

## (1) II. Seed Formation

Samuel Contreras  
Crop Science Department  
Pontificia Universidad Catolica de Chile  
Santiago, Chile

### (2) 1. Floral Induction

(3) Life cycles of all multicellular organisms may be divided into more or less defined developmental stages. In humans for instance, we distinguish infancy, childhood, adolescence, and adulthood with puberty as the transition period between non-reproductive and reproductive phases.

(4) In general, the life cycle of seed propagated plants may be divided into four phases illustrated in this figure:

- (5) 1) the embryonic phase,
- (6) 2) the juvenile vegetative phase,
- (7) 3) the adult vegetative phase, and
- (8) 4) the adult reproductive phase.

In plants, these changes do not occur throughout the individual, but in single, dynamic regions known as *shoot apical meristems*, (9) so the same plant may have areas with different growth phases. For example, this figure represents an ivy plant in which juvenile and adult phases are distinguished. The juvenile phase is a vine which, as it grows into vertical form, undergoes a transition into the adult phase.

(10) The *juvenile vegetative phase* starts with the germination of the seed and is characterized by vegetative growth. Juvenile plants are insensitive to stimuli that promote flowering and they cannot flower. In many annual species, this phase is short and difficult to identify. For example, in some cultivated cereals and legumes, the juvenile phase is imperceptible and the plants are ready to perceive the flowering stimulus as soon as they emerge.

(11) However, in other cases, especially in biennial and perennial species, the juvenile phase may be more clearly distinguished. In many of these species, the juvenile phase prepares the plants morphologically to later sustain reproductive structures such as flowers, fruits and seeds. This picture shows an example of carrot. This is a biennial species that typically has vegetative growth during the first season, accumulating reserves in the root, and (12) during the second season, the plant uses these reserves for reproductive growth, producing flowers and seeds, thus completing its life cycle. In the case of carrots, depending on the cultivar, the juvenile phase may last until plants have at least 8 leaves formed.

(13) The length of the juvenile phase varies among and within species and is not always represented by “chronological time”, but by the development or growth accumulation reached by the individuals. Because of this, conditions that retard growth, for example mineral deficiencies, low irradiance, water stress, defoliation and cold, tend to extend the juvenile phase. In some cases, adult shoots may even *rejuvenate* or revert back to

juvenility. For instance, the juvenile phase of an apple tree may be reduced from 3 years when grown in the field to 16 months when cultivated under conditions permitting continuous growth.

(14) In this table are examples of species with their typical time of juvenility.

Some of the possible factors mediating the transition from juvenility to the adult phases are: size, age, leaf number and growth conditions (Saupe, 2007).

(15) At the level of the shoot apex, the transition from juvenility to the adult phases can be affected by factors such as nutrients, gibberellins and other chemical signals transmitted from the rest of the plant.

At the end of the juvenile phase, vegetative growth tends to decrease and the growing points become sensitive to internal and external signals that may trigger flowering. When this happens, the plants have reached their adult vegetative phase and they have the potential to flower, a stage classified as being “*competent*” (Saupe, 2007).

Returning to carrot, after the transition from juvenile to adult vegetative stages, the plants are said to be competent and have the potential to respond to stimuli that promote flowering.

In order to flower, the vegetative meristems of competent plants need to change from vegetative to reproductive structures. From an ecological perspective, it is important that individuals in the same species in a population coordinate this transition at the same time. In this way, they ensure that pollination and production of fruits and seeds occur at the same time under a favorable environment, particularly with respect to temperature and water. The collective events triggering the production of flowers in the plant meristem are known as *floral evocation*. The developmental signals leading to floral evocation may be internal or external factors. Some common endogenous or internal factors include circadian rhythms, phase change, and hormones, while the most common external factors are photoperiod (day length) and temperature (vernalization).

(16) In the case of carrot, the external signal leading to flowering is vernalization or the accumulation of a period of time under low temperatures, which under natural conditions occurs during the winter. Once these flowering signals have been perceived by the plant apical meristems, the meristems are said to be “*determined*”. (17) This picture shows the determined meristem of a carrot plant early during the spring, after winter and vernalization of the competent plant is completed. During the spring, with the availability of higher temperatures and water, this carrot plant will be able to flower and complete its life cycle.

### **(18) External factors affecting flowering**

Photoperiod and temperature are the two primary environmental cues regulating plant flowering.

## **(19) Photoperiod**

Photoperiodism is the ability of an organism to detect day length thereby permitting seasonal responses, (20) i.e., at a particular time of the year. As seen in this figure, day length or photoperiod varies depending on the latitude and time of the year. So a response to photoperiod is an effective way to coordinate physiological events at a specific time of the year in a specific location or latitude. A diversity of plant responses are controlled by day length, including the initiation of flowering, formation of storage organs, and onset of dormancy. However, plant classification according to photoperiodic responses is typically based on flowering induction. (21) There are three main categories:

(22) 1) **Short day plants**, when flowering is promoted by short days, or days that are shorter than a *critical day length*. These are generally species from low latitudes (e.g., coffee, cotton, and rice) or species that flower in the late summer and fall such as chrysanthemum.

