

1 **(1) SEED DEVELOPMENT (Maturation)**

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6 **(2) 1. Introduction**

7 Induction of flowering and differentiation of flower parts are considered the starting points of
8 seed development. The diversity of floral structure and the interaction of its parts are of primary
9 importance in vegetable reproduction and cellular activity responsible for the events that
10 culminate in the formation of new individuals.

11 The process of seed development or maturation is controlled genetically and involves an
12 organized sequence of changes from ovule fertilization to the point in which the seed becomes
13 independent from the parent plant. These processes comprise successive stages that can be
14 viewed as preparation for successful germination. They are characterized by synthesis and
15 accumulation of food reserves followed by reserve mobilization during germination, leading to
16 the resumption of embryo growth to produce a seedling. The flowers in a plant or the same
17 inflorescence generally are not pollinated simultaneously, so the uniformity of seed maturation is
18 never achieved, especially when a plant population is considered.

19 **(3)** Initial studies of seed maturation were primarily directed at determining phenological
20 differences among species and cultivars and identifying consistent parameters to determine the
21 best time for seed harvest. The first studies proposed such criteria to identify seed maturity as: a)
22 The elapsed time from planting or seedling emergence or the period between flowering and
23 harvest and b) Seed moisture content and morphological characteristics of plants, fruits and/or
24 seeds during senescence. As a result, by using these criteria, there was no consistent mechanism
25 to define the main events at the beginning and conclusion of the seed maturation process.
26 Brenchley and Hall (1909), for example, defined seed maturation as a seed dehydration process
27 while still attached to the parent plant.

28 **(4)** Further research contributed significantly to the advancement of concepts about seed
29 maturation and maturity, and Delouche (1971) made the most significant contribution by
30 considering seed maturation as a process comprising a series of morphological, physical,
31 physiological and biochemical changes that occur from ovule fertilization to the time when seeds
32 become physiologically independent of the parent plant, i.e., they reach physiological maturity.

33 (5) The seed development process, from ovule fertilization to physiological maturity, can be
34 divided into four phases according to Dure (1975) and Adams and Rinne (1980) as shown in this
35 figure. Phases I and II comprise cell division and expansion. Reserve accumulation occurs in
36 Phase III as seed dry mass increases. At the end of this phase, seed moisture loss is intensified
37 (Phase IV).

38 Pollock and Roos (1972) reported that, after fertilization, there is a period of seed structure
39 formation as a result of cell division, expansion and differentiation (histodifferentiation) in which
40 seed structure primordia are formed and future embryo parts can be visualized. During this phase,
41 there is a significant increase in seed size forming the embryonic cells that receive assimilates
42 from the parent plant. During this period, seed moisture content remains constant and high. The
43 significant decrease in seed moisture content occurs at the end of maturation when changes in cell
44 membrane structure organization occurs as well as increases in enzyme synthesis in preparation
45 for successful germination. Recalcitrant seeds usually do not show this transition period between
46 maturation and germination.

47

48 (6) 2. General Parameters to Characterize Seed Maturation

49 During the 1960s and 1970s, there was a significant effort by seed technologists to clarify the
50 maturation process and to define the primary changes occurring during seed development. Most
51 studies were conducted by determining different seed maturation parameters within plant
52 populations to estimate crop performance instead of establishing a research priority that
53 emphasized differences in seed performance from individual flowers or plants.

54 In this sense, research usually involved seed sampling at pre-defined intervals followed by the
55 determination and identification of specific morphological plant and seed characteristic
56 parameters associated with the maturation process. Using this morphological approach, it was not
57 possible to identify seed physiological maturity (as in studies involving individually marked
58 flowers) because this would occur when all seeds in the sample had completed development,
59 which does not occur simultaneously. However, this approach allowed identifying seed
60 physiological maturity on a plant community basis such as occurs during field crop production.

61 The following changes occur during seed development:

62

63

64 **(7)** 2.1. *Seed moisture content*

65 Ovule moisture content at the time of fertilization is approximately 80% (fresh weight basis),
66 both for monocots and dicots. That value decreases during maturation although it remains
67 relatively high throughout most of the maturation period because water is the vehicle for
68 transferring nutrients from the parent plant to the developing seeds.

69 **(8)** The initial phase of dehydration is slow, and is accelerated from the time the seeds reach
70 maximum dry weight; at that time, seeds possess 35% to 55% moisture content for orthodox
71 monocot and dicot seeds, respectively, produced in dry fruits. This decrease in moisture content
72 proceeds until hygroscopic equilibrium is attained. From that point on, moisture content changes
73 are associated with variations in relative humidity. However, seeds produced in fleshy fruits
74 have a lower decrease in moisture content than seeds produced in dry fruits as demonstrated in
75 this figure.

76 Developing recalcitrant seeds do not show marked changes in desiccation at the end of
77 maturation, possessing moisture contents usually over 60% (fresh weight basis).

78

79 **(9)** 2.2. *Seed size*

80 The fertilized ovule is a small structure with respect to final seed size. As mentioned, the first
81 two phases of embryogenesis involve intense cellular division and expansion. These determine
82 the progressive increase in seed size that becomes maximum during the first half of the seed dry
83 weight accumulation period. For example, soybean seeds usually reach their maximum size at
84 stage R6 (see figure).

85 **(10)** Thereafter, there is seed size reduction that varies depending on the species and the
86 intensity of seed drying at the end of the maturation period. For example, seed size reduction in
87 leguminous seeds is more obvious than in cereal seeds.

88 **(11)** This table shows examples of the time required for seeds of some species to attain their
89 maximum seed size during maturation.

