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(2) Physiology of Seed Germination

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(3) In the germination process, the seed's role is that of a reproductive unit; it is the thread of life that assures survival of all plant species. Furthermore, because of its role in stand establishment, seed germination remains a key to modern agriculture. Thus, especially in a world acutely aware of the delicate balance between food production and world population, a fundamental understanding of germination is essential to crop production.

(4) Definition of Seed Germination. Various definitions of seed germination have been proposed, and it is important to understand their distinctions. The the seed physiologist, germination is defined as the emergence of the radicle through the seed coat. Such a definition says nothing about other essential structures such as the epicotyl or hypocotyls that become the above ground parts of a successful seedling. To the seed analyst, germination is "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions." This definition focuses on the reproductive ability of the seed, an essential objective in agriculture. Does it have the capacity to produce a normal plant? Others consider germination to be the resumption of active growth by the embryo resulting in the rupture of the seed coat and emergence of a young plant. This definition presumes that the seed has been in a state of quiescence, or rest, after its formation and development. During this period of rest, the seed is in a relatively inactive state and has a low rate of metabolism. It can remain in that state until environmental conditions trigger the resumption of active growth. Regardless of which definition is preferred, it should be emphasized that one cannot actually seed the process of germination unfold. Thereforem all definitions include some measure of seedling development, even though this occurs subsequent to the germination event.

(5) Morphology of Seed Germination. Based on the fate of the cotyledons, two kinds of seed germination occur, and neither appears to be related to seed structure. These two types are illustrated by the germination of bean and pea seeds. Although these seeds are similar in structure and are in the same taxonomic family, their germination patterns are quite different.

Epigeal Germination. Epigeal germination is characteristic of bean and pine seeds and is considered evolutionarily more primitive than hypogeal germination. During germination, the cotyledons are *raised above the ground* where they continue to provide nutritive support to the growing points. During root establishment, the hypocotyls begins to elongate in an arch that breaks through the soil, pulling the cotyledon and the enclosed plumule though the ground and projecting them into the air. Afterwards, the cotyledons open, plumule growth continues and the cotyledons wither and fall to the ground.

(6) *Hypogeal Germination.* Hypogeal germination is characteristic of pea seeds, all grasses such as corn, and many other species. During germination, the cotyledons or comparable storage organs *remain beneath the soil* while the plumule pushes upward and emerges above the ground. In hypogeal germination, the epicotyl is the rapidly elongating structure. Regardless of their above-ground or below-ground locations, the cotyledons or comparable storage organs continue to provide nutritive support to the growing points throughout germination.

(7) Requirements for Germination.

Water. Water is a basic requirement for germination. It is essential for enzyme activation, breakdown, translocation, and use of reserve storage material. In their resting state, seeds are characteristically low in moisture and relatively inactive metabolically. That is, they are in a state of quiescence. Thus, quiescent seeds are able to maintain a minimum level of metabolic activity that assures their long-term survival in the soil and during storage.

Moisture availability is described in various ways. *Field capacity* moisture is about optimum for germination in soil; however, germination varies among species and may occur at soil moistures near the *permanent wilting point.* Most seeds have a critical moisture content for germination to occur. For example, this value in corn is 30%, wheat 40% and soybeans 50%. Once that critical seed moisture content is attained in the seed, sufficient water is present to initiate germination and the seed is committed to that event and can not turn back. If the internal moisture content decreases below the critical moisture content, seeds will essentially decay in the soil.

(8) *Gases.* Air is composed of about 20% oxygen, 0.03% carbon dioxide, and about 80% nitrogen gas. If one provides different proportions of each of these gases under experimental conditions, it soon becomes clear that oxygen is required for germination of most species. Carbon dioxide concentrations higher than 0.03% retard germination, while nitrogen gas has no influence.

