

(1) WATER RELATIONS IN SEEDS

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(2) 1. Introduction

Water possesses physical properties that make it the ideal biological solvent because it functions as the primary component of living tissues and is essential for life existence. In the specific case of seeds, the processes of development and germination are typical examples that emphasize the importance of water as an active participant and/or determinant of events contributing to plant survival for consecutive generations.

The essential element needed to convert a seed from a dry, quiescent structure into a germinating seed is water. As a result, water relations play a critical role in the regulation of various seed metabolic processes.

Seed moisture content is an important parameter related to different aspects of seed performance. For example, knowing the optimum seed moisture content for harvesting and seed cleaning minimizes mechanical damage and/or bruising of the seed.

The natural desiccation period occurring during seed maturation represents another scenario in seed/water relations. The ability to survive after water removal, an important aspect for preservation of seed viability and vigor of orthodox seeds during storage, depends on a series of physiological factors that are opposite to the dependence of high quantities of water during seed development. In this case, decreases in water content do not upset normal seed metabolism that occurs during rehydration since this is related to desiccation tolerance.

Therefore, studies on seed/water relations should consider both seed developmental processes and those occurring in dehydrated tissues because water is fundamental for both processes, in addition to its important roles in other physiological events such as respiration, dormancy, deterioration, etc.

(3) 2. Functions of Water in Seeds

Water is the main constituent in living plant cells, representing at least 70% of the protoplasm weight of metabolically active cells. The organization of cellular structure (both macro- and

33 micro-molecular) and the overall chain of biochemical anabolic and catabolic processes
34 (biological processes) of an organism depend on the presence and activity of water.

35 The reduction of water availability causes significant decreases in the rate of solute diffusion
36 to metabolic sites where intense enzymatic activity occurs. This is because water is a solvent and
37 a medium in which diffusion of solutes and biochemical reactions take place in plant cells.

38 At the same time, water contributes to solute diffusion and chemical reactions within the cell,
39 determining the function of different tissues and, consequently, the whole individual. Therefore,
40 water is a significant participant and/or product of various biochemical reactions that characterize
41 seed metabolism. As a result, water is involved in every dynamic process in a living cell.

42 Water effects on phospholipid structure may play an important role in membrane organization
43 and its selective permeability. The integrity of membranes is essential for the maintenance of
44 seed viability. In the presence of water, membranes perform crucial functions for the survival of
45 any hydrated organism. By regulating the flow of materials between subcellular compartments,
46 membranes control intermediary metabolism by regulating enzyme activities and maintaining
47 cellular compartmentalization. Any disruption of the dried membrane system has undesirable
48 consequences once the seed imbibes water for germination (Priestley, 1986).

49 Literature emphasizes the existence of critical water contents for the initiation and
50 continuation of different stages of seed metabolism. For example, the stability of cellular
51 membranes is maintained only when seed moisture content is at least 25,0% (fresh weight basis).
52 At the same time, seed germination occurs only when endospermic seeds possess 30% to 35%
53 water and non-endospermic seed must possess a cotyledonary water content of at least 40%.

54 Water is usually the limiting factor for the germination of non-dormant seeds, affecting the
55 percentage, speed and uniformity of emergence. It is directly related to reserve mobilization,
56 energy production through respiration, enzyme and hormonal activity, and dilution of the
57 protoplasm to increase metabolism for successful embryonic growth.

58 The influence of seed water content on the rate and speed of deterioration during storage is
59 also important. Although this relationship is well known, our knowledge about seed water status
60 and its influence on metabolic reactions during storage still requires further research. It is known,
61 for example, that many reactions occur faster in dry seeds than in those of seeds at higher
62 moisture contents, including the absence of free water associated with lipid autoxidation

63 reactions. Water also influences quantitative chemical reactions (kinetic control) as well as
64 qualitative reactions (do the reactions occur or not?).