(23) 2) **Long day plants**, when flowering is promoted by long days, or days that are longer than a *critical day length*. In general, these are species of high latitudes that flower in spring.

(24) 3) **Day neutral plants**, when species are insensitive to day length and flower under any photoperiodic condition. In general, these are species with wide latitudinal distribution such as tomato.

(25) The response to long and short photoperiods may be **qualitative**, when plants only flower under the specific photoperiod, or **quantitative**, when flowering is accelerated or increased by the specific photoperiod. Additionally, there are species that may distinguish between *shortening* and *lengthening* days and fall in one of the following categories:

(26) 1) **Long-short day plants**, these are species that flower after a sequence of long days followed by short days, i.e., when days are shortening in the late summer and fall.

2) **Short-long day plants**, these are species that flower after a sequence of short days followed by long days, i.e., when days are lengthening in early spring.

(27) Despite the fact that these categories refer to day length as the criterion for classification, it actually is the length of the night that determines flower induction. This means that short day plants flower when the night length exceeds a critical dark period. As a result, interruption of the dark period by a brief light treatment prevents flowering. In the case of long day species, plants flower when the night length is shorter than a critical period. In some long day species, shortening the night with a light break induces flowering.

(28) Through several experiments where the dark period was interrupted by light of different wavelength composition, the involvement of phytochrome as the primary photoreceptor mediating flowering responses to photoperiod was demonstrated. This

figure summarize the effects of light breaks over flowering response of short and long day plants. Breaks of red light during the dark period induce flowering in long day plants, and this effect is reversed by a break of far-red light. In short day plants, a break of red light during the dark period prevents flowering, and this effect is reversed by a break of far-red light.

According to previous research studies, the perception site for the photoperiodic stimulus in short and long day plants is their leaves. This implies that a diffusible compound is involved in the translocation of the flowering stimulus from the site of perception to the site of action, i.e., from the leaves to the meristem. Experimental data about the transporting rates of this flowering stimulus suggest that it is transported through the phloem.

### **(29) Vernalization**

In general, vernalization may be defined as the induction of flowering in winter annual, biennial and perennial species through exposure to low temperatures. Without exposure to cold, plants that require vernalization will not flower (qualitative response) or will take longer to flower (quantitative response). (30) As seen in this example with *Arabidopsis thaliana*, some plants form a rosette with no elongation of the stem when they require vernalization and they do not receive a cold treatment. In this example, the plant on the right was exposed as a seedling to 4°C for 40 days and flowered 3 weeks after the end of the cold treatment, when it had about 9 leaves on the primary stem.

Let's remember the example of carrots, where vernalization is required for flowering of competent plants in this biennial species.

(31) From an ecological perspective, vernalization is an effective mechanism that allows many species to flower once the winter is over, helping to ensure successful pollination and seed development under more favorable temperatures. In order to sense the vernalization stimulus, plants must be in their adult phase. For example, many herbaceous perennial species must have a determined number of leaves, usually around 10, before they are sensitive to the vernalization stimulus. However, there are some species, such as many winter cereals, in which the cold stimulus may be perceived by imbibed or germinating seeds.

(32) Vernalization may occur under a range of temperatures between -6 and 14°C (Erwin 2005). For most species, optimal vernalization temperatures are between 1 and 7°C, with specific optimums depending on the species. The perception site for the vernalization stimulus is the apical meristem and the time required for completing vernalization depends both on the species and vernalization temperatures. Less time is required when vernalization occurs at temperatures closer to the optimum. Some species such as *Brassica campestris pekinensis* and *Raphanus sativus* require short vernalization periods of less than 30 days. Other species such as *Apium graveolens*, *Secale cereale* and *Alstromeria* hybrids commonly requires 6 or more weeks for vernalization.

(33) Immediately after the cold temperature before flowering, there is a period during which plants may be devernalized. Devernalization is the loss of vernalization response by exposure to devernalizing conditions, usually high temperatures. As shown in this figure, the longer the period of cold treatment, the more permanent or stable the vernalization effect. In this example, imbibed seeds of winter rye (*Secale cereale*) were exposed to 5°C for different lengths of time, then immediately given a devernalizing treatment of 3 days at 35°C. The longer the duration of the cold treatment, the greater the number of plants successfully vernalized.

(35) *Interaction between photoperiod and vernalization:* In many species, photoperiod and vernalization interact to induce flowering. The most common case is a requirement of cold followed by long days, conditions that in many species ensure the occurrence of flowering during early summer at high latitudes. In general, the requirements for long days (both number of days and critical day-length) after vernalization decrease as the days of cold treatment increase.