(9) *Temperature.* Seed germination is a complex process involving many individual reactions and phases, each of which is affected by temperature. The effect on germination can be expressed in terms of cardinal temperature: that is *minimum, optimum,* and *maximum* temperatures at which germination will occur. The minimum temperature is sometimes difficult to define since germination may actually be proceeding but at such a slow rate that determination of germination is often made before actual germination is completed. The optimum temperature may be defined as the temperature giving the greatest percentage of germination in the shortest time. The maximum temperature is governed by the temperature at which denaturation of proteins essential for germination occurs. The optimum temperature for most seeds is between 15 and 30^{o}C. The maximum temperature for most species is between 30 and 40^{o}C. Not only does germination have cardinal temperatures, but each stage has its own cardinal

temperature; therefore, the temperature response may change throughout the germination period because of the complexity of the germination process.

The response to temperature depends on a number of factors, including the species, variety, growing region, quality of the seed, and duration of time from harvest. As a general rule, temperate-regions seeds require lower temperatures than do tropical-region seeds, and wild species have lower temperature requirements than do domesticated plants. High-quality seeds are able to germinate under wider temperature ranges than low-quality seeds.

(10) Pattern of Seed Germination. Most seeds undergo a specific sequence of events during germination. Prior to germination, seeds are in a "maintenance" phase that is often characterized as dormancy being imposed by ABA, metabolic blocks or some other agent hindering the transition to germination. (11) At some point, the seed becomes sensitive to the presence of "trigger" agents. A "trigger" agent such as light or temperature alterations shift the balance of inhibitors to favor promoters such as gibberellins. A "trigger" agent can be defined as a factor that elicits germination but whose continued presence is not required throughout germination. (12) In contrast, a "germination" agent is a factor that must be present throughout the germination process. An example is gibberellic acid. The major sequence of events leading to germination is imbibition, enzyme activation, initiation of embryo growth, rupture of the seed coat and emergence of the seedling.

(13) *Imbibition.* The early stages of imbibition or water uptake into a dry seed represent a crucial period for seed germination. It is the first key event that moves the seed from a dry, quiescent, dormant organism to the resumption of embryo growth. Thus, any consideration of seed germination physiology and its resultant impact on stand establishment should focus initially on water uptake. The extent to which water imbibition occurs is dependent on three factors: (1) composition of the seed, (2) seed coat permeability, and (3) water availability

Composition of the seed. Seeds typically possess extremely low water potential attributed to their osmotic and matric characteristics. These potentials may be as low as – 400 MPa. The low water potentials are a consequence of the relationship of water with components of the seed. The Q_{10} value of imbibition for most seeds is 1.5 to 1.8 indicating that imbibition is a physical process not dependent on metabolic energy and related to the properties of the colloids present in seed tissues. (14) This is supported by the observation that imbibition occurs equally in dead and live seeds. Note in this figure that pea seeds have been heat killed and their rate of water uptake over time is essentially the same. The higher temperatures caused slightly greater rates of water increase due to the lower viscosity of water as temperatures increase. The principal component of seeds responsible for the imbibition of water is protein. (15) Proteins are zwitter-ions that exhibit both negative and positive charges that attract the highly charged polar water molecules. In contrast, this figure demonstrates that starches such as amylose have little affinity for water while lipids that have no charges on the molecular structure have no affinity for water. Thus, high protein containing seeds will imbibe more water than starch or oil containing seeds.

(16) Seed coat permeability. Entry of water into seeds is greatly influenced by the nature of the seed coat (or pericarp). Water permeability is usually

greatest at the mycropylar area where the seed coat is ordinarily quite thin. The hilum of many seeds also permits easy water entry. Mucilages extruded from seed coats such as with this Salvia seed increase imbibition as do the cellulose and pectins located in the cell walls.

(17) Availability of water. The environmental forces that determine the rate of water imbibition by seeds are complex. The ability to imbibe water is dependent on cell water potential and is a result of three forces:

1. Cell wall matric forces (represented by $\Psi_{m)}$. Cell walls and intracellular inclusions such as mitochondria, ribosomes, and spherosomes are characterized by the presence of membranes. These membranes possess charges that attract water molecules and contribute to the total cell water potential
2. Cell osmotic concentration (represented by Ψ_{Π}) . The greater the concentration of soluble compounds, the greater the attraction for water.
3. Cell turgor pressure (represented by Ψ_p) As water enters a cell, it exerts a swelling force on the cell wall called *turgor pressure*. Unlike the cell wall matric forces and osmotic concentration that attract water molecules into a cell, turgor pressure, which is a result of the restraining force of the cell wall, tends to retard water absorption.