90

91 **(12)** 2.3. *Seed dry weight*

92 After sexual fusion, the developing seeds begin to increase in weight as a result of nutrient
93 accumulation and water uptake. Seed fill is initially slow because cell division and elongation are
94 occurring during this stage as shown in this figure. Soon after, dry mass accumulation increases

95 until seeds reach their maximum dry weight **(13)**. At that time, which is often difficult to
96 precisely identify, seeds are considered physiologically independent from the parent plant.

97 **(14)** This Table shows examples of seed moisture content of different species at the time they
98 achieve maximum dry weight showing a wide variation among species. As a result, this table
99 emphasizes the need to carefully determine seed moisture content since it may change quickly at
100 the termination of the maturation process depending on ambient environmental conditions.

101
102 **(15)** 2.4. *Germination*
103 Seeds of various cultivated species are able to germinate a few days after ovule fertilization as
104 shown in this table **(16)**. In this case, germination refers to protrusion of the primary root, not the
105 formation of a normal seedling because histodifferentiation has not been completed and reserve
106 accumulation is still incipient at this phase. Therefore, this germination does not lead to the
107 production of vigorous seedlings.

108 **(17)** Theoretically, it is possible to consider that the percentage of germinable seeds increases
109 during maturation, reaching a maximum around the time when seeds attain maximum dry weight.
110 This is only found in species where dormancy does not occur, because the imbalance in the
111 germination promoters/inhibitors induced during the reserve accumulation period may directly
112 affect seed germinability.

113
114 2.5. *Vigor*

115 Seed vigor changes are usually parallel to nutrient reserve transfer from the parent plant. This
116 means that the proportion of vigorous seeds increases during maturation, reaching a maximum
117 near to or at the same time as seed maximum dry weight.

118
119 **(18)** 2. Determination of Physiological Maturity

120 The identification of the time of physiological maturity has been a controversial subject
121 among different authors studying seed maturation. Among the differing physiological maturity
122 concepts, three are dominant:

123 a) Physiological maturity is identified as maximum seed dry matter accumulation.

124 b) Physiological maturity is reached when there is no further significant increase in seed dry
125 weight.

126 c) Physiological maturity occurs when seeds reach maximum dry weight, germination and
127 vigor.

128 **(19)** According to the prevailing concept, seed development ceases when physiological
129 maturity is achieved, but this idea remains controversial because this expression is frequently
130 used with different meanings. For example, the first concept of physiological maturity was
131 proposed by Shaw and Loomis in 1950 as the stage in which the seed possesses maximum dry
132 weight and yield. Physiological maturity has also been termed “relative maturity” by Aldrich in
133 1943, “morphological maturity” by Anderson in 1955 and “mass maturity” by Ellis and Pieta
134 Filho in 1992 while others prefer to use terms such as “harvest maturity”, “agronomic maturity”,
135 and “time of harvest” to identify this same stage.

136 Consequently, physiological maturity and harvest time are distinct events. Seed maturation is a
137 process characterized as an organized sequence of several physiological events culminating at
138 physiological maturity, while harvest time is a decision based on technological and economic
139 parameters associated with seed yield/quality.

140 Physiological maturity can be determined on an individual seed or plant community basis.
141 Individual seeds in the plant community usually reach physiological maturity at different times
142 making definitions of physiological maturity on a community basis less precise. For example, an
143 individual soybean seed reaches maximum dry weight at a seed moisture content of
144 approximately 50% whereas reproductive growth stage R7 is the commonly accepted indicator of
145 physiological maturity in a soybean plant community. However, the average seed moisture
146 contents of individual seeds in that plant community at R7 usually range from 40% to 65%.
147 These examples illustrate that the variation in timing of seed maturity in most crops depends on
148 the reproductive growth characteristics of the individual seed.

149 **(20)** This figure shows the various parameters generally evaluated in seed maturation research
150 and further support these observations.

151 **(21)** The determination of physiological maturity usually requires differing measurements of
152 seed dry weight during the seed filling period which is often affected by sampling variation. This
153 makes it difficult to determine the precise time of physiological maturity, i.e., to identify the
154 moment when the transfer of plant reserves to the seed ceases (TeKrony and Egli, 1997).

155 This is perhaps the primary reason why some authors suggest the identification of
156 physiological maturity as the “period in which no more significant increases occur in seed dry

157 weight". Seed dry weight is usually determined at different intervals during maturation. The most
158 frequent method is drying seeds in an oven immediately after harvest followed by weighing the
159 dried seeds for a moisture content determination. This results in problems for obtaining
160 repeatable results, especially at the end of seed maturation when increases in seed weight are less
161 marked and can only be detected with more sensitive oven and scale equipment and extremely
162 careful experimental technique. These difficulties can be illustrated by the table from Neubern
163 and Carvalho in 1976 who studied the maturation process in bean seeds. The oscillations in seed
164 dry weight made the precise identification of the transfer of dry matter from the parent plant to
165 the seed uncertain.

166 **(22)** Determinations of seed moisture and dry mass content by the oven method often result in
167 a low level of precision in detecting small variations in seed dry weight that produce misleading
168 results. To reduce this problem, increasing the number of statistical replications, using an
169 adequate experimental design as well as reducing harvest intervals can be possible solutions.

170 Probably the most accurate method of determining physiological maturity is the measurement
171 of ^{14}C assimilate uptake by the developing seed. This was demonstrated by TeKrony et al. (1979)
172 with soybeans, Lee et al. (1979) with oats and Hunter et al. (1991) with maize seeds.

