

(1) Consortium slide

(2) Seed Germination II – Factors Affecting Seed Germination

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(3) Substantial progress has been made in our understanding of physiological mechanisms and factors in seeds that confer the ability to germinate under a wide range of conditions. The objective of this module is to describe various factors affecting seed germination including a wide range of internal mechanisms, environmental factors and chemical promoters. Germination and seedling establishment are the end result of a complex and interactive process involving a number of physiological, morphological, environmental and cultural factors. The discussion of these factors as individual topics needs to be put in context of this interacting environment. Germination is completed at various times for the individual seeds within a population which leads therefore to the distribution of germination times and a characteristic cumulative germination curve for any given seed lot. Aspects of internal seed physiology include the topics of seed vitality, the genetic potential of a seed to germinate, seed maturation, and seed dormancy.

(4) In most seeds, a desiccation period which leads to embryo quiescence, is naturally observed between development and germination. The sequence of events associated with seed development, germination, and growth are illustrated using pea (*Pisum sativum*), as a model. Various levels of development including histodifferentiation, maturation, or cell expansion, desiccation and seed drydown are involved prior to the germination and growth phases. During histodifferentiation, cell division, cell expansion and reserve deposition occur, and the seed is typically desiccation intolerant. As the seed approaches

the end of the development period where drydown has proceeded adequately, the seed is typically classified as desiccation tolerant. Upon exposure to germination conditions, including appropriate temperature ranges, water, and atmospheric conditions, the seed then again becomes desiccation intolerant as metabolism is renewed and reserve and storage materials are broken down for seedling growth.

(5) Seeds typically do not germinate during development on the parent plant, but undergo a process of maturation which includes the desiccation stage before being shed. Alfalfa seeds typically will not germinate (filled circles – Fig. A) until excised from the pod late in development after maturation drying has occurred, shown here as stages 6, 7 and 8. However, isolated embryos – filled squares in the Fig. A - will germinate from an early stage of development especially if placed on a nutrient medium. When embryos were placed on water - open circles in Fig. A -, the response was closer to that of the whole seed. For seeds which develop in a fully hydrated environment such as those of tomato, germination of isolated embryos- open circles in Fig B - can again occur at an earlier stage of development if they are placed on nutrient medium. Intact tomato seeds – black circles in Fig B - typically take about 60 days after pollination (DAP) to reach full maturity but will germinate at near 90 % levels by about 40 DAP.

(6) Endogenous hormones are often involved with seed development. Seed ABA content commonly increases during development and then declines during later maturation or the drying phase. Again, for seeds that develop within fleshy fruits (e.g. cucurbits, tomato) ABA content plus low water potential of the fruit tissues will both prevent precocious germination or vivipary. In this figure, wild-type *Arabidopsis* seeds are shown in the open circles, an ABA-deficient mutant is shown in the triangles and the downward triangles indicate an ABA-insensitive mutant response. Developing seeds of wild-type *Arabidopsis* do not germinate on water upon being dissected out of the silique. Seeds of the mutant lacking ABA germinate precociously under identical conditions as do those of the mutant that contains more ABA than the wild-type but which are insensitive to it. Mutants of other species, including tomato show similar responses.

(7) Viviparous mutants have seeds that germinate precociously, that is, while still on the mother plant. Mutants of this type have been reported for several species including various cereals (e.g. maize, wheat, rye and barley) and those that produce seeds in fleshy

fruits such as lemon, orange, tomato and melon, plus Arabidopsis. The effect of fluridone (an ABA biosynthesis inhibitor) on the germination of two lines of maize during the seed development is shown here. Developing kernels were sprayed with fluridone at 9 to 14 days after planting for the Tx 5855 line or at 5-15 days after planting stage for the Va 35 line. These plants were then allowed to mature to 45—50 days after planting before germination was tested. As can be seen by the different responses of these two lines, the effectiveness of fluridone varies with time of application during seed development.

(8) Seeds are not capable of withstanding desiccation or drydown at all stages during their development, but acquisition of desiccation tolerance is usually considerably earlier than the maturation drying itself. Germination results are shown of dried or fresh intact seeds of developing castor bean which were harvested at various days after pollination (DAP) and placed on water. Fresh seeds not subjected to drying do not germinate until 50 DAP, and even these seeds do not achieve normal growth (e.g. full germination) until 60 DAP. When excised seeds are first desiccated, germination is seen by 25 DAP, and the growth of seeds dried prematurely from 35 days onward exhibit normal post-germinative seedling growth.