(18) It should be remembered that water always moves to a more negative water potential and that the water potential of pure water is zero. The soils in which seeds are planted also exhibit their own water potentials. The physical properties of soils determine the retention and conductivity of water. For example, it is well know that soils heavy in clays are able to absorb water more vigorously and retain it longer than those possessing high quantities of sand. In effect, the seed and soil water potential must compete with the soil water potential for imbibition to occur. Initially, as in this example, the difference between seed and soil water potential is quite large. The seed is at – 14 MPa and the soil at – 3 MPa. Thus, water flows into the seed. However, as imbibition continues, this difference is reduced in the immediate vicinity of the seed. If it were not for the conductive ability of soils, imbibition would be quickly halted. However, most soils exhibit a high degree of hydraulic conductivity that replenishes the available water surrounding the seed as it continues the process of imbibition. This is important since seeds are sessile and a continuous flow of water is essential for maximum imbibition.

(19) Associated with seed and soil water potential is the degree of seed-soil contact. The greater the intimate contact of the seed with the soil, the greater the amount of water imbibed. At least three mechanisms have evolved to improve seed-soil contact as demonstrated in this table. Differing kinds of seeds were exposed to decreasing water potentials using the matric forces of sintered glass plates that retained more water on their surface and germination under these conditions determined. Some seeds possess mucilage which is extruded from the epidermal cells of the seed as it imbibes water. The mucilage serves to increase the contact of the seed with the soil by increasing the number of pathways through which water may be absorbed by the seed that leads to increasing germination. Another mechanism to enhance seed-soil contact is to increase the amount of seed contact with a specific volume of soil. This can be accomplished by altering seed coat configuration. Seeds possessing textured seed coats are more likely to have a greater seed-soil contact than smooth-coated seeds, and thus they will imbibe water more rapidly

resulting in greater germination. A final factor is seed size. Small seeds possess a greater surface are to volume ratio than large seeds. This greater surface area permits them to have access to a greater amount of water than larger seeds, which means they will hydrate and germinate more rapidly.

(20) There are also differences among species in their ability to germinate at differing soil water content values. Seeds rarely attract water beyond 10 mm in most soils. In this table is the germination of five species at differing soil water contents. Note that Agropyron and Triticum species are able to germinate at lower soil moisture contents than the others. Also, it is apparent that roots grow at lower soil water contents than shoots.

(21) Imbibition is not uniform. It would be expected that the process of water uptake would be uniform throughout the seed. Yet studies to examine this process in soybean and corn seeds have demonstrated this is not the case. For example, the presence of an intact soybean seed coat is important to slow the uptake of water into the seed and it can delay water uptake for the first eight hours of soaking. The seed coat also directs water movement both tangentially and radially into the embryonic axis. The seed coat is the thinnest on the back of the seed, so water first moves into this structure as demonstrated by the wrinkling or swelling of the seed coat at this position. (22) The radial movement of water is attributed to the presence of a radical pocket that possessed a high incidence of hourglass cells. (23) These cells may increase the water storage capacity around the radicle tip ensuring a ready source of water for turgor pressure essential for germination. (24) The same appears true in many grass seeds which possess a pericarp that completely surrounds the seed except at the pedicel end. This open, porous structure results in a more rapid hydration of the embryo that progressively moves from the radicle to the coloeoptile end as demonstrated by staining in nitroblue tetrazolium chloride. Note at 6 hours, the radicle tip has already begun to turn color while the coleoptile region still remains white. (25) A slower, more progressive wetting front simultaneously moves through the seed coat and into the endosperm that is not complete even after 48 hours imbibition.