173 **(23)** Changes in seed color or other visual changes in seed or fruit structure are also excellent
174 morphological indicators of physiological maturity. **(24)** For example, physiological maturity was
175 closely associated with the change in color from green to yellow in soybean seeds. In maize, the
176 progress of seed development is accompanied by decreases in seed moisture content, the
177 development of a black layer **(25)** in the seed placental-chalazal region and a progressive
178 solidification of the milky endosperm beginning at the seed apex and ending at the base. The
179 formation of the black layer and the location of the milk line at 75% of the seed's length with a
180 solidified endosperm are parameters associated with seed maximum dry weight.

181 **(26)** In addition, similar visual changes in seed or fruit color associated with physiological
182 maturity have been reported for other agricultural and horticultural crops, such as tomatoes in this
183 figure as well as cucurbits, pepper, etc. Research has shown that a short period of post-harvest
184 fruit storage can enhance physiological seed quality; in tomatoes, this happens if the fruits are
185 harvested at the green-mature stage and stored until the pericarp is completely red (Dias et al.,
186 2006).

187 (27) Debate is also found in the research literature regarding the simultaneous expression of
188 maximum germination, vigor and seed dry weight at physiological maturity. Harrington proposed
189 that these three parameters occurred at the same time and could be considered together as
190 markers of physiological maturity. However, although some studies have confirmed this
191 hypothesis, several observations have shown biochemical changes leading to metabolic
192 “adjustments” after seeds reach their maximum dry weight.

193 For example, TeKrony and Egli (1997) studied the seed maturation of nine cultivated species
194 (grain crops, vegetables and fleshy fruits) and concluded that maximum physiological quality
195 occurred at or before maximum dry weight for those seeds harvested as dry seeds, but after this
196 time for species with seeds developing in fleshy fruits as shown in this table. They emphasized
197 that rapid drying of seeds from fleshy fruits just after harvest could affect results and provide
198 erroneous information regarding physiological quality in comparison to those seeds in fleshy
199 fruits that continued maturation for a short time after harvest while attached to the fruit or
200 inflorescence structure (28). The importance of this observation was similarly demonstrated by
201 Adams and Rinne (1980); they found that fast drying of high moisture content soybean seeds
202 enhanced cell membrane disorganization and promoted a decrease in the effectiveness of repair
203 mechanisms, thus impairing seed germinability and vigor. They also suggested that seed
204 maturation studies should allow seeds to be retained on the inflorescence or in the fruit after each
205 harvest because fast drying was deleterious to membrane organization and led to an abrupt
206 interruption of protein and enzyme synthesis as shown in this table.

207 (29) In contrast, for seeds produced in fleshy fruits, such as tomatoes, a series of physiological
208 events occur during fruit maturation, including tissue degradation and accumulation of sugars and
209 organic acids, that lead to reduced water potential in the fruit. These solutes within the fruit create
210 an osmotic environment that results in little free water thus preventing seed germination.
211 Generally, at the end of the seed maturation process, seeds inside the fruit lose water slowly until
212 osmotic equilibrium is attained.

213 As a result, the existence of these differing studies about physiological maturity create
214 confusion in the literature that can be attributed primarily to the wide variation in experimental
215 designs and varying concepts of physiological maturity. Thus, the definition of physiological
216 maturity based on seed maximum dry weight should be considered a reference point to

217 characterize the end of seed development and the physiological independence of the seed from
218 the parent plant.

219

220 **(30)** 3. Seed Physiological Maturity and Harvest Time

221 Physiological maturity identifies the moment seeds possess or are close to their maximum
222 physiological potential. As a result, the decision to identify this time as the optimum time for
223 harvesting a seed production area should be logical.

224 Any harvest delay from the point in which the seed becomes independent of the mother plant
225 may have drastic consequences on seed quality as a result of seed exposure to less favorable
226 environmental conditions for relatively long periods of time resulting in rapid deterioration of the
227 seeds. The occurrence of dry periods can also influence seed quality and yield, primarily in
228 species that exhibit fruit dehiscence (legumes, forestry species, some vegetables) or when seeds
229 are typically shattered to the soil (tropical forage grasses, rice). However, even in species that do
230 not fit these patterns and are protected against problems caused by external agents, such as maize
231 seeds within the ear, the quality and quantity of seed loss can be significant. **(31)** For example,
232 this table shows the loss in yield caused by delayed harvests of maize seeds.

233 **(32)** One of the problems that causes a decrease in seed performance after physiological
234 maturity is variability in dry and humid periods with high temperatures during the final phase of
235 seed maturation. This predisposes the seed to an increase in deterioration rates and the occurrence
236 of seed coat injury as a consequence of cotyledonary expansion and contraction after a series of
237 moistening and drying cycles. This frequently occurs in soybean seed production fields as
238 illustrated in these figures. Seed coats first become wrinkled and, if the conditions persist,
239 ruptures occur. **(33)** The tetrazolium test can identify the symptoms of this type of damage as
240 illustrated in these pictures. **(34)** This table shows the detrimental effects of 14 and 28 day harvest
241 delays on soybean seed performance.

242 **(35)** Harvest delays and excessive seed drying while the seed is still attached to the parent
243 plant also predisposes seeds to mechanical injuries that promote a significant decrease in physical
244 purity and performance.

245 However, it is not possible to harvest seeds at physiological maturity due to their high seed
246 moisture content that makes mechanized harvesting difficult and would promote significant
247 physical damage and crushing of seeds during threshing to separate the seeds from the parent

248 plants. As a result, seed moisture content and other morphological characteristics of plants and
249 seeds have been used as primary markers to identify the proper harvesting time.

250 As mentioned previously, harvest is usually performed some time after physiological maturity
251 is achieved and this is a delay in relation to the time when seeds have reached maximum quality.
252 The longer the delay occurs while seeds are attached to the parent plant in the field, the greater
253 the probability of seed quality loss as a consequence of exposure to less favorable field
254 conditions.