65 Water is also an important parameter that contributes to important technological and
66 economical management decisions at pre- and post-harvest. For example, knowing the optimum
67 seed moisture content for harvesting and seed processing minimizes mechanical injury in seeds
68 that negatively impacts seed germination and vigor. In addition, seed drying requires precise
69 controls so that seed moisture content is not lost too rapidly thus reducing its quality or too
70 slowly thereby increasing seed drying costs or exposure to high seed deterioration.

71 The survival of a seed in storage depends upon its moisture content more than any other
72 physiological factor. This dependence can be attributed to the fact that physiological reactions
73 increase quantitatively as water content increases. In addition, identifying the optimum seed
74 moisture content for seed storage both increases storability and reduces contamination by storage
75 fungi. It is known that metabolic activity and damages promoted by insects and microorganisms
76 are influenced by seed water content.

77 As a result, it can be concluded that deteriorative reactions proceed at higher moisture levels
78 and can be reduced at lower moisture levels. However, studies have shown that some important
79 reactions occur more readily in the dry than in the wet state, and the nature of these reactions vary
80 with water content.

81 Even in seed testing, knowing seed moisture content is essential to avoid imbibitional injury
82 during germination and it also assists in standardizing results in the conduct of accelerated aging
83 and conductivity vigor tests.

84

85 **(4) 3. Seed Water Content**

86 The change in water content of tissues and organs is often used as an indicator of seed
87 hydration or dehydration. However, insufficient attention has been given to problems associated
88 with the use of water content as an indicator of dehydration stress. For example, the
89 identification of the quantity of water present in seeds has been described using expressions such
90 as “seed moisture content” or “seed water content”. The latter should be preferred since the
91 substance evaluated is water.

92 Water content on a fresh weight basis is widely used in seed physiology and technology
93 literature and is adopted by seed testing associations worldwide for the expression of seed

94 moisture content. Seed water content is expressed by percentage (%) on a *fresh weight basis* and
 95 can be described as the percentage mass fraction of water in the total seed mass and expressed by
 96 the formula:

$$97 \quad M.C.(\%Fwb) = \frac{Fw - Dw}{Fw} \times 100, \text{ in which}$$

98 M.C. (% Fwb) = moisture content on a fresh weight basis; Fw = seed fresh weight;

99 Dw = seed dry weight

100
 101 Seed moisture content on a fresh weight basis is not a linear expression of water content in
 102 tissues because fresh weight appears in both the numerator and the denominator in the equation.
 103 When moisture content (% Fwb) is used to monitor the loss of water during desiccation, the
 104 decrease in moisture content does not necessarily reflect the extent of dehydration. The change in
 105 water content during drying is, in fact, related to the change of the reciprocal of tissue fresh
 106 weight.

107 For example, when a tissue at 80% M.C. (Fwb) is dried to 70% and 60% water content, the
 108 tissue loses 41.7% and 62.5% of its initial water weight, respectively, not just 12.5% and 25% as
 109 expected by the values of M.C. (% Fwb) as demonstrated by Sun (2002).

110 Moisture content on a *dry weight basis* measures the mass ratio between water and the dry
 111 mass in seed tissues, and is often expressed on a g of water per g dry weight ($\text{gg}^{-1} \text{dw}$) basis and
 112 is calculated by the formula:

$$113 \quad M.C.(Dwb) = \frac{Fw - Dw}{Dw} \text{ gg}^{-1}$$

114
 115
 116 Seed moisture content on a dry weight basis is a linear expression of water content and the
 117 change of moisture content ($\text{gg}^{-1} \text{dw}$) during drying is proportional to the loss of water in a tissue.
 118 On the mass basis, a tissue at a M.C. of $0.80 \text{ gg}^{-1} \text{dw}$ is hydrated twice as much as the tissue with a
 119 $0.40 \text{ gg}^{-1} \text{dw}$ (Sun, 2002).

120 At moisture contents below 15% (Fwb), it makes very little difference if moisture content data
 121 are expressed on a fresh or dry weight basis. However, in the case of recalcitrant seeds that
 122 usually contain considerable amounts of water, there are marked differences in values when
 123 expressed on a fresh or dry weight basis, with the latter providing the highest values.

