Seed Development and Maturation

K. V. Sripathy and Steven P. C. Groot

Abstract

In plants, a fascinating set of post-fertilization events result in the development of a dispersal unit known as a seed. During the maturation phase, seeds accumulate storage reserves and acquire desiccation tolerance, followed by an increase in seed vigour during maturation drying. Physiological (or mass) maturity may be attributed to the stage of seed maturation when maximum seed dry matter accumulation has occurred, marking the end of the seed-filling phase. The stage of maturity at harvest is one of the most important factors that can influence the quality of seeds. Recent studies established that seed vigour and longevity continue to increase even after physiological maturity, signifying the importance of the late maturation phase for maximizing seed quality. Among the plant hormones, abscisic acid (ABA) has been studied extensively for its role during seed development and maturation. Apart from ABA, gibberellic acid (GA), cytokinin and auxin also play a critical role during the development of seeds. Desiccation tolerance in seeds begins much before the attainment of physiological maturity. Acquisition of desiccation tolerance is associated with embryo accumulation of oligosaccharides of the raffinose family, low molecular weight antioxidants, late embryogenesis abundant proteins and heat shock proteins coupled with structural changes at the cellular level. To obtain seeds of maximum quality (in terms of germination, vigour and longevity), harvesting needs to be performed at or slightly after harvest maturity a period at which seed moisture content stabilizes with environmental factors. In this chapter, an attempt has been

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made to present the current understanding of seed development and maturation concentrating on various aspects viz. phases of seed development, the role of plant hormones, other factors affecting seed development, concepts of seed maturity, and its relevance to seed quality, maturity indices in crop plants and acquisition of desiccation tolerance in seeds.

Keywords

Seed development · Maturation drying · ABA · Germination · Seed moisture · CF sorting · Desiccation tolerance

1 Introduction

In plants, seed development commences with the initiation of flowering followed by formation of floral structures and effective pollination. In angiosperms, after fertilization, as a result of cell division, expansion and histo-differentiation, there is a stage in embryogenesis, in which seed structure primordia are formed and future embryo parts can be envisaged. Delouche (1971) defined seed development and maturation as a process comprising of a series of morphological, physical, physiological and biochemical changes that occur from ovule fertilization to the time when seeds become physiologically independent of the parent plant. Seed moisture content increases during the initial part of development after fertilization and later begins to decline until equilibrium is established with environmental factors. Harrington (1972) defined that a seed attains physiological maturity when the dry weight reaches its maximum and that after this stage, the flow of nutrients to seeds from the mother plant generally ceases. However, significant seed quality processes occur in seeds even after the end of seed filling. In this regard, seed physiologists consider the late maturation phase as an additional phase, to demarcate seed development into three main phases viz. embryogenesis, seed filling and late seed maturation. The late maturation phase is also often called the maturation drying phase, where seeds prepare themselves for survival after shedding by the acquisition of several protection mechanisms. The relative lengths of seed developmental phases differ between species (Leprince et al. 2017).

A significant decline in seed moisture content occurs at the end of maturation, whereas the acquisition of desiccation tolerance begins during mid and late maturation. At the same time, massive structural and physiological changes occur within the seed, with a strong reduction in metabolic activity and a transition to a quiescent and frequently dormant state at the end of late maturation. Recalcitrant seeds usually do not show this quiescent state. Seed maturation is one of the main factors of seed quality and a prerequisite for successful germination and emergence. The plant hormones play a crucial role in histo-differentiation, pattern formation and embryo maturation. Extensive studies have led to the understanding of the role of ABA during seed development and maturation as compared to other plant hormones. Environmental factors viz. soil fertility, soil water content, photoperiod, temperature

and position of the seed in the inflorescence or on the mother plant also affect the process of seed development and maturation. Most seeds degrade chlorophyll during maturation drying, hence chlorophyll fluorescence (CF) sorting is employed in some crops for upgrading seed lots exhibiting heterogeneity in seed maturity. Physiological maturity is marked as the time when seeds attain maximum dry weight and thereby, maximum yield when it concerns crop production. Seed quality for propagation purposes increases during the late maturation phase and reaches maximum vigour at a stage called harvest maturity, which in natural systems most often coincides with seed shedding. In a seed crop, to secure maximum quality, harvesting needs to be performed at the end of the late maturation, although care has to be taken for preventing the onset of pre-harvest sprouting that can be induced after maturation under moist conditions with non-dormant seeds. The maturity indicators presented on the plant or seed can often serve as a sign to determine the harvest maturity. The morphological, physiological and biochemical changes associated with seed development and maturation right from the stage of fertilization have been contemplated in this chapter.

2 Double Fertilization

Following the events of mega and microsporogenesis, the functional megaspore (n) undergoes three mitotic cell divisions and develops into an embryo sac also known as ovule (one egg cell and two synergids constitute the egg apparatus at the micropylar end, two polar nuclei at the centre and three antipodal cells at the chalazal end). Similarly, the haploid nucleus of functional microspore (n) (pollen) undergoes mitotic cell division to form bi-cellular pollen grain. The mature anthers dehisce and release pollen grains. After successful contact of pollen grains on the receptive stigma, it germinates and the pollen tube traverses along the length of the style. The haploid generative cell divides again to form two haploid male cells, also known as sperm cells or male gametes. In angiosperms, both male gametes participate in fertilization. One male gamete fuses with egg cell to produce diploid zygote (2n) likewise, the other male gamete fuses with two polar nuclei to form triploid nucleus, also known as primary endosperm nucleus (3n). Together, these two fertilization events in angiosperms are known as double fertilization (Fig. 1). The testa (seed coat) develops from the outer and inner integuments, which is an ovular tissue. The fertilized ovule forms the seed, whereas the tissues of the ovary become the fruit or pericarp, usually enveloping one or more seeds. Whereas the embryo and endosperm are a combination of maternal and paternal genetics, the testa and pericarp originate from maternal tissues and these provide the same genetic constitution for all seeds originating from the same mother plant.