The vernalization stimulus can be very stable. For example, once a meristem has reached its vernalization requirement, it will remain competent to flower for as long as 300 days in the absence of the inductive photoperiod.

#### (36) Chemical stimuli

There are natural and synthetic chemical compounds that cause floral induction in some species. (37) Among the natural compounds, gibberellins (GAs) are the more common plant hormone shown to affect flowering. Exogenous applications of GAs induce flowering under non-inductive conditions in long- and short-day requiring species as well as cold requiring species. However, it must be noted that GAs have been associated with flower inhibition in some short-day species.

(38) Other compounds that promote or increase flowering in some species are: indoleacetic acid, cytokinins, ethylene, naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, ethylene chlorohydrin, acetylene, and 2,3,5- triidobenzoic acid. (Copeland and McDonald, 2001)

#### (39) Floral Morphology

(40) A flower can be defined as an assemblage of sterile and fertile organs supported on a (41) structure known as a **receptacle**. (42) The sterile parts are the **sepals** and the **petals**. Collectively, the sepals and petals form (43) the **calyx** and **corolla**, respectively. The calyx and corolla together (44) are called the **perianth**. If sepals and petals are not differentiated, the components of the perianth are called **tepals**. The fertile, or reproductive organs are (45) the **stamens**, forming the **androecium** and (46) the **carpels**, forming the **gynoecium**.

After flower induction, the meristems shift from the formation of vegetative structures to the formation of reproductive structures or flowers. (47) The initiation of the floral organ differentiation begins at the margin of the meristem and floral organs form

sequentially in whorls from the outside inward: first the sepals, then the petals, then the stamens, and finally the carpels or ovary in the center.

(48) In most angiosperms, the stamen consists of a filament and a two-lobed anther. The anther contains pollen sacs or microsporangia where the microspores are produced.

(49) A flower may have one or more **carpels**. When there are two or more carpels, they may be separate (50) (apocarpus gynoecium) or fused (51) (syncarpus gynoecium). The term **pistil** (52) refers to a single carpel in an apocarpus gynoecium, or to the group of fused carpels in the syncarpus gynoecium. (53) Each pistil is typically composed of a **stigma**, the **style**, and the **ovary**. (54) The ovary is typically composed by the ovary wall and one or more locules. A **locule** is the cavity that contains the ovules. When the carpels are fused, the ovary is commonly partitioned into two or more locules. In this example of a tomato fruit, there are three carpels fused, so three locules are distinguishable. The region of the ovary where the ovules originate is called the **placenta**. (55) The placentation or arrangement of the placenta and ovules is characteristic of different groups of flowering plants and may be used for plant classification. In this figure, three common forms of placentation are shown. **Parietal** is when the ovules are borne to the ovary walls or to extensions of it. **Axile** is when a partitioned ovary exists and the ovules are borne to a central column of tissue. In the **free central** placentation, the ovules are borne to a central column of tissue not connected by partitions with the ovary wall. Another placentation form that is not shown in this picture is **basal**, in which a single ovule occurs at the very base of a unilocular ovary.

(56) There is great diversity in flowers and they may be classified according to their structures that comprise them. Flowers that have sepals, petals, stamens and carpels are said to be **complete**. Flowers that lack one or more of these for structures are said to be **incomplete**. (57) Another approach to classifying flowers is according to their sex, in which case they can be categorized as perfect or imperfect. **Perfect** flowers, also known as bisexual or hermaphrodite, have both stamens and carpels. **Imperfect** or unisexual flowers lack either the male or female organs so, depending on the part present, they can be staminate or carpellate (pistillate).

Plants can also be classified according to the types of flowers they have. (58) Plants that have perfect flowers, such as tomato and pepper, are said to be **hermaphrodite**. Species, (59) such as maize, that have staminate and pistillate flowers in the same plant are known as **monoecious**. Species with imperfect flowers in which staminate and pistillate flowers are present in different plants (60) are known as **dioecious**; asparagus is an example. In this case there are male and female plants and both types are required to produce seeds; the male plants are the pollen donors and the female plants are the pollen receptors and the plant on which seeds are produced. (61) Additionally, there are species in which imperfect and perfect flowers are present in the same plant. In this case, **andromonoecious** species have plants with perfect and staminate flowers, while **gynomonoecious** species have plants with perfect and pistillate flowers.

(62) Depending on the species, flowers may be present as single units or as part of an **inflorescence**, which is an arrangement of flowers on an axis. The stalk of a solitary flower is called a **peduncle**. In an inflorescence the minimum stalk is known as a peduncle and the stalk of each individual flower is called a **pedicel**. Inflorescences may be classified as **determinate** when the axis terminates in a flower or **indeterminate** when the axis terminates in a vegetative bud that continues growing and producing flowers (Copeland and McDonald, 2001). They also may be classified as **simple** and **compound**. In this slide are some examples of different types of inflorescences.