(9) In maturing seeds of several species (e.g. white mustard, soybean, brassica and maize tested to date), the concentration of certain sugars and oligosaccharides increase in association with the onset of desiccation tolerance and the early phases of water loss. In data shown here from developing soybean embryos, there is an increase in some sugars and oligosaccharides within embryos that were induced to become desiccation tolerant by slow drying (filled circles), but not in embryos maintained in the intolerant state (open circles).

(10) Different groups of transcripts and their protein translation products arise at discrete times during seed development and maturation. One group, which was first identified in cotton embryos was named LEA or late embryogenesis abundant proteins. LEA proteins occur as two distinct classes with one class increasing at the mid-point of cotton seed development, coincident with ABA peaks. The second LEA class increases at the maturation drying stage, about 45-50 days after planting. LEA transcripts are now

known to occur in embryos of many species including pea, soybean, canola, carrot, castor bean, Arabidopsis, and several cereals.

(11) Following the various steps of seed maturation, the seed now enters the situation of dormant or non-dormant status. Seeds are often dispersed from the parent plant with different levels of dormancy, linked to different conditions due to genotype effects and the environment during seed development. Frequently, this variation is also observed in seed color, seed size and coat thickness. Also note in this figure that for dormant seed various light, temperature, and after-ripening treatments may release the seed from dormancy and allow seedling development to occur. Secondary dormancy (such as thermodormancy in lettuce) can also occur which will then have to be alleviated for proper germination and seedling growth.

(12) Dormancy in many cases is observed only in the intact seed, due to a coat imposed and/or embryo dormancy. Note the wide range of grains, woody plants and other species in these two categories. Both types of dormancy can exist simultaneously, or successively in some species. In apple seeds, embryo dormancy predominates but later on a contribution is made by the covering tissues (endosperm and testa) and their removal reduces the amount of dormancy-breaking treatment or chilling that is required.

(13) In many cases the expression of seed dormancy shows a strong temperature dependence. For example, grains of several grasses and cereals such as wheat, barley and oats are dormant only at temperatures above a particular value, a condition which is known as relative dormancy. This 'critical' temperature will frequently shift with seed age or various treatments including cytokinins, nitrate, and gibberillic acids.

(14) Dormant seeds can slowly lose their dormancy by the process known as afterripening, perhaps requiring as little time as a few weeks (e.g. barley), or as long as 60 months in the case of some weed species such as curly dock (*Rumex crispus*). Speed of afterripening depends on the environmental conditions such as moisture, temperature and oxygen. Temperature can be used to accelerate the loss of dormancy in agriculturally important species such as barley, wheat and rice. For rice, note the rapid loss of dormancy in terms of days needed as temperatures increase from 27-37 °C and higher.

(15) Secondary dormancy typically develops in already dispersed mature seeds in response to unfavorable germination conditions; some examples are given here. Several categories of secondary dormancy are related to light requirements involving red light, far-red light, and prolonged white light as well as temperature extremes, excessive or below normal conditions and water stress. Secondary dormancy is of great importance in germination ecophysiology, since it develops in many species when exposed to unfavorable conditions.

**(16) Environmental Factors and Seed Germination.** The response of seeds to a given combination of environmental factors depends on their (a) origin, (b) genotype, and (c) any enhancements or cultural practices employed in crop seeding systems. Of all the many environmental factors involved, water availability, temperature, and oxygen availability are of paramount importance. For some species, light variables and smoke are also important for germination.

(17) The uptake of H<sub>2</sub>O by seeds is an essential early step in germination. Water taken up during imbibition is generally about 2 -3x the seed dry weight. The rate of water uptake declines with time, and declines more quickly in sandy soils due to their low water holding capacity. Water uptake by chickpea (*Cicer arietinum*) seeds in distilled H<sub>2</sub>O or soils at various moisture contents is shown here. Soil water contents are expressed as percentages.

(18) Other factors also play a role in the rate and extent of water uptake, regardless of the difference in H<sub>2</sub>O potential between the seed and soil. Seed size, seed shape, seed coat characteristics, and production of mucilage can all influence seed water uptake. Small seeds, mucilaginous seeds, and smooth-coated seeds are more efficient in H<sub>2</sub>O uptake, because their better contact with soil.

(19) The range of germination responses among species to water stress is also wide, from very sensitive (e.g. soybean) to more resistant (e.g. pearl millet). Seeds of more resistant species may have an ecological advantage, establishing seedlings in areas where water-stress sensitive species will be unable to germinate.

(20) Seed permeability to H<sub>2</sub>O is influenced by morphology, structure, composition, initial water content and temperature. Soybean embryos with higher initial moisture

contents imbibed faster than those with a lower (8%) initial moisture level. All embryos imbibed more water as temperatures increased. Rates of water uptake are critical to successful germination; if uptake is too slow, germ/emergence is reduced because seed may deteriorate. If uptake is too rapid, seeds may exhibit imbibitional damage.