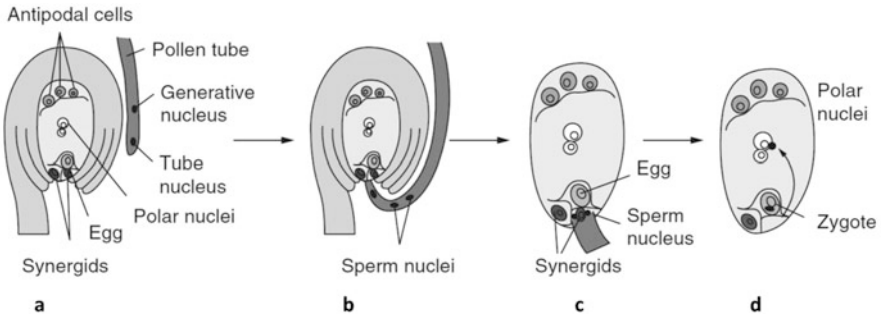

Fig. 1 Schematic representation of events during double fertilization in an angiosperm ovule. (**a**) Organization of cells in the functional megaspore and pollen tube prior to fertilization. (**b**) The pollen tube grows into the style and the generative nucleus divides to form two sperm cells inside the pollen tube. (**c**) Sperm cells are released into the embryo sac near the egg apparatus. (**d**) One sperm cell fuses with the egg cell to form a zygote (2n) and the other sperm cell migrates and fuses with the central cell to form the primary endosperm nucleus (3n). (Source: Sliwinska and Bewley 2014)

3 Embryogenesis

After fertilization, embryonic development begins. In the first stage of embryonic development, the zygote divides to form two cells, the upper cell (apical cell) and the lower cell (basal cell). The division of the basal cell gives rise to the suspensor, which finally makes a connection with the maternal tissue, providing a path for nutrition to be transported from the mother plant to the growing embryo. The apical cell undergoes multiple mitotic divisions, giving rise to a globular-shaped proembryo.

3.1 Embryogenesis in Monocot

Embryogenesis in monocot occurs through four distinctive stages in succession. Proembryo is the first stage in the embryogenesis followed by globular, scutellar and coleoptilar stage.

Firstly, the fertilized egg cell (2n) undergoes one cycle of mitotic cell division to produce two diploid celled structure known as the proembryo. The lower basal cell of the pro-embryo undergoes further division to form a structure known as suspensor (not well developed in monocots) (de Vries and Weijers 2017). The upper apical cell undergoes mitotic cell divisions to form a 16 dipliod celled globular structure, this stage is referred as globular stage. At this stage, cells at one side of globular structure divide faster to form the embryonic axis. Whereas, mitotically divided cells at the other end results in a single cotyledon (scutellum), the stage is referred as scutellar stage. In monocot seeds, the scutellum is the interface between embryonic axis and endosperm. As the scutellum is derived from the apical cell of the proembryo, its

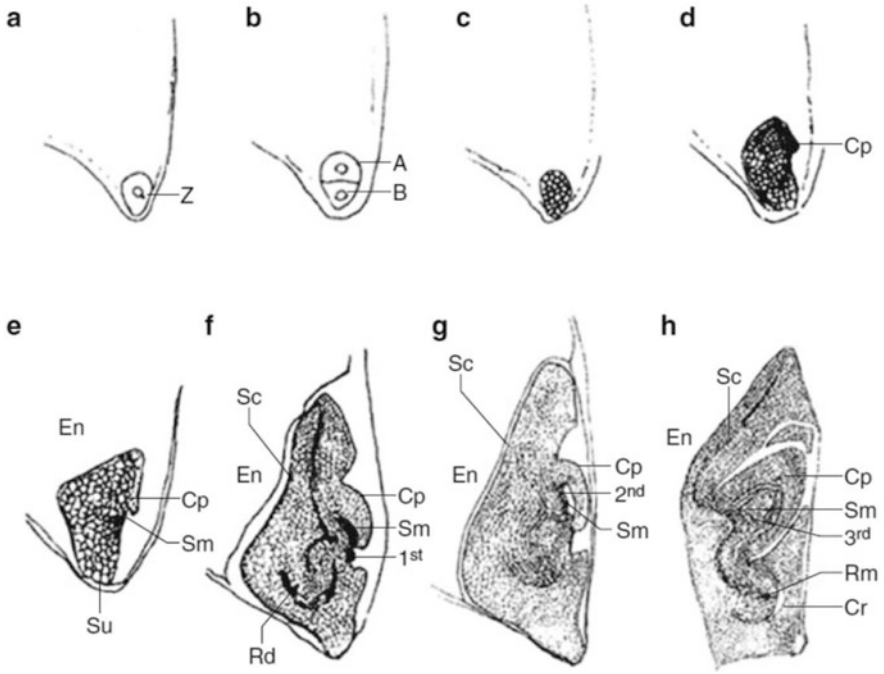

Fig. 2 Embryo development in a monocot (rice). (**a**) Formation of zygote (Z) post fertilization. (**b**) First mitotic division of zygote to form an apical (A) and a basal (B) cell, both undergo series of mitotic divisions to form multicellular (**c**) proembryo. (**d**, **e**) Differentiation of proembryo into a suspensor (Su), shoot meristem (Sm) and coleoptile (Cp). (**f**–**h**) The mature embryo is formed with distinct scutellum (Sc), radicle (Rd), root meristem (Rm), coleorhiza (Cr) and third leaf (third). Proembryo stage (**b**, **c**); Globular stage (**d**, **e**); Scutellar stage (**f**, **g**); Coleoptilar stage (**h**). (Source: Bewley et al. 2013)

genetic constitution is same as embryo. In the later stage of embryogenesis, the embryonic axis differentiates and plumule and radicle can be distinguished (Fig. 2).

Meanwhile, the triploid endosperm nuclei undergoes repeated nuclear division, later free nuclei migrate to the periphery of the cell and cellularization occurs upon cell wall formation. The inner layers of cells develop into endosperm and peripheral cells form an epidermis-like layer called aleurone. The aleurone layer is the outermost layer of the endosperm but is very distinct from starchy endosperm cells in terms of morphology and biochemical composition (Becraft and Yi 2011). The aleurone cells remain metabolically active after maturity and its role during seed germination is vastly studied. During seed germination, the scutellum produces gibberellin, which triggers aleurone cells to produce enzymes for hydrolysis of starch (α amylase) and storage protein (proteases). In monocot seeds, the funicle is not well developed and functional. During the seed-filling stage, storage reserves (photosynthates) are transferred from source to triploid endosperm cells via transfer

cells and stored predominantly as starch. The endosperm cells expand with the accumulation of food material.