(63) Pollen and ovule formation

(64) Pollen formation (microsporogenesis)

Male gametes are formed within the pollen grains, and pollen grains are produced in the stamens, within the anthers. There are two processes involved in the production of the male gametes or microgametophytes; a) **microsporogenesis**, which is the formation of the microspores or single celled pollen grains, and b) **microgametogenesis**, which is the formation of the microgametophyte within the pollen grain.

(65) Microsporogenesis:

Microsporogenesis occurs in the anthers within the microsporangia or **pollen sacs**. Commonly, there are four pollen sacs in each anther. In each pollen sac are many microsporocytes or **microspore mother cells**. (66) These cells are diploid ( $2n$ ) and undergo meiosis to form a tetrad of four haploid ( $1n$ ) cells known as **microspores**. Usually, each of these microspores becomes a viable pollen grain. The inner layer of cells of the pollen sac is called the **tapetum**, which supplies nutrients to the developing pollen grains.

(67) Microgametogenesis

The formation of the microgametophyte within the pollen grain begins with the mitotic division of the haploid nucleus to form a **vegetative** and a **generative** nucleus. The generative nucleus forms the **generative cell**, which moves to the interior of the pollen grain. The vegetative nucleus forms a large **tube cell**. (68) Following this two-celled stage, the generative nucleus divides to form two haploid male gametes or sperm. This picture shows this process for maize; in this case, pollen grains were stained with mithramycin to show nuclei. In some species, the microgametophyte is produced before the division of the generative nucleus, as a two-celled pollen grain, while in others species, the generative nucleus divides before the pollen is released from the anther. In the example of maize, anthesis occurs 6 to 7 days after formation of the sperm cells.

(69) Typically, a mature pollen grain possesses an inner thin wall called an **intine**, which is composed of cellulose and pectin, and a tough outer wall called the **exine**. The exine is composed of a resistant compound, apparently derived from the tapetum, known as sporopollenin. The exine characteristics are particular for each species and are important for pollen grain recognition. Pollen walls carry diverse types of proteins, including enzymes and allergenic proteins (such as those causing hayfever). These proteins may be involved in recognition reactions such as some types of self-incompatibilities.

**(70) Ovule formation**

In the gynoecium, the female gametes are formed in the carpel or ovary. There are two processes involved:

- a) megasporogenesis, which is the formation of the megaspore within the nucellus (megasporangium), and
- b) megagametogenesis, which is the development of the female gametophyte or embryo sac, from the functional megaspore.

**(71) Megasporogenesis.** This process begins with a single diploid cell ( $2n$ ), the **megaspore mother cell** or megasporocyte, which is located in the **nucellus**, a specialized tissue of the carpel. **(72)** This cell undergoes meiosis and forms four haploid ( $1n$ ) cells or **megaspores**, which are generally arranged in a linear tetrad. **(73)** Normally, only one megaspore is functional and the other three degenerate.

**(74) Megagametogenesis.** The haploid nucleus of the functional megaspore undergoes three successive mitotic divisions to form an enlarged cell with eight haploid nuclei known **(75)** as the **embryo sac** (female gametophyte or megagametophyte). The eight nuclei arrange **(76)** themselves within the enlarging embryo sac and, in some cases, cell walls are formed resulting in three antipodal cells at one end, two polar nuclei (without cell walls) near the center, and the egg apparatus, composed of the egg cell between two synergid cells at the other end. In the egg apparatus, the egg cell is larger than the surrounding synergid cells and is a complete cell containing a haploid nucleus with surrounding cytoplasm enclosed in a thin cell wall.

Finally, the two polar nuclei fuse to form a diploid polar nucleus. The resulting structure, with six haploid cells and a diploid nucleus, is known as the mature female gametophyte, megagametophyte, or embryo sac.

**(77) Ovule formation.** While megasporogenesis and megagametogenesis are occurring, one or two enveloping layers known as the **integuments** originate from the nucellus. The integuments, which later become the **seed coat** or **testa**, surround the embryo sac leaving a small opening at one end known as the **micropyle**. The **funiculus** is the structure that connects the ovule to the placenta (ovary cell wall). The **chalaza** originates as an integumentary attachment and is usually located opposite the micropyle. In the embryo sac, the egg cell is located close to the micropyle and comprises most of the egg apparatus.

**(78)** In summary, a mature ovule is primarily composed of a central mass of maternal tissue surrounded by one or two integuments. Within the nucellus is the embryo sac, containing the egg cell from which the new embryo will originate after pollination and fertilization.

## (79) Pollination and Fertilization

(80) **Pollination** is the transfer of pollen from the anthers to the stigma. (81) Pollination may be classified as:

- a) **Self-pollination** is when the pollen that reaches the stigma comes from the same flower, plant, or clone, and
- b) **Cross-pollination** is when the pollen that reaches the stigma comes from a plant with a different genotype.