255 Another important consideration is that seed moisture content and plant morphological
256 characteristics can show wide variations in response to climatic changes at the end of the plant
257 life cycle, making these parameters less reliable in characterizing harvest delays. Therefore,
258 additional information is still required to provide a simple, fast and consistent identification time
259 when seeds reach physiological maturity. This would be valuable to establish a starting point
260 from when harvest would be considered delayed and reductions in seed quality are possible
261 emphasizing the need for prompt harvest.

262 Research has provided important contributions to guide the identification of physiological
263 maturity in seeds. Besides the “black layer” in maize and sorghum, the “milk line” in maize, and
264 seed color in soybean seeds, other characteristics can be considered. For example, the loss of the
265 green color in the glumes and pedicel of barley and oat caryopses and the flag leaf in wheat
266 plants serve as markers of physiological maturity. Similar traits are also used in vegetables such
267 as tomato, cucumber, pepper, in forestry seeds and forage grasses based on a fruit or spikelet’s
268 color, respectively.

269 Parameters have also been identified to recognize physiological maturity in legume seeds. It
270 has been shown that bean seeds are physiologically mature when pods have lost their green color
271 and at least 75% of the seeds reach the color characteristic of the cultivar.

272 **(36)** Flowering, pollination and seed maturation are not uniform processes within each plant
273 and, obviously, among plants in a crop community. It is particularly difficult to identify
274 physiological maturity and optimum time of harvest in crops with an indeterminate flowering
275 habit. Examples of this lack of flowering uniformity for primary, secondary and tertiary umbels
276 in carrot are shown in this picture **(37)** and its consequences on carrot seed quality are illustrated
277 in this table where germination percentage, first count percentage, and seed weight vary based on
278 umbel position **(38)**.

279 Therefore, visual identification of physiological maturity can help identify the time in which
280 seeds reach their greatest quality or are very close to this time. Based on this information, it is
281 possible to monitor seed drydown while the seeds remain attached to the parent plant and then
282 initiate harvest as soon as possible, i.e., at the time when seeds attain a moisture content adequate
283 for mechanical harvest while simultaneously reducing the risks associated with substantial losses
284 in yield and quality due to excessive harvest delays.

285

286 **(39)** 4. Reserve Accumulation

287 Seed development is a result of a sequence of genetically programmed events. After sexual
288 fusion, the developing seed begins to increase in weight as a result of nutrient and water intake
289 associated with rapidly accelerated cell division and elongation. Typically, in monocots, the
290 developing endosperm accounts for most of the weight increase. In contrast, in dicots, the
291 cotyledons are usually responsible for the most gain in seed weight during development.

292

293 **(40)** 4.1. *Translocation of Reserves from the Parent Plant*

294 Immediately after fertilization, seed development begins and the seed becomes the primary
295 recipient (“sink”) of assimilates transferred from the plant. As previously described, there are
296 four stages of seed development: cell division, cell elongation, reserve accumulation, and
297 desiccation.

298 The extent to which filling occurs in crops harvested for seed is an important agronomic
299 consideration and research has been conducted to determine the factors that influence yield. For
300 example, final weight per seed is a function of the rate of dry matter accumulation (seed growth
301 rate) and the time from beginning seed fill to maximum seed weight (duration of seed fill).
302 Weight per seed (seed size) and seed growth rate are not related to yield, but the duration of seed
303 fill is closely associated with yield.

304 There is a gradation in which the developing embryo absorbs the nucellus/endosperm during
305 embryogenesis of different species, ranging from total absorption such as in legumes and cotton
306 to almost no absorption such as in cereals. Apparently, dicots that completely consume the
307 nucellus/endosperm during embryogenesis utilize significantly higher levels of energy than do
308 monocots. The main nutrition of the developing seed is provided by photoassimilates translocated
309 via vascular tissues and by available nutrients absorbed from the soil.

310 Photoassimilates (sugars, amino acids, and other solutes) are transported from the parent plant
311 to seeds via the phloem by an osmotic gradient in which the water flow generates a reasonable
312 pressure that is reduced when these substances arrive at the seed.

313 **Development of a cereal seed** is a result of initial cell division followed by an influx of water
314 which drives cell extension followed by filling as the reserves are deposited within the
315 endosperm. Carbohydrates reach a maximum concentration in the vegetative parts of the parent
316 plant at anthesis (dehiscence of anthers), after which they start to decrease as they are
317 translocated to the growing seed.

318 Cereals can accumulate up to 90% of their nitrogen content before anthesis and this is
319 remobilized to the developing seeds as plant parts senesce. The final seed nitrogen content is
320 largely a consequence of the efficiency of this remobilization.

321 Photosynthetic rates exhibited by different parts of the plant are quite variable among grain
322 crops. In wheat and barley, net photosynthesis in the flag leaf and ear is relatively high, and these
323 regions provide the major nutrient sources to the seeds, although each may make quantitatively
324 different contributions in different crops and cultivars. In maize, sugars produced by the leaves
325 above the ear are translocated into the seeds, but those from leaves below the ear are primarily
326 directed to the roots.

327 **Development of a legume seed** shows substantial differences with respect to the sources of
328 carbon and nitrogen utilized for seed filling. Before flowering, legume plants utilize net
329 photosynthate for root and bacterial nodule respiration and carbon is utilized primarily in the
330 formation of new leaves and roots. In general, about 75% of the nitrogen accumulated in legume
331 seeds is derived from symbiotic N-fixing activity after anthesis, but there are species that have
332 most of their nitrogen needs being a result of nitrogen fixed before anthesis. Other legumes show
333 variations in these patterns such as peas that assimilate most of their carbon and nitrogen during
334 flowering and early fruiting.