3.2 Embryogenesis in Dicot

The embryogenesis in monocot and dicot seeds is mostly similar up to the globular stage. In dicots, fully functional suspensor is developed from basal cell of the proembryo which pushes the globular structure into the embryo sac and also aids in transfer of food reserves to globular cells.

A depression is formed at the tip of globular structure (heart-shaped stage), which is the initiation of cotyledon differentiation. Relative deepening of the depression at the tip and elongation of cotyledons gives the torpedo stage. The radical and hypocotyls are well developed at the cotyledonary stage (Fig. 3). The rudimentary suspensor absorbs food material from surrounding tissue and transfers it to the cotyledons. The food reserves are stored in the cotyledons predominantly as proteins or lipids. To aid in the transfer of photosynthates to cotyledons, a vascular strand runs through the funicle and connects at one part of the seed coat. From seed coat, nutrients are diffused to nucellus tissues and later absorbed by the suspensor. Removal of one cotyledon may show between both the cotyledons the presence of the early stages of the first true leaves, the plumule, as can be seen in bean seeds. However, with many other seeds, as in tomato and cabbage, the shoot apical meristem does not develop beyond a dome-shaped structure and the formation of leaf primordia is seen only after the commencement of germination.

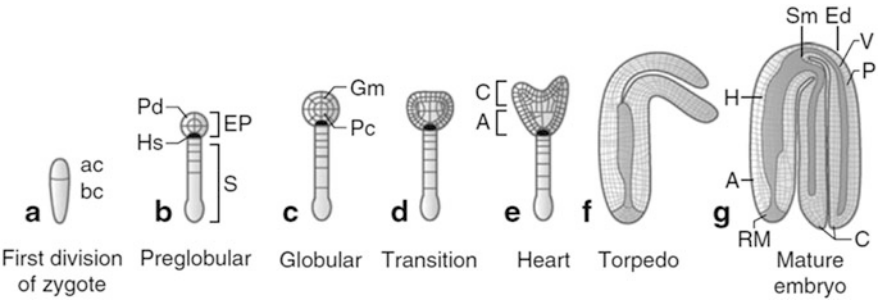

Fig. 3 (**a**–**g**) Embryo development in a dicot (Arabidopsis). (**a**) First mitotic division of zygote to form apical cell (ac) and basal cell (bc). (**b**) Further divisions lead to the formation of suspensor (S) and embryo proper (EP) at pre-globular stage, the protoderm (Pd) develops into epidermal layer and hypophysis (Hs) develops into root meristem (RM). Shoot meristem (Sm) differentiates from the apical-central region of the embryo. The ground meristem (Gm) of the globular stage develops into storage parenchyma cells (P) of cotyledons (C). The pre-cambium (Pc) forms vascular tissue (V). The axis (A) develops into radicle, plumule and hypocotyl (H). (Source: Bewley et al. 2013)

4 Acquisition of Desiccation Tolerance During Seed Maturation

After completion of series of cell division and cell differentiation, seed development shifts to the maturation phase that can be divided into early (reserve accumulation) and late maturation (maturation drying) (Fig. 4). During early maturation, the seed can acquire desiccation tolerance. Desiccation tolerance is defined as the ability of a living entity to deal with extreme moisture loss to levels below 0.1 g water per gram dry weight, or drying to relative humidity below 50%, and subsequent re-hydration without accumulation of lethal damage (Leprince and Buitink 2010). Based on the desiccation tolerance, seeds can be classified into orthodox and recalcitrant types. The maturation in the orthodox seeds is accompanied with a water loss up to 5–10% w/w, which allows them to sustain unfavourable environmental conditions, such as extremely high and low temperatures and drought. In contrast, recalcitrant seeds are sensitive to dehydration and desiccation leads to damage and loss of viability (Azarkovich 2020). In orthodox seeds, the mechanisms behind the onset of desiccation tolerance are activated at the early stages of maturation (Leprince et al. 2017). Later on, desiccation tolerance is lost during germination, at the moment of radicle emergence.

Desiccation tolerance is acquired by seeds through accumulation of an array of small molecules and proteins that enables them to maintain the structural integrity of critical cellular organelles, membranes and proteins so that they can persist during the dry state and resume their biological functions upon hydration (Bewley et al. 2013). The embryo accumulates specific molecules that are associated with the cells' ability to tolerate extreme water stress viz. low molecular weight antioxidants, oligosaccharides such as raffinose, stachyose, late embryogenesis abundant proteins (LEAs) and heat shock proteins (HSPs). Further, structural changes occur at the

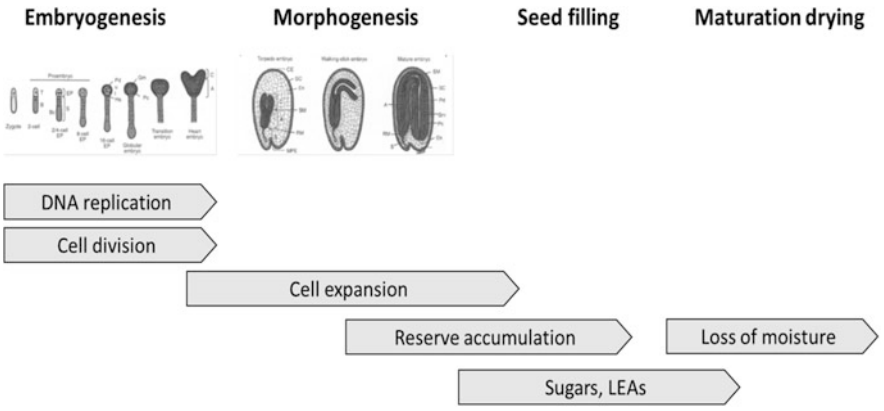

Fig. 4 Seed developmental stages signifying the series of events towards reserve accumulation, acquisition of desiccation tolerance and maturation drying. (Source: SPC Groot, Wageningen University & Research, The Netherlands)

cellular level such as folding of cell walls, condensation of chromatin and dismantling of thylakoids in chloroplasts (Ballesteros et al. 2020). These physiological and structural changes reduce metabolic activity while mitigating the mechanical stress of cell shrinkage during dehydration (maturation drying process). Changes at this stage correspond with a gradual increase in seed longevity (Verdier et al. 2013).