124 (5) The conversion of M.C. (% Fwb) to M.C. (% Dwb) is accomplished using the following
 125 equation (1), while Dwb is converted to Fwb using equation (2):

126

$$127 \quad M.C.(Dwb) = \frac{M.C.(Fwb)}{100 - M.C.(Fwb)} \quad (\text{equation 1}) \quad M.C.(Fwb) = \frac{M.C.(Dwb)}{100 + M.C.(Dwb)} \quad (\text{equation 2})$$

128

129 Priestley (1986) showed that use of nuclear magnetic resonance (NMR) demonstrated that
 130 seeds dried in an oven at 105°C may still retain 1.0% to 2.5% of their weight in water. This may
 131 cause distortions in moisture content results when these determinations are made on different wet
 132 or dry weight bases. This situation occurs primarily because water usually is not uniformly
 133 distributed within the seed, being greater in the embryonic axis than the storage reserve tissues.
 134 This is one of the reasons that there is an increasing interest in the determination of the seed
 135 water energy status that provides more precise understandings of the physiological processes that
 136 occur in seeds than information based solely on seed water content.

137

138 (6) 4. Properties of Water and Seed Hygroscopic Equilibrium

139 It well known that drying or freezing orthodox seed tissues preserves their chemical integrity
 140 and consequently their viability. As a result, it is important to understand the properties of water
 141 and how are they affected by seed moisture content and temperature. Seed moisture is always
 142 changing and this is because seeds are hygroscopic and readily absorb and desorb water based on
 143 the amount of water surrounding them.

144 As emphasized by McDonald (2007) water is composed of two light hydrogen atoms and one
 145 heavy oxygen atom as shown in this Figure. The oxygen nucleus contains 8 protons that allow it
 146 to attract 8 electrons. Two electrons fill the first shell with the remaining six electrons in the
 147 second shell. Since the second shell can hold a total of eight electrons, it has two empty spaces
 148 that can be filled with electrons from other atoms.

149 A hydrogen atom consists of a single proton and single electron. Thus, the two electrons in the
 150 two hydrogen atoms can be shared with the empty spaces in the second shell of the oxygen
 151 molecule. When this occurs, there is the formation of a covalent bond which requires
 152 considerable energy to break. Because of its larger size, oxygen pulls harder on the electrons
 153 than hydrogen resulting in a net negative charge around the oxygen atom and a positive charge

154 around the hydrogen atoms. This is referred to as a permanent dipole because there are
155 differences in electrical polarity around the oxygen and hydrogen atoms of the water molecule.
156 The permanent dipole has a significant effect on the reactivity of water with other compounds.
157 For example, because water is a small molecule with a large dipole moment per unit surface, it
158 can strongly be adsorbed on other polar substances.

159 (7) Thus, water quickly surrounds and separates the ions in salts with the positive hydrogen
160 atoms surrounding the negative salt ion and the negative oxygen atom surrounding the positive
161 salt ion as illustrated in this figure. In addition, this difference in charge results in an attraction for
162 molecules of the same substance as shown in the figure. (8) These positive and negative charges
163 on the water molecule permit an electrostatic attraction between the positive end of one molecule
164 and the negative end of another called “hydrogen bonding” as shown in this figure. As a result,
165 water has many opportunities to combine with different compounds and this affects the amount
166 of water found in a seed.

167 (9) The term “bound water” refers to water associated with a macromolecular surface and is
168 sufficiently structured so that its thermodynamic properties differ from free or bulk water. It is
169 not ready freezable (Leopold and Vertucci, 1989).

170 Seeds are hygroscopic anatomical and chemical structures having great affinity for water. This
171 affinity is expressed scientifically as water potential and seeds can have water potentials as low as
172 - 400 MPa (the lower the value, greater is the affinity for water) due to matric (attraction to
173 surfaces) and osmotic (attraction to charged compounds) forces. For example, water is attracted
174 to cellulose in cell walls. Since seeds are primarily storage organs, the affinity of water for
175 storage compounds such as proteins, carbohydrates and lipids is important to consider.