(21) As seeds begin to imbibe, a rapid leakage of solutes into the surrounding medium occurs. These solutes may stimulate the growth of soil microbes. Fig A shows... Initial solute leakage from pea embryos (Fig. B) lasts for ~ 30 min, and occurs only from the outermost cell layers.

(22) Some seeds (e.g. soybeans) leak proteinase inhibitors and lectins, compounds which may serve as protective agents against soil microbes and insects. Also, seeds of many legumes germinate poorly when hydrated without their testas; damage to the seed coat during harvest or planting can seriously reduce emergence and eventual yield.

(23) Temperature affects both the capacity for germination, and the rate of germination. Temperatures ranges for germination can vary widely by species; *Gypsophila* (solid line), leek (open circles), and *Lychnis* germination patterns are shown here.

(24) Species can exhibit widely different temperature minima and maxima. For experimental or descriptive purposes it may be useful to also define the temperatures at which 50% germination (GT50) occurs. The GT50 of several species can be affected by application of growth regulators. Kinetin raises the upper GT50 value for lettuce seeds (in light) from 31 to 40 °C, for example.

(25) When considering temp effects on germination, dormancy issues may arise. The unchilled *Delphinium* seeds in this figure are dormant at 20 °C and above, while the chilled seeds have no dormancy and germinate at 30+ °C.

(26) Although all seeds of a species (or cultivar, or seedlot) may germinate over a fairly wide range of temps, the time needed for maximum germination potential to be achieved varies with temperature. The maximum germination rate usually occurs over a narrow range, or at about 33 °C for the *Dolichos* (horse gram) species shown here.

(27) In this example, Brussels sprouts seeds were set to germinate over the temperature range of 4 to 42 °C, and germination percentages were recorded after 2 and 14 days. The highest germination rates were at temperatures ~ 25 °C (per 2 days counts), but all seeds were capable of germination at temperatures from ~ 10 to 35 °C.

(28) The consumption of O<sub>2</sub> by many seeds follows the basic pattern shown here, where embryo tissue respiration (fig. A) differs from that by storage tissues (fig. B).

Respiration in phase I increases linearly with tissue hydration. O<sub>2</sub> uptake in phase II increases slowly. Between phases II and III, radicle emergence occurs. A second respiratory burst is seen with the onset of phase III. Phase IV occurs only in storage tissues, coincident with reserve depletion and senescence.

(29) Oxygen levels in soil and greenhouse mixes are generally quite adequate for germination. Fine-textured (clay) soil particles may encase seeds under wet conditions, and lead to poor germination. Soil crusting (O<sub>2</sub> levels below 10%), deep planting, and flooding may also limit oxygen availability in extreme situations. Flooding-tolerant genotypes of maize, soybean and other crops have been identified for use in crop breeding programs as a partial remedy for short-term flooding situations.

(30) In flooded soils, O<sub>2</sub> concentrations can decrease to 1% or less (and CO<sub>2</sub> can increase to 5-8% in clay or muddy soils with decomposing organic matter). Flooding with warm water is also more detrimental to germination since O<sub>2</sub> solubility increases with decreasing temperature.

(31) Many seeds experience conditions of temporary hypoxia (or anoxia) during the lag phase (phase II) of germination. Ethanol and lactic acid can accumulate within the seed. Alcohol dehydrogenase and lactate dehydrogenase are responsible for both the synthesis and removal of ethanol and lactate, respectively. After germination has been completed and when conditions are more aerobic, Alcohol dehydrogenase – shown in this figure – and lactate dehydrogenase levels become negligible.

(32) The layers of tissue surrounding the embryo might limit gaseous exchange by the embryo in two ways: (1) O<sub>2</sub> entry is impeded, and (2) CO<sub>2</sub> escape may be hindered. The effects of tissue removal (e.g. testa, hull, pericarp, endosperm), seed coat puncturing, and oxygen-enriched atmospheres have different effects on the germination of species with 'coat-imposed' dormancy.

(33) In some species, seed coats have a powerful chemical inhibitory effect, rather than a physical restriction. The inhibitor ABA is present in many seed coat tissues; phenolic acids, tannins, coumarin, and other inhibitors are also listed here. In cases where repeated washing or leaching of the seed relieves dormancy, inhibitors are known to be removed.