LEA proteins have relative high content of glycine, alanine, glutamate, lysine, arginine and threonine, while low amounts of cysteine and tryptophan residues (Battaglia et al. 2008). Due to this primary nature, LEA proteins are stable in a broad temperature range. During cell dehydration, LEA proteins act as chaperons, i.e., involved in structural stabilization of denatured proteins and promote their refolding through intensive hydrogen bond formation (Smolikova et al. 2021). LEA proteins are also responsible for sequestration of ionic compounds, accumulating during cell dehydration, and protection of membrane proteins and enzymes from the deleterious effects of increased salt concentrations. Non-reducing sugars fill the free volume between large molecules, created during dehydration and the dehydrated cytoplasm forms a glassy matrix with very low molecular mobility (Ballesteros et al. 2020). Other structural adaptations that occur during this stage are chromatin compaction and nuclear size reduction, which are reversed during germination (van Zanten et al. 2011). Furthermore, metabolic activity is reduced and chlorophyll is degraded towards the end of seed maturation thereby minimizing the production of reactive oxygen species (ROS) (Pammenter and Berjak 1999). To protect the seed against oxidative damage, which cannot be repaired by enzyme activity under dry conditions, seeds accumulate during seed maturation many antioxidants, such as ascorbate, glutathione, polyols, tocopherols, quinones, flavonoids and phenolics (Kranner and Birtić 2005).

5 Seed Development and Maturation in Relevance to Seed Quality

Since protection mechanisms are mainly built during the late seed maturation phase, the stage of harvest becomes the most critical factor for seed quality and storability (Jalink et al. 1998; Demir et al. 2008). Harvesting seeds too early when there is inadequate development of essential structures and protection mechanisms may result in poor quality (Ekpong and Sukprakarn 2008). Similarly, harvesting too late may increase the risk of shattering and may decrease the quality of seed due to ageing. If harvesting is delayed, incidence of adverse environmental conditions such as rain and humidity may result in precocious germination (Elias and Copeland 2001). Additionally, under high humid conditions, delayed harvest of seeds can also result in infection by saprophytic fungi resulting in discolouration of seeds. Low quality of seeds can potentially decrease the rate and percentage of germination and seedling emergence, leading to poor stand establishment in the field and consequently yield loss, as has been found with many crops such as rice, corn, wheat, cotton, barley and garden pea. Therefore, it is necessary to examine and identify the

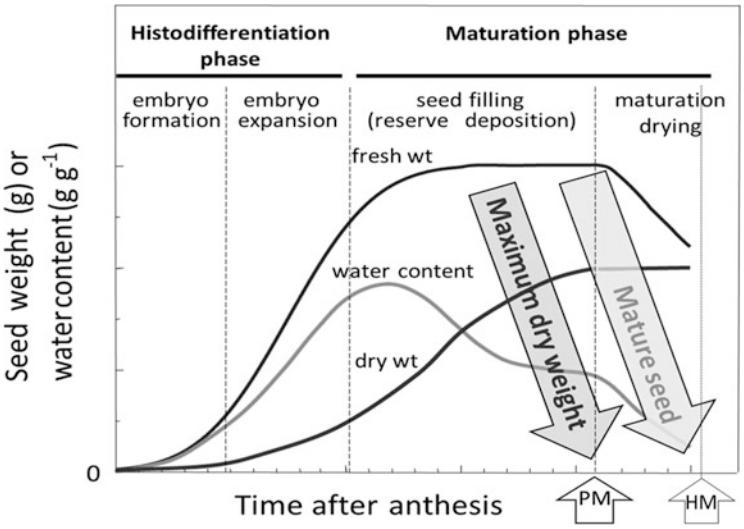

Fig. 5 Progression of seed fresh weight, dry weight and moisture content during seed development and maturation. *PM* physiological maturity, an index used for yield, *HM* harvest maturity, an index used for seed quality. (Source: SPC Groot, Wageningen University & Research, The Netherlands)

suitable stage of harvest (maturity indices) in crops for the production of high-quality seeds (Fig. 5).

Even though seeds attain maximum dry weight at physiological maturity, the maximum vigour, which is responsible for performance of seed under stress conditions, is not attained until the end of maturation drying. As seed production is dealing with a population of seeds, the relationship between germination, desiccation tolerance, vigour and longevity during seed development and maturation is expressed through a sigmoid curve (Fig. 6). Hence, bulk harvested seeds are always heterogeneous pertinent to seed maturity.

In crops viz. rapeseed and mustard, cole crops, pigeon pea, onion, carrot, etc. indeterminate flowering results in wide variation in seed developmental stages within the inflorescence (Singh and Malhotra 2007). During a bulk harvest, few over-mature, mature and immature seeds shall always persist in crops having indeterminate flowering habit (Fig. 7). In such cases, seed harvesting needs to be done at a stage when most of seed-bearing structures are mature or nearing maturity and flower initiation stops in the inflorescence. Prolonging the harvest beyond certain stage may result in seed shattering. With some crops, it is possible to cut the mother plant from the roots and let the plant, with developing fruits, slowly dry (in field or a threshing yard) before harvesting the seeds. This practice provides ample time with enough moisture for the less mature seeds to complete the process in their late maturation phase.