(82) Plants species are classified according to the relative frequency of self- or cross-pollination during seed production (Fehr, 1987). There is a continuum of variation among and within species, ranging from plants with almost 100% self-pollination to others with frequencies close to 100% cross-pollination. Even within the same genotype, there may be different frequencies of self- and cross-pollination depending on the environment where the plant develops. Despite this variation, a species usually may be classified as:

- i) self-pollinated or autogamous, when reproduction is predominantly by self-pollination under normal conditions, or
- ii) cross-pollinated or allogamous, when reproduction is mainly by cross-pollination under normal conditions.

The existence of different types of pollination has been of great importance for evolution and breeding of cultivated species. Additionally, management of pollination is a fundamental aspect of seed production, affecting both seed yield and quality, specifically genetic purity. In the Seed Production module “Fundamentals of seed production I: Genetics, breeding and seed production”, these and other aspects that consider the importance of pollination with breeding and seed production are discussed.

(83) After anther dehiscence, the transfer of pollen to the stigma may be accomplished by biological and/or physical vectors. Common physical vectors are wind and water, while common biological vectors are insects, birds and mammals.

(84) When pollen is released from the anther, it commonly is in a dry state. After it reaches the stigma, however, the pollen grain hydrates and a pollen tube grows through the style toward the ovule to accomplish double fertilization. As mentioned, the mature pollen grain is composed of a large tube cell nucleus and two gametes or sperm cells. Once in the stigma, the pollen tube elongates and the growth of the pollen tube through the style is facilitated by the presence of transmitting tissue. In most angiosperms, the styles have one or more strands of transmitting tissue extending from the stigma to the ovules. Depending on the type of plant, the pollen tube grows either between the cells of the transmitting tissue or within their thick walls. (85) In this picture, a germinated pollen grain with an elongated pollen tube directed by the tube nucleus followed by the two sperm cells is seen. (86) Once in the ovary, the pollen tube penetrates the ovule through the micropyle. By this point, the tube nucleus is exhausted and no longer functional. The two sperm nuclei are released into the embryo sac. One sperm nucleus fuses with the egg cell nucleus, forming a diploid zygote, while the other sperm nucleus fuses with the

diploid polar nucleus to form the triploid endosperm cell. This process is known as **double fertilization** and is characteristic of angiosperms. During this process, the synergid and antipodal cells degenerate leaving the zygote and endosperm to continue the development of the seed. The formation and development of an embryo from a zygote is known as **embryogenesis**.

#### (87) Embryogenesis

Although the overall patterns of embryogenesis are similar in angiosperm plants, the details of this process vary widely among species. Here, we will discuss the general patterns of the process in monocot and dicot seed formation.

(88) Embryogenesis in dicots. The first division of the zygote results in a **basal** (closer to the micropyle end) and a **distal** (axial or apical; closer to the chalazal end) cell. The basal cell, after an ongoing series of transverse divisions, forms a **suspensor**, which is a structure that anchors the developing embryo to the embryo sac, pushes it into the endosperm, and provides one of the mechanisms for the transfer of nutrients from the surrounding tissue to the embryo. During the initial phase of embryogenesis, when there is no clear differentiation between the embryo and suspensor, the entire structure is called a **proembryo**. (89) The embryo *per se* originates from successive vertical and transverse divisions of the distal or axial cell, which soon forms a **globular** structure. (90) The globular stage is followed by the **heart stage**, which is characterized by a flat embryo with a depression that begins to show bilateral symmetry. (91) Once the cotyledons and the discernible hypocotyl elongate, the embryo is in the **torpedo stage**. (92) Later, during the **cotyledonary stage**, the embryo continues enlarging and elongating to complete growth.

(93) Embryogenesis in monocots. In monocot species, after the first cell division of the zygote, the basal cell does not divide, but forms a terminal or haustorial cell of the suspensor. The embryo and other cells of the suspensor are initiated from the division of the distal cell. After subsequent divisions of the distal cell, a globular mass of cells is formed; this is similar to events in dicots, but one difference is that the globular embryo becomes cylindrical in shape and only one cotyledon is formed. (94) In some monocots, such as onion, the single cotyledon enlarges more than the rest of the embryo and becomes the dominant structure. (95) In other species, such as grasses, the single cotyledon forms the **scutellum**, a structure that remains in the seed following germination and serves as an organ that absorbs nutrients from the endosperm and transfers them to the growing embryonic axis. Note how, in this case, the embryonic shoot or plumule is enclosed by a **coleoptile**, and the radicle is enclosed by a **coleorhiza**.

(96) In both mono- and dicot species, the apical-basal pattern of the embryo appears during the transition from the globular stage to the differentiation of the cotyledon(s). (97) In the mature embryonic axis, a shoot, the cotyledon(s), hypocotyl, and radicle are distinguishable. Apical meristems are found at the tip of the shoot and radicle. These meristems are composed of cells capable of dividing and forming the root and shoot systems of the new seedling during germination and emergence. As a result, their integrity is fundamental for the seed to produce a normal plant.