176 *Proteins.* Proteins are polymers of amino acids and amino acids can possess positive (at the
177 amino terminus) and negative (at the carboxyl terminus) charges on the molecule as well as some
178 hydroxyl groups. These negative and positive charges are ideal for hydrogen bonding with water.
179 In addition, water is also responsible for maintaining the tertiary structure of many protein
180 molecules. As a result, any portion of the seed containing large quantities of proteins will adsorb
181 large quantities of water.

182 *Starch.* Starch is a polymer of long or branched chains of glucose molecules. It is generally
183 composed of amylose which possesses α -1,4 bonds and amylopectin which possesses both α -1,4
184 and α -1,6 bonds. The α -1,6 bonds give this compound a branching structure. Hydrogen bonding

185 can occur on the hydroxyl groups of the starch molecule. Thus, starches have an affinity for
186 water in seed storage tissues, but not as great as proteins.

187 *Lipids.* Storage lipids are predominately polymers comprised of glycerol and three fatty acids.
188 Because the three fatty acids have no polar groups (they are primarily carbon/hydrogen bonds),
189 the compound is non-polar and, resultingly, there is no hydrogen bonding. Thus, there is no
190 attraction for water and lipids and water do not mix. Consequently, lipid storing seeds have little
191 attraction for water.

192

193 *Seed hygroscopic equilibrium*

194 **(10)** Hygroscopicity refers to the water holding capacity characteristic of each substance with
195 proteins having a high affinity for water and they are thus considered hydrophilic. In contrast,
196 lipids have fewer attraction points with water and the association only occurs by hydrogen
197 bridges making lipids hydrophobic. Consequently, the chemical composition of seeds affects its
198 water holding capacity.

199 Atmospheric air is composed primarily of gases and water vapor. Even though it is present in
200 a relatively small proportion of the air, water vapor has a great influence on seed physiological
201 activities because of its direct relationship with seed water content.

202 The quantity of water vapor in the air varies according to the temperature; when this vapor
203 quantity reaches a maximum that can be contained in the air, it is called the *saturation humidity*
204 (dew point). The saturation humidity is constant under a specific temperature and, as temperature
205 increases, the air increases its water vapor holding capacity as shown in this table (Harrington,
206 1972). This quantity generally doubles for each 10°C increase in the air temperature. However,
207 air is not usually saturated and contains only a fraction of the maximum value.

208 **(11)** Relative humidity is the ratio of the amount of moisture in the air to the amount the air
209 can hold at the same temperature if saturated. Therefore, when relative humidity is 65%, this
210 means that the air contains 65% of the maximum water vapor holding capacity at that
211 temperature.

212 As temperature increases, air can hold more water at saturation (dew point) and the relative
213 humidity values decrease if the amount of water in the air remains constant. Therefore, increases
214 in air temperature without altering the quantity of water vapor in the air causes a decrease in the
215 relative humidity, and the opposite occurs when air temperature decreases. At the same time,

216 when temperature remains constant, a reduction in the relative humidity is only possible when
217 water vapor is removed by dry air circulation.

218 Atmospheric pressure results from the sum of partial pressure of gases and vapor pressure,
219 expressing the energy created by the molecules impact on a static surface. But, water within a
220 seed is in a liquid or vapor state. When it is in the vapor state, vapor pressure against the cell
221 walls is directly proportional to the vapor quantity existing in the seed and corresponds to a
222 specific water potential.

223 Seed water or moisture content is directly related to relative humidity of the surrounding air.
224 Seeds and the atmosphere are two systems in permanent water vapor exchange; the direction of
225 vapor movement depending on the difference between seed and air water potentials or vapor
226 pressure. The flow of water is from the system with a higher to one with a lower vapor pressure
227 or water potential until energetic equilibrium is established between seed and the air. In other
228 words, when there are differences between these pressures, a gradient is established that
229 generates vapor movement from seed to atmosphere or vice versa until hygroscopic equilibrium
230 is reached. As a result, for each relative humidity there is always a corresponding seed water
231 content at a specific temperature, i.e., a point of *hygroscopic equilibrium*.