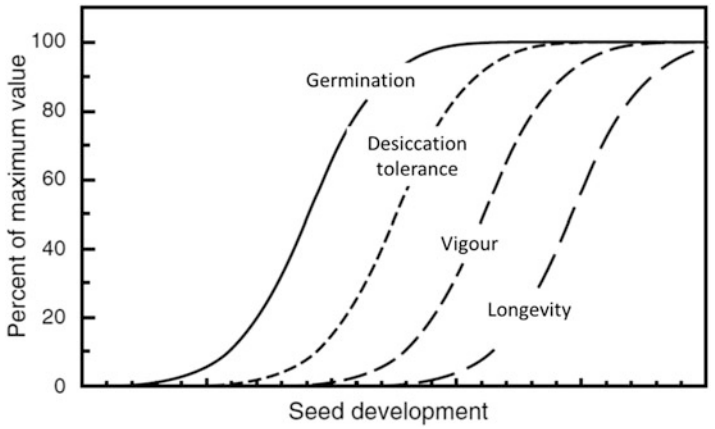

Fig. 6 Pattern of development of seed quality attributes during seed development and maturation. Seed vigour and longevity progressively developed during the late maturation phase and reaches maximum during harvest maturity. (Source: Bewley et al. 2013)

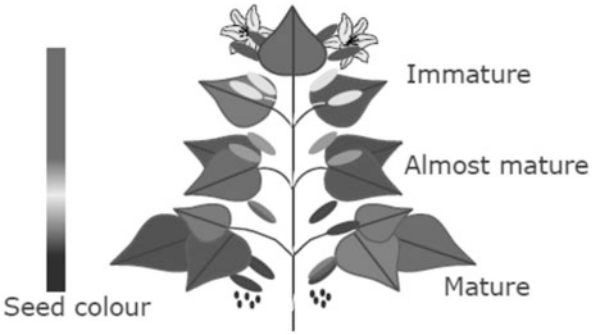

Fig. 7 Varied seed developmental stages observed in plants as a result of indeterminate flowering behaviour. (Source: H. Jalink, Wageningen University & Research, The Netherlands)

5.1 Hormonal Regulation of Seed Development and Maturation

Plant hormones are signal molecules that are produced in the plant and are active at very low concentrations. The hormones abscisic acid (ABA), gibberellins (GAs), auxin (IAA), cytokinins, ethylene and brassinosteroids regulate cellular processes in targeted cells, which may or may not be the cells in which they are synthesized. The most important role of plant hormones is to control and coordinate cell division, growth and differentiation (Hooley 1994).

Measurements of endogenous hormone concentration have suggested the high transient expression of cytokinins, GAs and IAA during the early phase of seed development (Bewley et al. 2013; Davies 2013). In studies with tomato, GAs were found essential to produce fertile pollen, but pollination of a GA-deficient (female) mutant with mutant pollen, obtained upon spraying the male plant with GAs, resulted in the development of normal-looking healthy seeds that only needed GAs for germination (Groot and Karssen 1987; Groot et al. 1987). Fruits could

develop on GA-deficient mutant mother plants, but they remained small without fertilization and seed development. The transport cells (that aid transfer of nutrition from source to sink) like those of the suspensors are important for nutrition of early embryos. Studies with *Phaseolus* have shown that exogenous GAs can substitute for a detached suspensor in promoting embryonic growth, suggesting that the suspensor may normally provide GAs as well as nutrients to the developing embryo. Cytokinins have also been implicated in promoting suspensor function, but may be even more significant in promoting endosperm growth and grain filling via promotion of cell division (Bewley et al. 2013). In contrast, during early embryogenesis, auxins play a major role in establishing the embryonic body plan via effects on apical-basal polarity or pattern formation (transition of embryo from globular to heart shape and cotyledon separation at later stages) and vascular development (Vogler and Kuhlemeier 2003).

During maturation, seeds of most species acquire the capability to endure desiccation. The maturation phase begins when the embryo and endosperm have accomplished the morphogenesis and patterning stages (Wobus and Weber 1999). This phase is categorized by a growth arrest, followed by the synthesis and accumulation of reserves, whose degradation upon germination will provide nutrients to the growing seedling before the photosynthetic capacity is fully acquired (Baud et al. 2002). Early and mid-phases of maturation are controlled by the action of ABA, initially synthesized in the maternal tissues and later on, although to a lower extent, in the embryo and endosperm (Nambara and Marion-Poll 2003). Seed maturation coincides with an increase in seed ABA content; consistent with the fact that ABA induces expression of a cyclin-dependent kinase inhibitor (ICK1) that could lead to cell cycle arrest (Finkelstein et al. 2002).

In the later stage, a decline in ABA level occurs and synthesis of LEA proteins follows, which is characteristic to the late maturation phase. Maturation is not always an obligatory process, if ABA effects are eliminated by removing the embryo from the seed would lead to development of seedlings (Berger 2003). But due to their low vigour, planting these immature seeds in the field will not result in the development of a healthy seedling.

The ethylene pathway studies in relation to seed development and maturation are extremely limited. In plant tissues, ethylene affects chlorophyll metabolism (Matilla 2000). Because chlorophyll loss is triggered during the final stages of embryogenesis (during acquisition of seed vigour), this process may be affected by ethylene. Mustard and canola seeds produce significant amounts of ethylene during embryogenesis, specifically in the early pre-desiccation stages (Child et al. 1998). Hence the role of ethylene can be attributed as minor during seed development and maturation and may be associated with the embryo de-greening process.

6 Physiological Maturity, Mass Maturity and Harvest Maturity

Seed development and maturation passes through a series of distinct (or overlapping) events such as histo-differentiation, embryogenesis, morphogenesis, reserve accumulation and maturation. Developmental stages could be monitored through relative changes in traits such as seed moisture content, dry weight accumulation, acquisition of desiccation tolerance, development of germinability, attainment of maximum vigour and longevity at harvest maturity. Two stages of maturity have been defined viz. physiological (or mass) maturity and harvest maturity. Physiological maturity is the end of the seed-filling period (Harrington 1972), whereas harvest maturity is the point of time that coincides with the end of maturation drying.

Physiological maturity was defined as the seed developmental stage at the end of seed filling (Shaw and Loomis 1950), when seed dry weight is at its maximum. Physiological maturity is more relevant for agronomic purpose to highlight the seed developmental stage beyond which none of the agronomic intervention could increase seed yield, because the funicular functionality is lost and nutrients cannot be transferred from mother plant to seed (Ellis 2019). Harrington (1972) hypothesized that at physiological maturity stage seed quality is greatest since seed quality improves during the seed-filling phase reaching a maximum but seeds do deteriorate thereafter. This concept of physiological maturity was supported by investigators for more than two decades in many crop species. Still and Bradford (1998) identified that physiological maturity was said to occur some days after maximum seed dry weight was attained in two Brassica seed crops, retracting the definition of Shaw and Loomis (1950). Black et al. (2006) abridged the definition of physiological maturity from that of Harrington's, to the stage of development at which a seed, or the majority of a seed population, has reached its maximum viability and vigour. Finch-Savage and Bassel (2016) also detached the definition of physiological maturity from the original to define it solely as the point of maximum seed quality. This led to some distortion or inconsistency in the use of term physiological maturity per se. Moreover, by earlier researchers the final stage of seed maturation, i.e., maturation drying has not been taken into consideration to be important to development of seed quality.