(98) In dicot embryos, the apical meristem of the shoot appears between the two cotyledons.

(99) In monocot embryos, the apical meristem of the shoot arises on the side of the cotyledon (scutellum) and is completely enclosed in the coleoptile.

(100) Endosperm and perisperm development

(101) *Endosperm development*

The endosperm originates from the fusion of one of the male gametes (sperm nucleus) and the two polar nuclei of the embryo sac resulting in a triploid ( $3n$ ) nucleus. There are three main types of endosperm development: nuclear, cellular, and intermediate.

a) In the **nuclear**, or non-cellular type, the endosperm undergoes several free nuclear divisions, thus generating many free nuclei. Cell walls may or may not form later. Some examples are apple, wheat, and squash.

b) In the **cellular** type there is no free nuclear phase, the cell walls form immediately after each nuclear division. *Magnolia* and *Lobelia* are examples of species with this type of endosperm development.

c) While the nuclear and cellular types occur in both monocots and dicots, the **intermediate**, or **helobial**, type of endosperm is only found in some monocots. In this case, there is free nuclear division, but cell wall formation accompanies nuclear division in some parts of the endosperm as well. Coconut is an example of a seed with this type of endosperm development.

In general, during early seed development, the endosperm grows by acquiring nutrients from adjacent tissues, thus becoming a strong sink within the ovule. Later, at maturity, the endosperm fate will depend on the species. (102) In some cases, such as in many dicots (e.g., beans and cotton), the endosperm develops briefly and then is totally absorbed by the developing embryo. In these seeds, the cotyledons are typically the main source of nutrients for embryo growth during germination and emergence. (103) In other cases, such as tomato, pepper, and lettuce, the endosperm is not totally absorbed by the embryo, but there is a remaining layer of endospermic cells that surround the embryo of the mature seed.

(104) During seed development of many monocots, e.g. Poaceae species as well as a few dicots, e.g. castor bean, the endosperm is not absorbed by the embryo, but becomes the main nutrient source for embryo growth during germination and emergence. In these species, the endosperm usually attains its maximum growth at physiological maturity and remains as a major structure of the seed. In cereals, the mature endosperm is composed primarily of starch grains and proteins. These proteins are in protein bodies that lose their membranes in the mature, dead endosperm and the proteins form an amorphous matrix, that is known as **gluten** in wheat and rice seeds. The outermost cell layers of the endosperm remain alive in the mature, dry seed and are known as the **aleurone layer**. This is an important structure that functions as both a storage tissue and as a source of hydrolytic enzymes. These enzymes are activated and secreted during germination to break down storage tissues and make nutrients available to the growing embryo.

**(105) Perisperm**

The **perisperm** is a nutritive diploid tissue that originates from the nucellus. It is unusual for the perisperm to be the only storage tissue in the seed, and, when present, it usually shares a nutrient storage function with the endosperm or cotyledons. Amaranthaceae, Chenopodiaceae, Polygonaceae, and Cactaceae are examples of families with species that possess seeds with a perisperm serving as a storage tissue.

**(106)** Seeds storing nutrients in the endosperm or perisperm are called *albuminous*, while those species lacking these storage tissues are called *exalbuminous*.

**(107) Seed coat**

In angiosperms, the embryo and storage tissue are typically surrounded by a seed coat. **(108)** In many species, the seed coat is comprised of the testa, which have developed from the ovule integuments. **(109)** However, there are many species in which the testa is relatively thin and undeveloped, and the seed coat is formed by other structures such as the ovary walls or pericarp. Note that in both cases, the seed coat is of maternal origin.

The development of the testa from the integuments begins after fertilization, apparently triggered by endosperm development. In studies conducted on soybean and Arabidopsis, there are tissues that appear during early and mid-development that do not persist into the mature testa.

**(110)** Seed coats have several functions during seed development and in the mature seed. Some of the functions of the integuments, or developing testa, during seed development are:

- a) Being a pathway for transport and conversion of amino acids and carbohydrates from the pericarp into the ovule for the development of the embryo, endosperm, and the testa itself.
- b) Temporary storage of compounds for later use by seed coat cells.
- c) Involvement in gas exchange.
- d) Possible supply of growth compounds to the growing embryo and maternal organs,
- e) Protection of the embryo and endosperm from desiccation and mechanical injury.

**(111)** Some of the functions of the seed coat in the mature seed are:

- a) Protection of the embryo and endosperm against physical and biological damage. In general, in the soil or in storage, those seeds with strong, impermeable seed coats lose viability and vigor more slowly than those with weaker seed coats. However, it must be noted that seed coats may also harbor seed-borne microorganisms, including saprophytic and pathogenic species.
- b) Sometimes seed coats develop special structures that assist dispersal. This is an important consideration in seed ecology studies.
- c) Water uptake regulation. In some cases, the seed coat completely impedes water absorption and causes seed dormancy. In other cases, it regulates the rate of water absorption, preventing too rapid intake of water that could cause cell damage.
- d) Regulation of seed dormancy by supplying substances that impede germination or prevent leaching of inhibitory compounds from the seed.