232 **(12)** This observation helps us understand how seed moisture content fluctuates during open-
233 air storage. For example, during the course of a day, relative humidity is lowest at the highest
234 temperatures in the afternoon and highest at the lowest temperatures at night as illustrated in this
235 figure. Seeds absorb water based on relative humidity surrounding them so the greatest amount of
236 water available to a seed occurs at night.

237 **(13)** A simple “rule of thumb” can describe the relationship between relative humidity and
238 seed moisture content.

239 If atmosphere vapor pressure > seed vapor pressure, then SMC increases

240 If atmosphere vapor pressure < seed vapor pressure, then SMC decreases

241
242 Seed moisture content at the hygroscopic equilibrium increases as relative humidity increases
243 and vice versa. The predominant chemistry of a seed influences its isotherm. The examples in
244 this Table show that cereals high in starch achieve a higher equilibrium moisture content value at
245 the same relative humidity than seed high in oil. This phenomenon is due to the lack of attraction
246 of oil containing seeds for water. Since high oil-content seeds have little affinity for water, the

247 relatively low water values suggest that the water present in these seeds is primarily free water.
248 This is the reason high oil seeds come to equilibrium with relative humidity at lower seed
249 moisture content. Generally, more water is attracted to proteins, then starch, and finally little to
250 oil.

251 The knowledge of hygroscopic equilibrium allows the establishment of principles for seed
252 drying, packing and storage. It is affected by various factors, such as the proportion of seed
253 reserves, relative humidity and air temperature, permeability of seed coats, and physical integrity
254 of seeds

255 **(14)** Water uptake by seeds increases or decreases at differing relative humidities as shown by
256 this isotherm curve (equilibrium moisture content) in a sigmoidal fashion suggesting three types
257 of water binding. The interaction between water and seeds is most easily summarized by a
258 consideration of sorption isotherms, which are obtained by measuring equilibrium moisture
259 content as a function of relative humidity at constant temperature.

260 Isotherms describe the equilibrium relationship between the amount of water sorbed by the
261 seed at specified vapor pressures or relative humidities at a specified temperature. This figure
262 shows a typical water sorption isotherm for wheat and rice seeds at 25°C from 0 to 100% relative
263 humidity. Note there are three phases in this reverse sigmoidal curve (Vertucci and Leopold,
264 1989). Beginning at low moisture contents, the initial stage of water uptake in seeds is believed a
265 result of water deposition on polar sites present in storage compounds.

266 It is already known that water in dry seeds is structured and non-freezable. Structured water
267 can be separated into regions with distinctive affinities in which the region with the lowest water
268 content has the highest affinity for water.

269 In region I, when percentage relative humidity is below 20%, water has almost no mobility
270 and is believed to be “chemi-sorb” to macromolecules through ionic bonding. As a result, the
271 few clusters of water molecules present are attracted to the polar sites on proteins and starches
272 and form a monolayer of water tightly bound to the storage molecules. There are, therefore, in
273 this region very strong interactions between water molecules and the charged carboxyl and amino
274 groups of proteins, lipids, cell walls, and other constituents.

275 Region II, a region of water avidity between 20 and 60% R.H., is the region of deflection and
276 is a straight line showing that the increase in seed moisture content in this phase is linearly
277 dependent on the increase in relative humidity. This phase is represented by deposition of a

278 second layer of water due to hydrogen bonding on the first layer of more tightly bound water
279 present on the proteins and starches in Phase I; this water condenses over the hydrophilic sites of
280 macromolecules. Hydrogen bonding of water to the hydroxyl groups of starch and to the
281 hydroxyls and amides of proteins tends to be particularly evident.

282 Region III of abundant water binding is similar to Phase I in that there is another rapid
283 increase in seed moisture content beginning at 60 to 70% relative humidity and 15% seed
284 moisture content. This phase is the addition of water to more layers of water forming bridges over
285 hydrophobic sites of macromolecules and is considered to be free water that can easily be
286 removed from the system. This is considered a region of predominantly mobile or free water in
287 which capillary and osmotic forces become significant (Priestley, 1986).

