA** (1986) *Seed Aging.* Comstock Publishing Associates, Ithaca, New York
21. **Roberts EH** (1973) Predicting the storage life of seeds. Seed Sci Technol **1:** 499–514
22. **Roberts EH, Ellis RH** (1989) Water and seed survival. Ann Bot **63:** 39–52
23. **Rockland LB** (1969) Water activity and storage stability. Food Technol **23:** 1241–1251
24. **Rupley JA, Gratton E, Careri G** (1983) Water in globular proteins. Trends Biochem Sci **8:** 18–22
25. **Sanches R, Melo WLB, Colombo MF** (1986) Effects of hydration in the oxygenation and autoxidation of carbonmonoxyhemoglobin powder. Biochim Biophys Acta **874:** 19–22
26. **Vertucci CW** (1989) The effects of low water contents on physiological activities of seeds. Physiol Plant **77:** 172–176
27. **Vertucci CW, Leopold AC** (1987) Oxidative processes in soybean and pea seeds. Plant Physiol **84:** 1038–1043
28. **Vertucci CW, Leopold AC** (1987) Water binding in legume seeds. Plant Physiol **85:** 224–231
29. **Vertucci CW, Leopold AC** (1987) The relationship between water binding and desiccation tolerance in tissues. Plant Physiol **85:** 232–238
30. **Wolfe J, Leopold AC** (1986) A spectrum of desiccation *In* AC Leopold, ed, Membranes, Metabolism and Dry Organisms. Cornell Press, Ithaca, New York, p 2