(26) *Enzyme Activation.* Dry seeds are characterized by a remarkably low rate of metabolism that is undoubtedly attributable to their low moisture content that can be a low as 5 to 10% in unimbibed seeds. As soon as the seed becomes imbibed, however, marked changes in metabolism occur. A triphasic pattern of water uptake has been demonstrated during the germination of most seeds. Phase I occurs in dead or live seeds, an immediate release of gases is observed. It is not dependent on metabolism and is the result of matric forces and their attraction for water molecules inside the seed. (27) Enzyme activation begins during Phases I and II of imbibition. During Phase II, there is a lag period in water uptake, but the seed undergoes many processes essential for germination. (28) Finally, in Phase III, root elongation is observed. The root becomes functional during this phase and is responsible for the increased water uptake noted in Phase III. (29) Interestingly, if respiration rates are observed, a similar triphasic pattern is found demonstrating the importance of water for enzyme activation. Here, pea seeds are followed with their seed coats intact (solid dots) or removed (open dots). When the seed coat is removed, respiration is more rapid because water uptake occurs more quickly. Note how there are three phases of respiration increase associated with the three phases of water uptake. The fourth phase in this graph is related to activities associated

with lack of light and a decline in respiration due to the lack of photosynthesis since these seeds were germinated in the dark.

(30) Respiration. Respiration is the break down of sugars to produce energy molecules such as ATP. Based on these respiration findings, ATP production should also possess a triphasic pattern. In this study of imbibing lettuce seeds, the top graph documents the uptake of water and oxygen over time. Lettuce seeds initiate radicle protrusion based on this graph at 16 hours. In the lower graph, the production of ADP, ATP and total nucleotides is presented. Notice the expected triphasic increase for ATP and total nucleotides. Phase III occurred at 16 hours imbibition or the time of radicle protrusion.

(31) Breakdown of Storage Tissues. Generally, enzymes that break down carbohydrates, lipids, proteins, and phosphorous-containing compounds are the first to be activated during Phase II of water uptake by seeds. The controlling mechanism for directing this storage tissue degradation has not yet been clearly elucidated.

In monocots, the following diagram depicts the transitions in chemistry with germination. Each circle represents the activity of a particular enzyme. Since the embryonic axis requires energy for growth, storage compounds must be hydrolyzed to soluble forms, translocated from the endosperm to the embryo, and transformed to energy molecules that can be immediately utilized by the embryonic axis. The endosperm initially becomes rich in soluble products such as glucose and maltose. These are then absorbed by the scutellum. In the scutellum, glucose and maltose are transformed by a series of *in situ* enzyme reactions to form sucrose. Sucrose itself is not hydrolyzed within the scutellum because the essential enzymes are not present. The sucrose molecule is then transported to the adjacent embryonic axis as the principal energy molecule for growth.

(32) In dicots, the hormonal regulation of storage product degradation is not as clear as monocots. This may be due to the absence of an aleurone-like tissue that synthesizes hydrolytic enzymes. Additionally, the role of hormones in dicot seed germination has been debated. In some instances, gibberellins are known to trigger hydrolytic enzyme synthesis, but the degree of activation is never as great as that noted in cereals. Some believe that germination is mediated by the growing embryonic axis. As the axis continues to grow, it incorporates breakdown products into the synthesis of new compounds. This reduces the concentration of compounds in the cotyledons, which in turn stimulates the hydrolysis of other storage reserves for use by the embryonic axis. Should this stimulation prove to be too great, and hydrolyzed storage products begin to accumulate, a feedback mechanism may be operative that retards further storage reserve hydrolysis. The mobilization and transfer of nutrients as shown in this diagram is through the conductive tissue of the cotyledons to the growing embryonic axis. Like grass seeds, storage compounds must initially be hydrolyzed to a soluble form before they can be translocated.

(33) The final sequence of events is radicle protrusion. At this point the seedling is an autotrophic organism capable of growth without additional reserves. Germination is also ended by visible protrusion of the radicle and subsequent growth is attributed to seedling development.

(34) Metabolism of Germination.

Studies have been conducted on the developmental changes that occur in seeds as they initiate embryo growth. Generally, the only substances taken up by the seedling during these early stages are water and oxygen. Because the seedling is underground and not photosynthetically active, there is a loss of CO_2 and leakage of seed materials that result in an overall loss in dry weight. As the seedlings initiate photosynthesis and have the capability to fix CO_2 into sugars, an increase in seedling dry weight occurs and there is a shift in sink strength within the seedling based on growth.