(34) Only 7 species are known to germinate under anoxia – including 4 species of *Echinochloa* (barnyard grass), and rice – while several aquatic plant species germinate better under reduced O<sub>2</sub> concentrations than in air (see Table 1). O<sub>2</sub> requirements for germination may depend on other environmental factors (e.g. temperature, osmotic pressure of the germination medium, light) as well.

(35) Germination of fatty seeds (e.g. lettuce, sunflower, cabbage, soybean, and tomato) is completely inhibited when O<sub>2</sub> concentrations approach 2%. This figure shows that tomato seed germination at 25 °C steadily decreased as atmospheres went from ambient (21%) O<sub>2</sub> in curve 5, to 15% (curve 4)....10% (curve 3)....5% (curve 2)....and 3% in curve 1. Mainly starchy seeds (e.g. rice, wheat, maize, sorghum, pea) are able to germinate in atmospheres containing less than 1% oxygen.

(36) Scarification or coat removal promotes the germination of many nondormant seeds in hypoxia, since isolated embryos require less oxygen than intact seeds. In most cases, isolated embryos of apple, oat, sunflower and melon germinated well in 1 to 3% oxygen. However, as for whole seeds, their O<sub>2</sub> requirement increases when temperature increases. In this figure curve 1 represents apple embryos, and curve 2 represents oat embryos.

(37) Light influences germination in two ways; it can promote or inhibit. Seeds of many species are stimulated to germinate by light, but there is some debate as to whether this is a direct effect on germination itself, or a dormancy-breaking effect. Light responses are important to seed bank dynamics and other seed ecology responses where optimal timing for seedling establishment is linked to this environmental trigger. The photoreceptor responsible for most types of light-responses in seeds is phytochrome. Phytochrome is a family of chromoproteins where the protein part (phy A-E, and possibly F in tomato) of the molecules are encoded by different genes. (Black, 2006)

38) Examples of species whose seeds are sensitive to light are shown in this table. Light requirements also depend on associated factors such as temperature, depth of dormancy, seed coat integrity, seed development/maturation environment, water status, and the presence of chemicals (e.g. nitrate).

39) Levels of photodormancy in seeds vary among genotypes, and seed lots of a given genotype or cultivar. Photoconversions of the red-absorbing phytochrome, Pr (inactive form) to the far-red-absorbing Pfr (active form) usually take place when seeds are

hydrated above 8% seed moisture content. Critical periods for light environment effects on lettuce seed germination and storability can occur after physiological maturity (PM) has been achieved.

40) Lettuce (cv. 'Tango') germination data of seeds produced from plants grown under various daylength conditions are shown here. L= LD conditions throughout seed development, and S=SD conditions throughout; S6=6 d of seed development in SD, then LD, S12 = 12 d of seed development in SD, then LD, and so on. Even though physiological maturity (PM) is seen for this lettuce cv. by 11 days after anthesis, note how seeds from S6 and S12 treatments show a similar germination response to seeds from the full L (LD) treatment.

41) Prolonged exposure to relatively high fluence-rate white light (sunlight or lamps) also inhibits germination of many species. This effect is attributed to the blue region of the visible light spectrum, and is likely mediated thru cryptochrome. Two cryptochromes (cry1, cry 2) exist in higher plants; cry 1 is light-stable, while cry 2 is light labile, being rapidly degraded in blue light. White light photoinhibition is magnified for seeds under water stress (e.g. very negative water potentials). Evidence from tomato seed studies suggest that ABA biosynthesis is promoted at low H<sub>2</sub>O potentials, and that blue light increases tissue sensitivity to ABA. (Black, 2006)

42) In addition to water, temperature, oxygen and light, a fifth environmental factor influencing germination is smoke. First reported in 1990, the stimulatory effects of smoke on more than 100 species of heathland ('fynbos') and prairie plants from South Africa, Australia and North America have been noted. Butenolide compounds are found in smoke from burning plant materials, and these compounds have the needed attributes for germination effects; they are (1) stable at high temperatures, (2) water soluble, (3) biologically active across a broad range of concentrations, and (4) capable of promoting the germination of a wide range of species from fire-prone environments.

43) Chemical Promotion of Seed Germination. Changes in endogenous hormones are often occurring while the developing seed is accumulating its major storage reserves. Auxin, gibberellins, cytokinins and ABA can all play important roles in the regulation of seed growth, development and eventual germination. Ethylene is also involved in the germination response of seeds from some higher plants.

44) The major auxin in developing seeds is indoleacetic acid (IAA); IAA is formed from tryptophan and is not derived from the parent plant. IAA is most abundant in developing plant tissues (e.g. immature seeds, fruits). In pea (*Pisum sativum*) -shown in this figure- and seed of most plants studied to date, auxin is first found in the endosperm – solid line - and is later detectable in the embryo – dotted line - after the endosperm has been reabsorbed. The final decline in free IAA is the result of metabolic conversions to bound forms and other products. IAA is commonly conjugated to sugars, sugar alcohols and amino acids (asp, glu, ala).