335 In general, carbon assimilated before flowering is proportionally much less readily available to
336 the developing fruits than is nitrogen assimilated before flowering. Thus, storage reserve
337 formation in the seed depends primarily on assimilates formed during fruit formation and seed
338 yield is therefore extremely sensitive to adverse environmental conditions.

339 Sucrose is the primary form of sugar translocated from the sites of photosynthesis to the
340 developing seed. The major pathway for transport from vegetative parts to the seed is via the

341 phloem. Developing cereal seeds have no direct vascular connection with the parent plant, and a
342 short-distance transport mechanism operates to move the assimilates from the vascular tissues to
343 the region of reserve deposition, i.e., the endosperm. In temperate cereals, such as wheat and
344 barley, assimilates are supplied via the vascular tissue running in the furrow or crease located at
345 the pericarp surface and must first pass through the funiculus-chalazal region, then through the
346 nucellar projection, and finally through the aleurone layer before entering the starchy endosperm.
347 In monocot seeds, the major carbohydrate in the endosperm and the entire seed is starch. The
348 carbohydrate content increases rapidly as the endosperm develops at the expense of the testa-
349 pericarp tissue where only slight increases in carbohydrate content are observed. Sucrose and
350 reducing sugar levels, initially high in the young endosperm, decrease rapidly as the starch
351 content rises and the endosperm matures.

352 **(41)** In maize and several other tropical cereals that represent **endospermic seed**
353 **development**, assimilates are unloaded from the phloem terminals located at the base of the
354 caryopsis, the pedicel, where specialized transfer cells facilitate movement from this maternal
355 tissue into the base of the developing endosperm.

356 Invertases are present at the base of the maize seed which cleave sucrose to glucose and
357 fructose. These products are taken up actively into the endosperm cells and resynthesized to
358 sucrose prior to utilization in starch biosynthesis and other metabolic pathways. The uptake of
359 amino acids into developing cereal seeds has received less research attention, but it is generally
360 accepted that asparagine and glutamine are the primary translocated forms of amino acids from
361 the parent plant to the seed. Formation of other amino acids must occur within the developing
362 seeds.

363 **(42)** During the early stages of development, **non-endospermic legume seeds** obtain their
364 nutrients from the liquid surrounding the embryo in the embryo sac, other assimilates required
365 later for reserve deposition in the cotyledons are translocated from the parent plant **(43)**. This is
366 facilitated by a vascular strand which branches from the vascular tissue running through the pod
367 and then passes through the funiculus into the integuments. Passage of assimilates through the
368 funiculus and from the seed coat into the cotyledons by diffusion is aided by the presence of
369 transfer cells.

370 About 95% of the carbon translocated in the phloem of the seed coat is in the form of sucrose.
371 The major forms of nitrogen in the phloem are asparagine and glutamine. The relative abundance

372 of amino acids present in the seed coat phloem is affected by the form of nitrogen supplied by the
373 parent plant; those acquiring nitrogen by nodule fixation import more asparagine than those
374 utilizing nitrate from the soil.

375 In some legumes, such as certain soybean cultivars, the translocated sucrose produced by
376 photosynthesis in the leaves and pods may be stored temporarily as starch in the pod prior to
377 remobilization and later transferred to the developing seed. It has been suggested that hormones,
378 particularly abscisic acid, play important roles in the regulation of assimilate flow into the
379 developing seed by controlling phloem unloading or establishing sink strength.

380

381 (44) 4.2. *Deposition of reserves in developing seeds*

382 Developing seeds receive a relatively small number of substances from the parent plant. As a
383 result, most others must be synthesized. One of the special characteristics of this process is that
384 this synthesis must occur in different cellular compartments, one specific for each substance.
385 Carbohydrate accumulation precedes that of lipids and proteins because carbohydrates are
386 precursors or take part indirectly in the synthesis of lipids and proteins at a later time in most
387 seeds.

388 In the *Poaceae*, the highest concentration of carbohydrates occurs at anthesis. On the other
389 hand, in the *Fabaceae*, photosynthesis peaks at the beginning of flowering and it is then that the
390 transfer of assimilates from the parent plant to seeds is accelerated. This period corresponds to
391 the R5 developmental stage in soybeans.

392 The coordinated activity of different genetically regulated enzymatic systems determines the
393 direction of reserve synthesis. Most mature seeds contain at least two or three appreciable storage
394 reserves during seed development and, to a large extent, these are synthesized concurrently in
395 precise quantities and ratios. One feature which contributes to this observation is that this
396 synthesis takes place in different cellular compartments, i.e., starch and fatty acids in plastids, and
397 proteins in the cytosol and endoplasmic reticulum. Different enzymes and differential expression
398 of genes regulate this synthesis process.

399 (45) **Starch** is stored as amylose and amylopectin. Synthesis of amylose occurs first, followed
400 by later modification to produce amylopectin. Sucrose, the sugar translocated to the seed, is the
401 precursor substrate for starch formation. Starch is not the only carbohydrate stored in cereal and

402 legume seeds, and even when it is present, it may not be in the major form of stored polymeric
403 sugar.

404 In cereals, the walls of the dead cells of the mature starchy endosperm are composed of
405 considerable quantities of **hemicelluloses** and glucans. In some seeds, including some legumes,
406 the major storage product is deposited in the cell wall as hemicelluloses as well as in coffee and
407 ivory nut seeds where galactomannans may be deposited in the cell walls.