(112) Seed coat structure and thickness vary considerably among species and even within species depending on the environmental conditions in which the seed developed. For example, in soybean, it has been observed that seeds that develop under higher temperatures had thicker seed coats (Keigley and Mullen 1986, *Crop Science* 26: 1212-1216).

As mentioned previously, the seed coat may be primarily composed by the testa derived from the integuments, or by the testa and the fruit wall or pericarp. In seeds formed inside indehiscent fruits, such as nuts and drupes (e.g., peach), the main protective structure is usually the entire pericarp (e.g., in **nuts**) or the endocarp, which is the inner layer of the pericarp (e.g., **drupes**). In this case, the testa is generally poorly developed and thin, without differentiation of any seed coat tissue. (114) Caryopses and achenes are other examples of simple, dry, single-seeded indehiscent fruits in which the pericarp is the primary protective structure of the seed coat. **Caryopses** are characteristic of grasses (Poaceae family) in which the testa are firmly united with the pericarp. **Achenes** are similar, but the seed coat does not adhere to the pericarp; species such as lettuce and sunflower have seeds with this type of seed coat.

The structure of the seed coat is diverse and, when not formed by the pericarp, (115) is mainly derived from the ovary integuments and to various extents by the raphe and the chalaza. The **raphe** is a ridge along the body of the seed formed by part of the funiculus that is adnate to the ovule. (116) This mechanically resistant structure usually possesses one or more layers of sclerenchyma originated from the integuments. Sclerenchyma cells are variable in form and size and usually have thick secondary walls with various accumulations of lignin, suberin or chitin. In addition to their mechanical resistance, the seed coat may provide additional protection against predators by containing toxic compounds such as phenolics. Tannins are a class of phenolic compound commonly found in seed coats. Tannins give a dark coloration to mature seed coats, which may be used as an indication of seed maturity. Phenolic compounds also increase rigidity of the seed coat by cross-linking polysaccharides that provide additional protection against microorganisms. Additionally, because of the antioxidant properties of phenolic compounds present in seed coats (particularly flavonoids), a protective effect of these compounds against solute leakage, damage, and oxidative stress has been suggested (Debeaujon et al. 2000. *Plant Physiology* 122, 403- 413).

### (117) **Fruit development**

A basic knowledge of fruit development and morphology is important to understand seeds and seed formation. (118) A **fruit** may be defined as the ripened ovary of the flower containing one or more seeds. However, in some cases what is commonly known as a fruit is not derived exclusively from the ovary, but from other structures (119) such as the receptacle of the flower (e.g., strawberry) or from the entire inflorescences (e.g., pineapple).

(120) A typical fruit is derived from the ovary wall or pericarp. There are three layers that compose the pericarp: (121) the exocarp (or epicarp), which is the outer layer, the

mesocarp, which is the middle layer, and the endocarp, which is the inner layer. The relative development of each of these layers in different species contributes to the overall fruit structure and morphology. For example, in a peach, which is a drupe, the endocarp is typically hard and represents the main protection of the seed, the mesocarp is fleshy and represents most of the fruit volume, and the exocarp is a thin layer that represents the peel of the fruit.

There is a great diversity in fruit morphology among species so fruits can be classified based on different factors.

(122) A basic classification can group fruits into three types: simple, aggregate and multiple fruits.

a) **Simple fruits** are derived from a simple or compound ovary with only one pistil. (123) This group can be further subdivided into fleshy and (124) dry fruits, and dry fruits may be dehiscent (at maturity, they split open and release the seeds) and indehiscent (do not split open at maturity).

(125) Achene, caryopsis, nut and samara are examples of simple dry indehiscent fruits.

(126) Capsule, pods and siliques are examples of simple dry dehiscent fruits.

(127) Berry, drupe, and pepo are examples of simple, fleshy fruits.

(128) b) **Aggregate fruits** develop from a flower with numerous simple pistils. Raspberry and strawberry are examples of aggregate fruits. Note that in the case of strawberry the individual seeds are contained in achenes and most of the fruit is composed of an enlarged receptacle.

(129) c) **Multiple fruits** are derived from an inflorescence. Each flower from this inflorescence produces a fruit and all the fruits from the inflorescence mature into a single mass. Examples of this type of fruit are pineapple and fig.

(130) Seed structure

At this point, we have reviewed how the different seed structures originate from the ovule and, in some cases, the ovary from the onset of double fertilization. (131) This slide summarizes this information:

The ovary of the flower originates in the fruit. In some cases, such as achenes and caryopses, the pericarp is closely associated with the seed and represents a main component of the seed coat.