45) The gibberellins comprise a large family of compounds that can regulate seed germination, reserve mobilization in cereal grains, and stem growth. The first GA identified was isolated from a rice plant infected with the fungus *Gibberella fujikuroi*, which causes ‘foolish seedling’ disease. The first plant gibberellin (GA<sub>1</sub>) was isolated in 1957; since then over 125 naturally occurring GA-like compounds have been isolated from higher and lower plants (mosses, algae). The biosynthetic pathway of GA’s is complex, beginning with conversion from geranylgeranyl diphosphate (GGPP) to GA<sub>12</sub>-aldehyde.

46) Studies with *Arabidopsis* and tomato gibberellin-deficient mutants provide the basis for the ‘hormone balance theory’, the concept that dormancy and germination are regulated by the internal actions of hormones, predominantly gibberellin (promotive) and abscisic acid (inhibitory). Some types of seed dormancy are also based on changes in GA + ABA content or sensitivity.

47) Abscisic acid (ABA) is multi-functional (e.g. promotes dormancy and maturation, inhibits germination, promotes synthesis of certain storage protein) and is found in the seeds of many species, as well as in leaves, roots, tubers, ripening fruit and dormant buds. Like other growth regulators in immature seeds, ABA generally rises in concentration during seed development, reaches 1 or 2 peaks – as seen in this picture for developing wheat grains- then (usually) declines rapidly at seed dry down.

48) Cytokinins also have diverse effects on plants; most notably in promoting cell division. Cytokinins also occur in developing embryo (e.g. zeatin from maize kernels). Cytokinins are synthesized primarily in root tips and transported (in xylem) to aerial plant parts. Cytokinins can also be synthesized in shoot tips. The amount of cytokinins

increases markedly during seed development, - seen in this figure for developing lupine seeds- then declines with seed maturation.

49) The plant hormone ethylene ( $C_2H_4$ ) is made in most plant tissues, and is also induced by a range of stresses and wounding. Ethylene dynamics can interact with other hormones (e.g. Cytokinins, ABA), or it can self-regulate its own synthesis. Ethylene is biosynthesized from ACC (1-aminocyclopropane-1-carboxylic acid), which is in turn synthesized from SAM (s-adenosyl-L-methionine). Exogenous ethylene-generating compounds (such as ethephon) can be used in seed enhancement treatments for improved germination.

50) Exogenous ethylene stimulates the germination of various dormant and non-dormant seeds (see table). This compilation includes cultivated plants (e.g. lettuce, peanut, sunflower), trees (apple, beech), weeds, and parasitic plants (e.g. witch weed, *Orobancha*). This gas can break coat-imposed dormancy (cocklebur, curled dock), embryo dormancy (apple, sunflower), and can overcome thermodormancy (lettuce). Ethylene can also inhibit germination of some species, or have no significant effect. Ethylene enhances the action of light, and can interact with other factors such as temperature, oxygen, osmotic agents and ABA.

51) Hydrogen peroxide ( $H_2O_2$ ) was noted as a germination stimulant in addition to its early role as a fungicidal seed treatment.  $H_2O_2$  may accelerate early respiratory phases of germination, but exact mechanisms are still unknown. Seed analysts do employ an 'H<sub>2</sub>O<sub>2</sub>' test which results in more rapid radicle protrusion from the seed (SCST Training Manual).

52) Potassium nitrate ( $KNO_3$ ) is also used in seed/germination testing.  $KNO_3$  is linked to relieving light dormancy (by increasing sensitivity to light) in many species. Various other chemicals (e.g. metabolic inhibitors, anaesthetics) also promote germination by acting on dormancy or germination physiology (or both).

53) Germination stimulators are numerous, and of great interest because their use may contribute to a fuller understanding of the biochemical and physiological mechanisms involved in germination and/or dormancy. Ethanol, for example, may modify membrane characteristics (ala anaesthetics) or may be involved metabolically as a respiratory substrate since it increases the  $O_2$  uptake in oats (and wild oats).

54) A great variety of compounds are also known to inhibit germination (see table). These include gases, aldehydes, organic acids, aromatic acids, coumarin, alkaloids, tannins, proline, and many others. While a diverse set of inhibitors exist, their biological action is less clear. More studies as to physiological roles, and mechanisms of action by inhibitors will also contribute to our understanding of seed germination and dormancy.