288 An interesting observation about a water sorption isotherm is that if water is *desorbed* from a
289 seed by reducing the relative humidity of the surrounding environment, the seed does not come to
290 the same moisture content at equilibrium with the same relative humidity as it did on *adsorption*.
291 Note that the moisture content during desorption is slightly higher and this is believed the result
292 of water being trapped in tertiary protein structure and, thereby, more difficult to remove than
293 when first added to the unfolded protein. This phenomenon is called “*hysteresis*” and is an
294 important concept to keep in mind when large gradations in seed moisture content are
295 encountered.

296 It should be remembered that seed moisture content is often expressed on a whole seed basis
297 even though it is known that the chemistry of different parts of a seed vary in composition and,
298 therefore, their affinity for water.

299

300 (15) 5. Seed Water Potential

301 Seed water may be characterized by an energy status and is likely to be determined through
302 potential energy. Such energy status is conveniently described by the total **water potential**
303 which, in terms of energy per volume unit, can be expressed in pressure measurement units.

304 Total water potential allows the identification of whether a system is or is not water-balanced
305 as well as the estimation of the spontaneous water flow towards a water potential difference
306 between two systems such as seed/air or seed/soil.

307 The total water potential (ψ_w) is equal to the sum of matric potential (ψ_m), osmotic or solute
308 potential (ψ_s), and pressure or turgor potential (ψ_p), i. e., $\psi_w = \psi_m + \psi_s + \psi_p$.

309 The water potential depends on water status (energetic conditions) and the water position in
310 relation to macromolecules. Both interact to establish the energetic status characterized by water
311 potential.

312 The *matric potential* is the ability of matrices (cellular walls, protein bodies, soil particles,
313 etc.) to uptake and retain water. It is the most important component of water potential, being
314 represented by the sum of capillary forces and adsorption to colloids. The *osmotic or solute*
315 *potential* is determined by the osmotic concentration; the force of water attraction is proportional
316 to solution concentration. *Turgor or pressure potential* is created against the walls of turgid cells:
317 as water penetrates the cell, cell volume increases creating a pressure against cell walls.

318 Under normal atmospheric pressure, the seed osmotic potential is always negative because the
319 potential energy of water in the presence of solutes is lower than the energy of pure water
320 considered the standard. The same occurs with matric potential because the capillary and
321 adsorption forces are not negligible. In contrast, turgor pressure is positive. As a result, the
322 overall water potential is usually negative, except in turgid cells where it can be zero. Then, both
323 matric and osmotic potentials reduce the total water potential (becomes more negative).

324 The concept of water potential is an expression of the state of water energy. The diffusion
325 process occurs due to an energy gradient, from a region of larger potential (less negative) to
326 another region of lower potential (more negative). In dry seeds, matric and osmotic potentials
327 contribute to making the seed water potential very low (-100 to -200 MPa). This causes dry
328 seeds to rapidly increase their water uptake capacity.

329 **(16)** During seed hydration, seed water content increases which causes the seed water potential
330 to increase with subsequent decreases in the water potential of the substratum resulting in the
331 systems ultimately achieving hygroscopic equilibrium. When this equilibrium is reached, there
332 are no more differences in water movement (the water quantity that enters the seed is same as that
333 which leaves). At this time, seed parts may differ in water content, but if the water potentials
334 remain similar, there is no further water movement within the seed.

335 While leaf or stem water potential can be measured by either a pressure chamber or by a
336 thermocouple psychrometer, measurements of seed ψ_w and its components have been limited to
337 the psychrometric approach. More recently, this determination in plant tissues has been
338 performed using nuclear magnetic resonance (NMR) methods. Villela et al. (2003) obtained

339 consistent results of seed water potential using a multivoltimeter thermo par psychrometer
340 operating in the dew point.

341

342 **(17)** 6. Water Status in Seeds

343 The most commonly used parameter for evaluating seed water relations is seed moisture
344 content. However, this is a poor indicator of the physiological status of water during seed
345 development, maturation, germination, deterioration and all other vital processes, since it is
346 changing continuously following variations in relative humidity and provides only information on
347 the water quantity present in the seed, but no information about its availability or its energy
348 status.