408 There is considerable variation in the major fatty acid constituents of stored **lipids** in seeds.
409 Sucrose entering the developing oil seed is used for the synthesis of storage glycerols and protein.

410 In general, the synthesis of storage **proteins** is initiated at similar, but not identical, times
411 during seed development and they are accumulated at about the same rate. There are some
412 exceptions, and in some species the appearance of new storage proteins during late development
413 (even in the early stages of seed desiccation) has been noted. The final quantity of stored protein
414 varies markedly and there is usually a characteristic major reserve protein within a species.

415 **(46)** In monocots, especially in cereal seeds, carbohydrates are polymerized in the endosperm,
416 while the proteins are accumulated in the aleurone layer. Cell division in the endosperm in
417 developing maize seeds is essentially completed 28 days following pollination. This figure shows
418 that at 28 days after pollination, the endosperm has maximum DNA content, indicating
419 completion of cell division. RNA content has also reached a maximum value, and the initial rapid
420 phase of protein synthesis has begun to plateau.

421 The second increase in endosperm protein content at 40 days after pollination is a result of the
422 formation of specialized storage proteins. It has been generally recognized that different proteins
423 are synthesized at different stages in development and these reserve proteins are synthesized at
424 later stages of seed development.

425 **(47)** Most reserves accumulated by developing legume seeds are stored in the cotyledons and
426 represent about 90% of the cotyledon dry weight. The carbon recycling from seed respiration and
427 retained by the pod walls contributes very little to seed fill.

428 **(48)** The synthesis of different protein occurs at the same time during seed development and,
429 in general, accumulation rates are very similar. The final content of each protein may be variable,
430 but some proteins predominate according to species.

431 **(49)** This figure shows that, in pea seeds, most proteins synthesis occurs during cellular
432 elongation and ceases when late maturation drying is completed. The DNA and RNA contents in

433 the cotyledons also increase during cellular expansion. Messenger RNAs direct the synthesis of
434 storage proteins and their values are greatest during the period of maximum storage protein
435 deposition. Consequently, as seeds initiate the final maturation drying period, mRNA quantity
436 declines as well as protein synthesis.

437 **(50)** Dry matter accumulation in legume seeds increases when pods reach their maximum
438 length. The data from Dornbos and McDonald (1986) presented in this table show that the critical
439 period of reserve accumulation in soybean seeds occurs between the R5 to R7 stages during
440 which plants are sensitive to environmental stresses that can influence seed yield and
441 physiological potential.

442 **(51)** While the growing seed is accumulating major storage reserves, changes are also
443 occurring in the content of other important chemical substances such as the growth regulators or
444 **hormones** – auxins, gibberellins, cytokinins, and abscisic acid (ABA). These substances play
445 important roles in the regulation of biosynthesis and metabolism of developing seeds. The
446 endogenous growth regulators may be involved in several processes such as: seed growth and
447 development, including: a) the arrest of growth prior to maturation; b) accumulation of storage
448 reserves; c) growth and development of exogenous seed tissues; d) storage of key compounds for
449 later use during germination and seedling growth.

450 During seed development, there is a wide variation in cytokinin, auxin, abscisic acid and
451 gibberellins levels. The maximum level of each hormone does not occur at the same time, but the
452 hormonal concentrations during seed development generally follow a standard pattern.

453 The period of cell division and enlargement in both the embryo and endosperm is also a period
454 when cytokinins are at their highest concentration and are promoting cytokinesis (cell division) in
455 meristematic plant tissues. Gibberellins are important for cell expansion, activation of enzyme
456 synthesis, and to monitor reserve accumulation, while ABA is associated with synthesis
457 processes and also the arrest of embryo growth to avoid precocious seed germination while the
458 seed remains attached to the parent plant. Auxins assure that normal reserve assimilation occurs
459 during seed development.

460

461 **(52)** 5. Environmental Factors Affecting Seed Development

462 The environment in which seeds form affects their development. This is often illustrated by
463 changes in seed size and weight as well as seed germination, vigor, and pathological health.

464 Components of the environment that influence seed performance include soil fertility, water,
465 temperature, light, and seed position on the plant (Copeland and McDonald, 1995).

466 Responses to environmental stress during seed development are diverse and complex,
467 although the effects are generally deleterious and result in a decline in seed number, weight, and
468 physiological potential. Stressful conditions can occur at any time during seed development and
469 the degree of damage depends on time (stage) of occurrence, type, intensity and duration of
470 stress.

471

472 5.1. *Soil fertility*

473 In general, plants that have been fertilized with the three major elements (N, P, K) produce
474 larger seeds than those which have not been fertilized. The increase in seed size is due to a
475 enhanced seed development rate during the seed filling period as a consequence of increased
476 nutrient availability. This has been shown for various species such as soybean and tomato.

477 According to Copeland and McDonald (1995), when the effects of individual fertilization
478 elements on seed development are considered, nitrogen has the greatest influence on seed size,
479 seed germination, and vigor.

480 Cultural practices usually influence these effects, depending on the intensity of competition
481 among plants. For example, row spacing and plant population per area are determined by
482 research that includes several production variables including the nutritional crop requirements.

483

484 5.2. *Water*

485 Water deficits reduce plant metabolism and seed development. Research has reported
486 decreases in leaf area, photosynthetic rate, and other effects that promote flower abortion and
487 negatively influence assimilate production and translocation to developing seeds; one of the most
488 important effects is the decrease in carbohydrate supply caused by a reduction in photosynthesis
489 rate. Prolonged droughts and reduced soil water availability cause decreases in seed size,
490 particularly when these effects occur during seed filling. If water deficits occur during flowering,
491 its primary effect is on a reduction in seed number.