Lately, many researchers have confirmed that maximum seed quality does not occur until sometime after physiological maturity in cereals (Rao et al. 1991; Ellis and Pieta Filho 1992; Ellis et al. 1993; Sanhewe and Ellis 1996; Ellis 2019), Brassica (Still and Bradford 1998) and vegetable crops (Demir and Ellis 1993; Jalink et al. 1998). Ferguson (1993) established that in different cultivars of peas, maximum seed quality was attained 14–19 days after the stage of maximum seed dry weight and seed quality did not decline before harvest maturity. In these studies, seed quality continued to improve for considerable time and maximum seed quality was achieved, after attainment of physiological maturity, just prior to (or at) harvest maturity. Assuming the fact that, the definition of physiological maturity (stage of development marked with maximal seed dry weight, germinability, vigour and viability) had become compromised and misleading, the term mass maturity was

proposed to designate the end of the seed-filling phase alone (Ellis and Pieta Filho 1992). Mass maturity represents the stage of seed development that Shaw and Loomis (1950) termed as physiological maturity. The physiological maturation is represented for individual seed and this maturation will not be the same for all seeds in the population, due to differential flowering habit.

The term harvest maturity represents the stage of maturity at which a seed crop is ready for harvest. The maturation drying phase ends at harvest maturity. The moisture content of the seed at this stage is significantly lower than at mass maturity. For obtaining seeds of maximum quality (germination, vigour and longevity), crop should be harvested at or slightly after harvest maturity; a period at which seed moisture content equilibrates with environment humidity. Black et al. (2006) defined harvest maturity as stage of development at which a seed, or majority of the seed population is best suited to harvesting in high quality and yield, considering its storage, its handling characteristics to minimize mechanical injury, and potential field losses due to inefficient collection by harvesting equipment. In practice, harvest maturity dates vary among the crops.

6.1 Seed Maturity Indices in Relation to Harvest Maturity

Indicators of maturity that could predict the right stage of harvest in a given crop are termed as maturity indices, although, for a bulk harvesting, a quick estimation of maturity at field level is quite challenging. Harvest maturity can be determined in a variety of crops by visual indicators, such as apparent visual changes in seed, fruit, panicle or through testing seed brittleness. Below is the list of common visual indicators associated with harvest maturity in few field and horticultural crops (Table 1).

6.2 Trackable Parameters During Seed Development and Maturation

Significant efforts were made during 1960s and 1970s by seed technologists across the globe to study the maturation process and primary changes associated with seed development. The research was oriented towards determination of morphological characteristics presented in either plant or seed during maturation process. This approach allowed an identification of harvest maturity on a plant population basis. Chlorophyll degradation resulting in a less green colour of the seeds was noticed. In the late 1990s, chlorophyll fluorescence was discovered as a very sensitive marker for seed maturity that can also be used on individual seed basis (Jalink et al. 1998). Given below are the plant characteristics that could be monitored during seed development process.

Table 1 Harvest maturity indicators for select field and horticultural crops

Crops	Seed maturity indicators as a criterion for harvesting
Field crops	
Rice	Nearly 85% of panicle turn straw-coloured and seeds are in hard dough stage in the lower portion of panicle
Wheat	Seeds in hard dough stage and yellowing of spikelets
Sorghum	Yellow-coloured ears with hard seeds
Pearl millet	Ears turn compact and hard seed comes out upon pressing
Finger millet	Hard seeds are embedded in brown-coloured earhead
Maize	Husk colour turns pale brown
Red gram	Nearly 80% of pods exhibit brown colour
Black gram/green gram	Seeds become hard and pods turns to brown or black colour
Cowpea	Brown-coloured seeds are observed in light straw-coloured pods
Groundnut	Pods become dark, patchy appearance inside the shell and upon pressing the kernel, oil is observed on fingers
Cotton	Black-coloured seeds exposed in fully opened boll
Horticultural crops	
Onion	Seeds become black on ripening in silver-coloured capsules
Carrot	Second and third order umbel turn brown
Radish	Pods become brown and parchment like
Turnip	Plants turn to brown and parchment colour
Peas	Pods become parchment like
Beans	Earliest pods dry and parchment like and remaining have turned yellow
Eggplant	Fruit turn to straw yellow colour
Tomato	Skin colour turn to red and the fruits are softened
Cucumber	Fruit becomes yellowish brown in colour, and stalk adjacent to the fruit withers for confirming actual seed maturity
Squash, Pumpkin	Rind becomes hard and its colour changes from green to yellow/orange or golden yellow to straw colour
True potato seed	Berries of potato become green to straw coloured and soft
Anise	Tips of fruits greyish green in colour
Celery	Pick the mature umbels periodically when they turn brown

Source: Malarkodi and Srimathi (2007), Singh and Malhotra (2007)

6.2.1 Seed Moisture Content

In both monocots and dicots, ovule moisture content at the time of fertilization is very high. The moisture content decreases during maturation process, but still it remains relatively high throughout most of the maturation period because water is the vehicle for transferring nutrients from the parent plant to the developing seeds. Moreover, enzymes, including those needed to produce the storage compounds, can be more active at high water contents. Dehydration was observed to be slow during the initial phase and gets accelerated after seed attains mass maturity. This decrease in moisture content proceeds during the maturation drying phase until hygroscopic equilibrium is attained with the environment. From that point onwards, changes in

seed moisture content are the function of environmental factors. However, developing recalcitrant seeds do not show significant changes in desiccation during maturation phase and possess moisture levels usually over 60% on fresh weight basis.