The ovule, present inside the ovary, forms the seed. Different components from the ovule form different seed structures. The integuments form the seed testa or seed coat and the nucellus forms the perisperm. In the embryo sac, the fusion of the egg nucleus and the sperm nucleus forms the zygote that ultimately becomes the embryo of the seed. The other sperm nucleus fuses with the two polar nuclei to form the endosperm.

Depending on the relative development of each one of these structures, different types of mature seeds may be found. For instance, (132) a legume seed has an embryo with two large cotyledons that provide the main food storage structure, and the testa are the primary constituents of the seed coat. In this case, the endosperm is completely absorbed by the developing embryo and is not present in the mature seed.

(133) In maize seeds, the seed is actually a fruit, the caryopsis. In this case, the seed coat is formed primarily by the pericarp. The endosperm is the main nutrient reserve for the seed and is mostly dead tissue, with the exception of the aleurone layer. The single cotyledon is the scutellum and is located between the embryo and the endosperm. An embryonic axis is composed of a plumule surrounded by the coleoptile at one end and a radicle surrounded by the coleorhiza at the other end.

(134) A sugar beet seed can be used as an example of a seed in which the main nutrient storage tissue at maturity is the perisperm. Note that, in this case, the endosperm is present as a layer surrounding the embryo at maturity, but it has little relevance as a nutrient storage resource.

(135) Seeds of castor bean (*Ricinus communis* L.) are examples of dicot seeds in which the main tissue for storage of nutrients is the endosperm.

### (136) Apomixis and polyembryony

(137) Until now, we have seen that seed formation relies on the formation of female and male gametes that undergo double fertilization, thus this is a form of sexual reproduction. However, some plants have the ability to reproduce asexually through seeds by the phenomenon known as **apomixis**. In this case, embryogenesis occurs without fertilization, and the progeny genotype is identical to that of the mother plant; in other words, it is a clone. (138) Three different types of apomixis have been described: diplospory, apospory, and adventitious embryony. In this figure, each type is presented and contrasted with the sexual formation of the seed, in which case the capital F represents the occurrence of double fertilization.

a) **Diplospory** is produced by the formation of the embryo sac without meiosis or reduction division, so it forms from modified development by mitosis of the megaspore. Examples of species with this type of apomixis are dandelion and Kentucky bluegrass.

b) **Apospory** is when the embryo forms from the nucellus, which is diploid maternal tissue. In this case, the normal sexual embryo sac may remain in the seed. Millet is an example of a Poaceae species having apospory apomixis.

c) **Adventitious embryony** is when formation of the seed occurs from development of the nucellus or the integuments without formation of an embryo sac. This is an uncommon form of apomixis, but it is found in *Citrus* species.

Diplospory and apospory types are known as gametophytic apomixis and the adventitious type is known as sporophytic apomixis. A fourth type of apomixis has been described, mixed apomixis, which combines more than one of these types of apomixis.

(139) **Polyembryony** is the simultaneous formation of more than one embryo within a seed. It may occur when more than one egg cell is fertilized within a single ovule, which is the case in many gymnosperms. Another form of polyembryony is when one embryo is

sexually formed and others are formed by adventitious embryogenesis. This is the case in some *Citrus* species.

### References

- Bewley J.D. and M. Black. 1994. *Seeds: Physiology of Development and Germination*. Second edition. Plenum Press, N.Y.
- Black, M., J.D. Bewley, and P. Halmer. 2006. *The Encyclopedia of Seeds: Science, Technology and Uses*. CAB International.
- Bradford, K.J. 2004. *Seed Production and Quality*. Course syllabus, spring- 2004.
- Copeland, L.O. and M.B. McDonald. 2001. *Principles of Seed Science and Technology*. Fourth edition. Kluwer Academic Publishers. Boston.
- Erwin, J. 2005. Factors affecting flowering in ornamental plants. In *Flower Seeds: Biology and Technology* (eds. M.B. McDonald and F.Y. Wong). CAB International.
- Esau, K. 1977. *Anatomy of Seed Plants*. Second edition. John Wiley & Sons.
- Hartmann, H.T., D.E. Kester, F.T. Davies, and R.L. Geneve. 2002. *Plant Propagation: Principles and Practices*. Seventh edition. Prentice Hall.
- Krarup, C. and I. Moreira. 1998. *Hortalizas de estación fría. Biología y diversidad cultural*. P. Universidad Católica de Chile, VRA, Facultad de Agronomía e Ingeniería Forestal, Santiago, Chile. [http://www.puc.cl/sw\\_educ/hort0498](http://www.puc.cl/sw_educ/hort0498)
- Raven, P.H., R.F. Evert, and S.E. Eichhorn. 1999. *Biology of Plants*. Sixth edition. W.H. Freeman and Company, N.Y.
- Taiz, L. and E. Zeiger. 2002. *Plant Physiology*. Third edition. Sinauer Associates, M.A.