492 In cereals, water stress during development of the inflorescence reduces the number of flower
493 primordia, resulting in a reduction in the total number of seeds formed. If the stress occurs at the
494 time of anthesis and fertilization, pollen production is negatively affected and the ability to form

495 receptive stigmas can also be retarded. This effect is of particular concern in maize seed
496 production.

497 **(53)** Cell division and elongation following fertilization determine the size and reserve storage
498 capacity of the seed, and both can be adversely affected by water and heat stress. Duration of
499 seed fill is shortened at temperatures over 30°C in several species, such as wheat and soybean.
500 This is probably a consequence of accelerated development popularly called “forced maturation”,
501 and, since the rate of seed fill does not increase proportionally to the larger number of cells
502 produced, seed yield is reduced at maturity. A similar situation likely occurs under conditions of
503 water stress.

504 **(54)** Water deficits during pod fill in soybean seeds may cause a significant reduction in yield
505 due to the formation of smaller, lighter seeds and, depending on the cultivar, wrinkled and
506 deformed seeds as shown in this figure. These problems are also accentuated by high
507 temperatures associated with water deficits.

508

509 **(55)** 5.3. *Temperature*

510 High temperatures during seed development produce smaller seeds, while low temperatures
511 retard seed growth. Seed germination and vigor are also adversely affected by exposure to low
512 temperatures during development. Oil seeds usually show reduced quantitative and qualitative
513 changes in lipid reserves when low temperatures occur during the later stages of maturation. For
514 example, seed development is negatively affected by temperatures below 22°C in maize, soybean
515 and wheat.

516 **(56)** High temperatures are considered the principal reason for the “forced maturation” of
517 soybean seeds. This causes a faster translocation of nutritional reserves, prevents the complete
518 degradation of chlorophyll by the enzyme chlorophylase, and results in the formation of greenish
519 seeds as seen in this figure. This phenomenon is also caused by water deficits or desiccant
520 application at inappropriate times during maturation. **(57)** The occurrence of greenish seeds is
521 undesirable because this abnormality results in decreases in seed germination and vigor as
522 documented in this table.

523

524

525

526 **(58)** 5.4. *Light*

527 The seasonal distribution of solar radiation is a fundamental factor in assuring adequate plant
528 development. In general, reduced light to the parent plant results in smaller seeds. This effect has
529 been reported in carrot, pea, soybean, corn and others as a result of decreases in photosynthesis.

530 The formation and retention of flowers in various species are related to global radiation so the
531 planning of sowing time and plant population per area is based on many factors, but light
532 availability is considered of most importance.

533

534 5.5. *Seed position on the plant*

535 The position in the inflorescence can affect seed development rate. For example, distal seeds
536 in a wheat spike have slower growth rates and shorter seed filling periods than proximal seeds.
537 Corn seeds at the tip of the ear are smaller than those at the base which has been attributed to
538 inadequate photosynthate supply. Soybean pods located in lower plant branches are produced
539 before those located in the upper nodes and are affected by different environmental conditions
540 during development and this causes differences in seed performance.

541 Smaller seeds are also produced from smaller fruits or those that mature later in the growing
542 season or are exposed to unfavorable environmental conditions. The usual consequence is
543 decreased seed germination and vigor.

544

545 **(59)** 6. Metabolism Reversion from Development to Germination

546 For most seeds, desiccation or maturation drying is the terminal phase of seed development. It
547 leads to a state of metabolic quiescence which links maturation to germination. Subsequent
548 hydration of mature seeds results in germination and, as a result, it has been suggested that this
549 loss of water from seed tissues plays a role in the transition from a developmental program to a
550 program oriented towards germination and growth.

551 Seeds do not usually germinate while still attached to the parent plant, but may undergo a
552 germination process after being removed from their surrounding structures and placed in water,
553 depending on the maturation stage. In many species, seeds do not germinate when removed from
554 the plant until they undergo late maturation or are at a desiccation stage.

555 **(60)** An important aspect of seed development deals with the general metabolic reversal that
556 takes place in nutrient storage tissue, i.e., the endosperm or the cotyledons at the end of the

557 maturation process. Cells that have been synthesizing significant amounts of protein and
558 carbohydrates or lipid reserves during embryogenesis completely reverse this process and start a
559 very rapid hydrolysis of these same materials during germination.

560 This reversal is generally not accompanied by cell division in these tissues and there is a
561 phenomenon of gene metabolic reversal taking place in an unchanging cell population.

562 Obviously, a certain amount of gene activation/deactivation is involved in such a reversal and
563 synthesis of all necessary cell constituents that accompanies cell division must occur.

564 During seed development, the concentration of abscisic acid (ABA) remains high during the
565 slow dehydration developmental period, thus ensuring a continuous reserve translocation from
566 plant to seeds and enzyme synthesis necessary that acts specifically in this anabolic processes.
567 Thus, the physiological mechanism by which seeds retain the embryo in a developmental state
568 without germination while still attached to the parent plant depends directly on ABA action.

569 This hormone ensures that the embryo remains in an embryogenesis mode instead of
570 germination mode while seeds are physiologically dependent on the parent plant by regulating the
571 metabolism of reserve accumulation and stimulating the production of mRNAs that codifies the
572 synthesis of enzymes and proteins fundamental to embryogenesis.

573 There are three kinds of mRNA in the developing embryo. These include those: a) involved in
574 the regulation of enzymes directing embryo development and germination; b) involved in the
575 regulations of specific enzymes related to seed development; c) involved in the regulation of
576 enzymes “stored” during late embryogenesis for utilization during the germination process.