(35) Because monocot and dicot seeds are different in their morphological structure, it is not surprising that the alterations these seeds exhibit are unique. This graph demonstrated the changes in dry weight of a dicot seed, *Vigna sesquipedalis*. Here, it is obvious that the major storage tissue, the cotyledons, is a source of energy during the first six days of growth for the developing hypocotyl and subsequently radicle, plumules and epicotyl which have become the new sinks in the emerging seedling.

(36) Monocot seeds generally display a germination pattern similar to that exhibited by corn. During the first 120 hours of germination, water uptake primarily occurs in the axis which is high in protein content with little water uptake in the scutellum which is high in oil content. (37) Overall, there is a decrease in seedling weight initially with a marked decrease in dry weight of the endosperm and a concomitant increase in the dry weight of the embryonic axis. The scutellum shows little change in dry weight throughout the process. (38) Soluble protein changes can be considered those associated with new enzyme formation. Note that there is a marked increase in soluble nitrogen formation throughout seedling growth. Initially, an increase in soluble nitrogen is found in the endosperm followed by a subsequent decrease in soluble nitrogen. This is an indication that enzymes are necessary initially to degrade the endosperm that later disappear as the endosperm digestion is complete. Enzymes then appear in the embryonic axis as it initiates growth. The scutellum produces only a moderate increase in soluble nitrogen throughout this process. (39) Nucleic acids increase in both the whole seedling and embryo axis. These demonstrate that cells are dividing in this particular structure as growth is initiated. There is no change in the endosperm because this tissue is dead while the scutellum shows only a slight increase likely associated with the nucleic acids being transcribed for the synthesis of lipases. (40) Lipid changes decrease during seedling growth because they are being degraded into fatty acids that are utilized during β-oxidation for the synthesis of energy. Most of these changes occur in the scutellum that is the primary oil storage structure in the corn seed. Little change in lipids is observed in the axis because this structure does not possess storage lipids.

(41) Castor bean or *Ricinus communis* serves as an example of the physiological changes that occur in an oil-storage seed. In this seed, most of the oil is stored in the endosperm because the cotyledons are thin and leaf-like. (42) To obtain energy from the lipids for seedling growth, mechanisms must be found where the lipids can be degraded to fatty acids for use in β-oxidation. This process is directed by the glyoxylate cycle that changes fatty acids ultimately to sucrose which is a non-reducing sugar. Glucose and fructose, two molecules used in the TCA cycle to generate energy in lipid containing seeds, are reducing sugars. The graph on the left demonstrates that sucrose is first detected in the cotyledons where it is translocated to the embryo. Then it is hydrolyzed

to glucose and fructose which are used to generate energy for seedling growth. (43) The fatty acids are used in the glyoxylate cycle to produce sucrose. The disappearance of fatty acids from the endosperm where the greatest concentration of oil exists is documented in the following graph. The enzyme responsible for the degradation of oils to fatty acids is lipase.

(44) In protein containing seeds, protein hydrolysis is accomplished by proteases likely synthesized under the direction of gibberellins. The graph demonstrates that much of the protein degradation occurs in the cotyledons and then declines as the protein levels are exhausted with little activity present in the embryonic axis.

(45) The availability of high inorganic phosphorous levels during the early stages of seedling growth is essential for the production of phospholipids as well as energy rich molecules such as ATP. Approximately 80% of the phosphorous found in seeds is associated with the molecule phytin. To obtain this phosphorous, the hydroxyl group of phytin must be hydrolyzed by the enzyme phytase to release the inorganic phosphorous. This graph demonstrates that as phytase levels increase, phytin levels rapidly decrease during germination.

(46) With respect to nucleic acids, the initial stages of radicle protrusion through the seed coat are caused by cell elongation followed by cell division. As a result, DNA synthesis is detected late after visible radicle protusion. Initial enzyme synthesis is guided by the presence of long-lived or stored mRNA that was synthesized during seed development and maintained in the seed until the germination event since the genetic code on DNA must be read and transcribed onto mRNA. During germination, the mRNA is translated into the synthesis of enzymes essential for germination.

(48)