349 **(18)** The superficial properties of macromolecules, particularly physiologically important
350 proteins, are modified by seed water level. Discrete changes in seed metabolic activity according
351 to water content are likely associated with discrete changes in the physical properties of water.

352 At different hydration levels, corresponding to water types, tissue water content has different
353 physical properties (Pammenter and Berjak, 2000). Water removal from a cell increases the
354 concentration of solutes and therefore causes an eventual decrease in the fluidity of the aqueous
355 medium thus affecting cellular metabolism.

356 Proteins are composed of amino acid sequences that present multiple sites for water sorption.
357 The connections between water molecules and proteins occur through associations with ionic and
358 polar sites. Commonly, the formation of hydrogen bonds between water molecules and amino
359 (NH_2), hydroxyl (OH), carboxyl (COOH) and other groups can be found. These multiple
360 connection influences protein configuration.

361 The characteristics of macromolecules in seeds can be described using a model for globular
362 proteins in which the sorption positions or sites have distinct affinities to water. When a dry
363 protein is hydrated, water links first to charged positions of the molecule and, in sequence, to the
364 hydrophilic positions shown in this figure. To completely hydrate the molecule, water forms
365 bridges on the exposed hydrophilic sections.

366 Considering this multiple macromolecule water sorption sites' model, Vertucci (1993) and
367 Vertucci and Farrant (1995) used a globular protein as a conceptual model to explain how water
368 interactions with different molecular surfaces can result in the different properties of water. They

369 described five types of seed water or levels of hydration in seed tissues, their corresponding
370 potential intervals and water contents according to the molecule mobility and phase properties.

371

372 **(19) Water type 5:**

373 Water type 5 has properties similar to a dilute solution and is detected at water potentials
374 greater than about -1.5 MPa and water contents greater than 41% (fwb) and it is not linked to the
375 macromolecule surface. At these high water contents and high water potential, it can be
376 considered as free water.

377 **Water type 4:**

378 Water type 4 is believed to be similar to a concentrated solution or capillary water and is
379 detected at water potentials between -1.5 MPa and -4 MPa corresponding to seed water contents
380 from 33% to 41%. As the moisture content decreases, this type of water takes on the properties
381 of water in a concentrated solution where the interaction of water and solutes becomes stronger
382 and the system deviates from “ideal” behavior. Type 4 water can be identified by its extremely
383 low melting temperature and is believed to be water that remains in pores or capillaries and does
384 not interact directly with protein surfaces.

385 **(20) Water type 3:**

386 Water type 3 is suggested to form bridges over hydrophobic moieties of amino acids necessary
387 to fully wet the molecule. It is detected at water potentials between -4 MPa and -11 MPa
388 (corresponding to water content from 20% to 33%). There is an indication of the presence of
389 freezable water. This water has some glassy characteristics at subfreezing temperatures that are
390 lost by further drying.

391 This type of water still has unique properties that distinguish it from a very diluted solution,
392 although it can promote moistening of the surface of macromolecules. Recalcitrant seeds, when
393 dehydrated up to water level 3, exhibit deterioration mechanisms similar to orthodox seeds
394 exposed to high levels of relative humidity.

395

396 **Water type 2:**

397 Type 2 water is believed to have strong interactions with the polar surfaces of macromolecules
398 or hydroxyl groups of solutes. It is detected at water potentials between -12 MPa and -150 MPa
399 (corresponding to water content from 7.5% to 20%).

400 At those water content levels, the solution becomes so concentrated that it is considered as
401 unfreezable water. This water forms a thin coat in the matrix surface and interacts strongly with
402 hydrophilic moieties of the macromolecules and has some solution properties, but it is also
403 extremely viscous. Type 2 water begins to have a solvent role and acquires properties closer to
404 free water.

405 The chemical reactions involving type 2 water are catalyzed by enzymes, but protein and
406 nucleic acid synthesis still does not occur.

407 At very low water contents, those characteristic of orthodox seeds, all the remaining water is
408 tightly associated with macromolecular surfaces; its mobility is reduced and it constitutes the so-
409 called “bound” or unfreezable water.