577 Synthesis of the first two kinds of mRNA remains high during reserve translocation and
578 assimilation. As seeds dehydrate, the predominate mRNA synthesis that codifies enzymes
579 involved in hydrolysis processes occurs. Thus, when seeds are exposed to favorable
580 environmental conditions for germination, these enzymes become active and resume immediate
581 metabolic activity before the initiation of “de novo” hydrolytic enzyme synthesis. **(61)** However,
582 at the late embryogenesis/desiccation period, ABA concentration is reduced or the seed becomes
583 less sensitive to ABA action, thus inducing reversion of metabolism towards germination.

584 **(62)** Therefore, there is a developmental stage in the seed that permits a general reversion of
585 metabolism of reserve tissues, i.e., cells that were involved in synthesis processes (mostly
586 carbohydrates, lipids and proteins) reverse these processes and become capable of initiating
587 hydrolysis of these same materials that requires gene activation/deactivation processes. Thus, a

588 gradual deactivation of enzymes involved in synthesis metabolism occurs and enzyme synthesis
589 involved in reserve mobilization during germination is stimulated.

590 In contrast, recalcitrant seed development does not follow this pattern since no significant seed
591 desiccation occurs during late embryogenesis, so these seeds pass almost directly from the
592 development phase to germination without exhibiting any period of quiescence. It has been
593 suggested that seed metabolism of these species is less sensitive to ABA at the end of maturation,
594 even though these seeds possess high moisture contents.

595

596 **(63)** 7. Desiccation Tolerance

597 The progress of seed maturation can be evaluated by different molecular, physiological and/or
598 biochemical parameters. As previously mentioned, seeds of various species can germinate a few
599 days after fertilization, while others require more advanced phases of seed development before
600 germination is possible.

601 Maturation drying is a normal terminal event in the development of many seeds, after which
602 they pass into a metabolically quiescent state. Seeds may remain in this dry state for several days
603 to many years and still retain viability.

604 However, seeds are not capable of withstanding desiccation at all stages during their
605 development. The acquisition of desiccation tolerance usually occurs considerably earlier in seed
606 development than during the latter maturation drying stage. The ability of seeds to tolerate
607 desiccation progressively improves during development and this is a consequence of
608 physiological and morphological changes that take place as development proceeds, perhaps
609 initiating the synthesis of protective substances in the latter stages of seed maturation.

610 For example, castor bean seeds removed from the fruit (capsule) and placed in water do not
611 germinate until 50-55 days after pollination, at the time of the initiation of maturation drying.
612 However, when seeds were first desiccated and then placed in water, germination was achieved
613 as early as 25-30 days after pollination. Seeds dried at 20 days after pollination did not germinate
614 and also did not survive, because they had not reached the desiccation tolerant stage during
615 development. Other species usually exhibit this same behavior. It has been observed, however,
616 that at the early stages of development, survival desiccation occurs only if the seed is slowly
617 dried.

618 **(64)** Seed development has two phases with respect to tolerance to fast drying. Seeds are
619 considered as desiccation intolerant during cell division and elongation as well as a portion of the
620 reserve deposition period. Desiccation tolerance is completely established from the stage where
621 most reserves are already deposited in the seed that corresponds to the period when a decrease in
622 ABA concentration is observed, there is cessation of storage protein synthesis and of the
623 initiation of enzyme and protein synthesis associated with germination, i.e., hydrolytic enzymes.

624 During seed maturation, cells are more sensitive to fast dehydration or hydration than to the
625 water loss or uptake *per se*. Consequently, the gradual loss of water allows the activation of
626 protective mechanisms, which induces a higher tolerance to cellular imbalances caused by
627 dehydration that result in considerable cellular membrane and internal structure disorganization.
628 As a result, if exposed to fast drying, seeds may require additional time to establish repair
629 mechanisms. When there is not enough time for these necessary readjusts to the maintenance of
630 cellular structure, considerable cell damage occurs that reduces seed performance. This means
631 that a period of slow dehydration at the end of maturation when the seeds are still attached to the
632 parent plant is important for the formation of desiccation tolerant seeds.

633 **(65)** Among the metabolic changes occurring in seeds prior to or during drying, there is the
634 appearance of two types of compounds considered essential for the protection of seed tissues
635 against desiccation: some disaccharides and oligosaccharides and specific types of proteins.

636 The disaccharide sucrose and the oligosaccharides raffinose and stachyose generally occur at
637 much lower concentration in the desiccation-intolerant stage when monosaccharides (glucose,
638 mannose, fructose, galactose) predominate. Slow drying at the end of seed maturation favors the
639 accumulation of soluble sugars (sucrose, stachyose, and raffinose) that also act as protective
640 compounds against desiccation. These sugars contribute to membrane stability by preserving the
641 hydrophilic environment associated with successful protein activity during dehydration.

642 In addition, different groups of transcripts and their protein translation products arise during
643 seed development. One group that appears late in embryogenesis and accumulates to a relatively
644 high concentration is the *late embryogenesis abundant* (LEA) proteins.

645 Other substances and protective mechanisms involved in seed tolerance to dehydration have
646 been identified such as: a) physical cell characteristics, such as reduced vacuole volume; b)
647 antioxidant systems that prevent cellular damage caused by free radicals; c) mechanisms that
648 prevent coalescence of membranes; d) action of repair mechanisms during rehydration; e) action

649 of “heat shock” proteins. Recalcitrant seeds are deficient in, at least, one or all of these protective
650 mechanisms.

651 It is clear that desiccation tolerance is a complex phenomenon, involving the expression of
652 several genes involved in the performance of cellular protection mechanisms and in the decrease
653 of cell damage. At the same time, this protection must be extended until the seed is exposed to
654 imbibition so that successful germination can ensue.

655

656 **More Information**

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