6.2.2 Seed Size

As a result of intense cellular division and expansion during initial phases of embryogenesis, seed size increases gradually. The ovule during fertilization is a smaller unit in comparison to final seed size. The seed size reaches maximum at the end of reserve accumulation phase. For instance, in soybean, the maximum-sized seeds were observed at the full seed stage (R6) (Du et al. 2017). Thereafter, reduction in seed size was observed depending on cultivar and intensity of drying process coinciding with late maturation phase. In case of legumes, seed size reduction is more obvious than in cereals.

6.2.3 Seed Dry Weight

Post fertilization, as a result of accumulation of food reserves and water uptake, progressive increase in weight is observed in developing seeds. During the early phase of seed development, the accumulation of food reserves occurs at slower pace due to intense cell division and elongation. Thereafter, dry matter accumulation increases until seed attains maximum dry weight at mass maturity stage.

6.2.4 Germination

Seeds of various cultivated species are able to germinate a few days after ovule fertilization. Here, germination refers to radicle emergence, not the formation of a normal seedling, because histo-differentiation has not been completed and reserve accumulation is still incipient at this phase. Therefore, this germination does not lead to the production of vigorous seedlings. In the absence of seed dormancy, generally, the proportion of germinable seeds in the plant increases during maturation and reaches maximum when seeds attain harvest maturity.

6.2.5 Vigour

During maturation drying, the seed prepares itself for survival in the dry state, when moisture levels are too low for enzymatic repair. For an optimal survival in the dry state several protection mechanisms are imposed, as mentioned in the above section. The percentage of vigorous seeds significantly increases during the later maturation phase, reaching a maximum around the time when seeds attain harvest maturity (end of maturation drying phase).

6.3 Chlorophyll Fluorescence (CF) Sorting vis-à-vis Seed Maturation

During photosynthesis reactive oxygen species are produced, which are directly scavenged enzymatically. But in a dry seed, enzymatic scavenging is not possible and formation of reactive oxygen species would result in oxidation of organic

molecules, including DNA and membrane lipids. In the late maturation phase, chlorophyll present in the seed is degraded (Ward et al. 1992). The amount of chlorophyll in the seed or seed coat will therefore serve as a marker for assessing the level of maturity. Chlorophyll in white-seeded *Phaseolus vulgaris* seeds is visible and may be detected and sorted using conventional colour-sorting equipment (Lee et al. 1998), but with most seeds, colour sorters are not sensitive enough to discriminate subtle differences in chlorophyll. At the end of the last century, it was discovered that chlorophyll levels could very sensitively be measured for individual seeds using its fluorescent properties (Jalink et al. 1998). That technique makes use of laser technology, narrow optical bandwidth filters, detection of chlorophyll-a in the seed coat, measuring the resulting chlorophyll fluorescence (CF), and linking it with the quality of the seeds. Chlorophyll-a in the seed coat is excited by laser radiation (at 650 nm) and the resulting fluorescence is measured instantaneously and non-destructively (at 730 nm). An exponential decrease in CF during maturation of seeds was found. This decline in CF signal was directly related to the germination performance under laboratory and greenhouse conditions. Equipment has been developed for analysing and sorting seeds individually based on their CF signal. Cabbage seeds with high CF signal are of lower quality and seeds with the lowest CF signal of better quality (Fig. 8). This analysis has been performed with seeds of many different species, including cabbage (*Brassica oleracea*) (Jalink et al. 1998; Dell'Aquila et al. 2002), tomato (*Solanum lycopersicum*) (Jalink et al. 1999), barley (*Hordeum vulgare*) (Konstantinova et al. 2002), carrot (*Daucus carota*) (Groot et al. 2006), and pepper (*Capsicum annuum*) (Kenanoglu et al. 2013).

6.4 External Factors Affecting Seed Development and Maturation

Environmental stresses can occur at any point of time during seed development. Responses to these stress factors are diverse and complex and largely dependent on the intensity and duration of stress, stage of incidence and the position of seed on the mother plant. The environment factors that influence seed development and maturation include soil fertility, water, temperature and light.

6.4.1 Soil Fertility

In general, plants that have been well nourished with the three major elements (N, P and K) produce larger seeds than those which have not been well nourished. The increase in seed size is due to enhanced seed development rate during the seed-filling period as a consequence of increased nutrient availability. Soils deficient in minor elements may cause seed quality issues. Calcium and boron deficiencies are known to cause cotyledonary discolouration in field beans. According to Copeland and McDonald (2001) when the effects of individual elements on seed development are considered, nitrogen has the greatest influence on seed size, seed germination and vigour.