410

411 **(21) Water type 1:**

412 Type 1 water corresponds to the theoretical level at which water strongly binds to
413 macromolecules as a structural component and maintains its integrity. It occurs at water
414 potentials less than -150 MPa ($MC < 7.5\%$) and has practically no mobility and does not possess
415 solvent properties.

416 This type of water is present in very dry seeds and, at this physiological level, metabolic
417 activity is restricted, although some peroxidative reactions may occur. Its removal can accentuate
418 seed deterioration because protection against the toxic effects of free radical and/or protein
419 denaturation is reduced.

420 Seed moisture contents corresponding to hydration levels 1, 2, and 3 vary according to the
421 species and seed chemical composition. However, the relative humidities in equilibrium with
422 these moisture contents are similar for different species. For example, seeds with type 1 water are
423 in equilibrium with relative humidity lower than 30%; type 2, from 30 to 85%, and type 3, from
424 85 to 92%, regardless of the species, type of tissue and its chemical composition.

425 **(22)** In summary, this table presents characteristics of the five types of water with respect to
426 water potentials and respective seed moisture contents (fresh and dry weight basis).

427

428 **(23) 7. Physiological Events Associated with Seed Moisture Content and Water Status**

429 As emphasized above, seed physiological activity is directly associated with its water content.
430 Therefore, research has focused on the identification of important physiological events and their

431 association with specific seed moisture content. Different metabolic processes can take place at
432 different hydration levels as influenced by the thermodynamic properties of water. Various
433 examples illustrate this.

434 **(24)** This table contains information about the association of different aspects of seed
435 performance, the type of water and seed moisture content is useful information for establishing
436 important procedures for seed harvesting, processing and storage.

437 At high water content, normal metabolism occurs and seeds can germinate. At hydration level
438 4, protein and nucleic acid synthesis is possible, but there is inadequate water for normal cell
439 growth and germination. At lower water contents, protein and nucleic acid synthesis is no longer
440 possible, but some respiration can be detected. At even lower water contents, only reduced levels
441 of catabolic events occur slowly.

442 Type 5 water is probably required for turgor in seeds and this means that germination can only
443 be completed if this type of water is present. The removal of type 5 water from immature and
444 germinated orthodox embryos and perhaps throughout development in highly recalcitrant
445 embryos can be lethal suggesting that these tissues cannot survive the mechanical stresses
446 associated with drying.

447 Respiratory activity is first observed in hydration level three. Mature embryos of orthodox and
448 many recalcitrant seeds can survive drying to water type 3, but do not survive prolonged periods
449 at this hydration level. Presumably this is because unregulated catabolic activities occur, which
450 lead to the degradation of macromolecules and the accumulation of toxins. The complete removal
451 of type 3 water is associated with membrane structural damage.

452 **(25)** Some catabolic reactions catalyzed by enzymes occur in the presence of type 2 water, but
453 when orthodox seeds are stored at moisture contents corresponding to this hydration level, seed
454 deterioration is reduced.

455 Removal of type 1 water appears lethal to seeds with intermediate storage behavior and most
456 orthodox seeds. Since water protects macromolecules from free radical attack on
457 macromolecules, it is expected that increased deterioration will occur with progressive removal
458 of water from first hydration level.

459 In contrast, seed sensitivity to various stresses can be associated with the type of hydration
460 level. For example, cooling seeds hydrated to the fourth and fifth hydration levels usually results
461 in lethal freezing injury. Mature recalcitrant seeds do not survive complete removal of type 3

462 water while seeds of intermediate storage behavior do not survive complete removal of type 2
463 water. In addition, seeds deteriorated under the usual conditions of the accelerated aging or
464 controlled deterioration tests are frequently within hydration level 3 where mitochondria are
465 functional.

466 These are some examples of the importance of the knowledge of seed physiological events
467 affected by water status. The information in this table is useful to a better knowledge of the
468 association of water to metabolic events in seeds and to the selection of seed handling during
469 different phases of the production system.

470

471 **More Information**

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