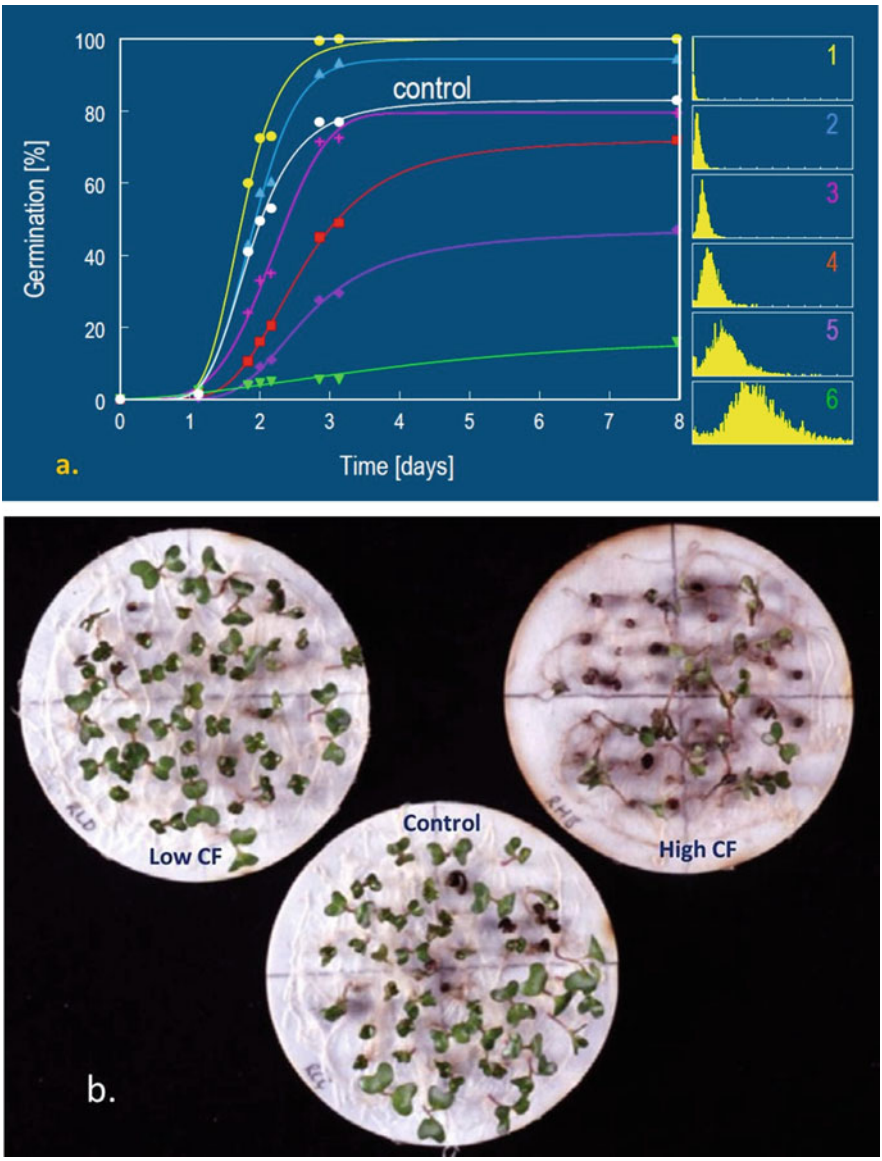

Fig. 8 CF sorting in relation to germination in cabbage. (**a**) Germination studies in six CF-sorted cabbage seed lots, peaks represented in the box on right-hand side indicate level of chlorophyll in seeds. (**b**) Differences in germinability among CF sorted seeds in Brassica. (Source: H Jalink, Wageningen University & Research, The Netherlands)

6.4.2 Water

Water deficits largely affect plant metabolic processes including seed development. The manifestations of water deficit are reduced leaf area, photosynthesis, excessive

flower drop and abortion of embryos coupled with poor photosynthate production and translocation to developing seeds. Prolonged droughts leading to poor water availability result in reduced seed size more particularly when these stress factors occur during the seed-filling phase. Similarly, if water deficit coincides with flowering, it results in excessive flower drop and poor seed set (Copeland and McDonald 2001).

6.4.3 Temperature

High temperatures during seed development produce smaller seeds, while suboptimal temperatures retard seed growth. Seed germination and vigour are also adversely affected by exposure to low temperatures during development. High temperatures are considered the principal reason for the forced maturation resulting in wrinkled and deformed seeds in some plants. This phenomenon is also caused by water deficits during maturation. The occurrence of greenish seeds due to forced maturation is undesirable because this abnormality translates into decline in seed germination and vigour (Copeland and McDonald 2001).

6.4.4 Light

The intensity and duration of solar radiation and its seasonal distribution is crucial for plant development. It is well established fact that diffused or reduced light to mother plant results in smaller seeds due to decreased photosynthesis (Copeland and McDonald 2001).

6.4.5 Seed Position on the Plant

The development rate with seeds is largely affected by its relative positioning in the inflorescence. For instance, wheat seeds located at the distal end of spike exhibit slower growth rate and shorter filling periods as compared to the seeds positioned at proximal end. Similarly, in maize, developing seeds positioned at the tip of ear are smaller in comparison to seeds located at the base which is attributed to poor supply of photosynthates. Further, in soybean, pods in the lower and upper branches are produced in different time frames and they experience different environmental conditions during development, and this results in differences in seed performance. The poorly filled seeds (smaller seeds) produced during fag-end of crop growth period exhibit decreased germination and vigour.

7 Conclusion

Induction of flowering and differentiation of flower parts are considered the starting points of seed development. The course of seed development and maturation is controlled genetically and involves an organized sequence of events starting from ovule fertilization to the point in which the seed becomes independent from the parent plant. Earlier studies on seed maturation were mainly focused on recognizing phenotypical differences among species and cultivars in order to identify reliable parameters to determine the best time for seed harvest. When flowers in the same

inflorescence are not pollinated at the same time, uniformity of seed maturation is never achieved, especially when a plant population is considered. Seed moisture content, seed size, germination, dry matter accumulation and seed vigour are considered to be the best parameters for evaluation of maturity status of developing seed. During the process of seed development and maturation, seeds of most crops acquire desiccation tolerance at mid or late maturation stages, which allow maintaining seed viability even after the loss of water up to 95%. The mechanisms behind the desiccation tolerance mostly rely on LEA proteins, small heat shock proteins, non-reducing oligosaccharides, antioxidants and structural changes at the cellular level. Orthodox seeds are tolerant to desiccation, which can be dried without loss of viability and the metabolic processes can be resumed upon subsequent rehydration. During late maturation, protection mechanisms needed for survival after shedding are strengthened to obtain maximum vigour at harvest maturity.

Seed is the basic unit of multiplication, but it should possess quality characteristics in terms of physical, physiological soundness, genetic purity and seed health. Hence, production of quality seed depends on genetic, environmental, edaphic and biotic factors prevailing in the production site. One of the environmentally influenced genetic factors deciding the quality of seed is the period and pattern of development and maturation of seed in any particular crop. Seed quality is a complex trait and high viability and vigour attributes of a seed enable the emergence and establishment of normal seedlings under a wide range of environments. In general, the seeds harvested at harvest maturity will have the greater seed yield and quality. In crops, the maturation will not be always uniform but there will be mingling of immature, matured and over-matured seeds based on the time of anthesis and fertilization. Hence, optimum timing of harvest for a given seed crop is necessary as beyond this point losses will be greater than the potential seed yield. The mechanism behind the intriguing events of development of seeds from point of fertilization till acquisition of desiccation tolerance, role of hormones and secondary metabolites and underlying genes controlling the whole set of events needs to be studied in a holistic manner. This might provide a new insight into the entire set of sequence or pathways associated with the various events during seed development stages.

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