2.4 Gènes contrôlant la maturation de l'embryon

Plusieurs changements caractérisent la phase de maturation de l'embryon:

- accumulation des réserves
- préparation à la déshydratation
- dormance
  (exception: graines vivipares)

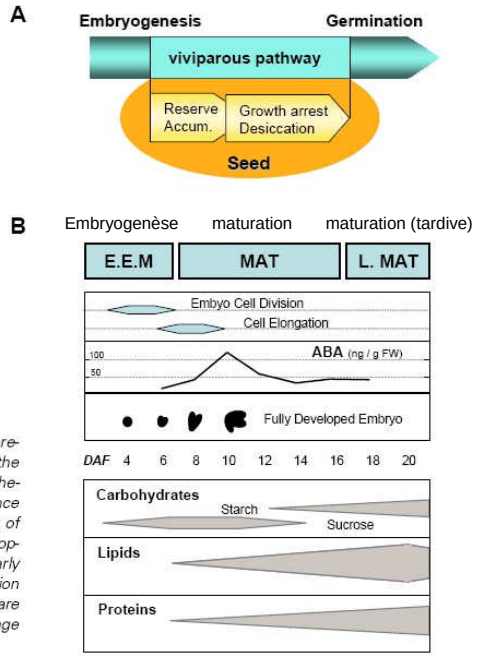


Fig. 1. Development within the seed. Phases and Features. (A) *Representation of embryo development under a viviparous pathway or within the seed, undergoing typical maturation phases of reserve compound synthesis and accumulation, growth arrest, acquisition of desiccation tolerance and entry into quiescence. Germination is indicated as the exit point of development from the seed.* **(B)** *Time course of* Arabidosis *seed development (adapted from Baud* et al., *2002), indicating major events during early embryo morphogenesis (E.E.M.), maturation (MAT) and late maturation (L.MAT) phases. Embryo globular, heart, torpedo and mature stages are represented in parallel to the ABA levels and abundance of storage compound in the seed.*

La tolérance à la déshydratation est due à l'accumulation de:

- saccharose (aussi raffinose) et autres métabolites
  → protègent les membranes en remplaçant les molécules d'eau
  → « vitrification » : limite réactions chimiques délétères
- **protéines LEA** (Late Embryogenesis Abundant): protéines hydrophiles
  - protection membranes
  - fixation d'ions
  - activités enzymatiques

Remarque: applications bio-technologiques → augmentation de la résistance des plantes à la déshydratation…

Les gènes essentiels de la phase de maturation sont identifiés par le phénotype des graines

- *LEC1* et *LEC2*
- *FUS3* : régulateur transcriptionnel
- A*BI3* : régulateur transcriptionnel
  → perception de l'ABA
- autres…

Embryons mutants retirés de la graine avant la déshydratation: développement normal
→ effet spécifique à l'embryon

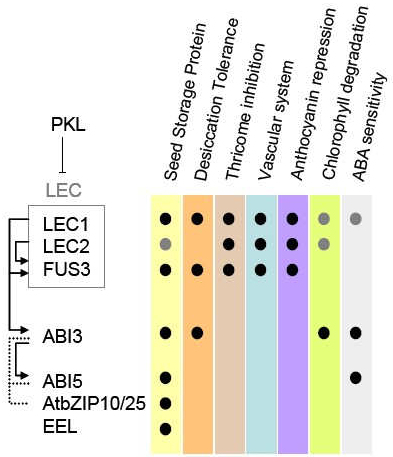


Fig. 3. Key regulatory genes identified in *Arabidopsis thaliana* affecting important traits of the maturation phase during seed development. *The contribution of the different genes to diverse aspects of seed development is indicated by dots in each particular case. Arrows show the activating or repressing relationships disclosed among them and dotted lines represent reported protein-protein interactions.*

L'examen détaillé des différents phénotypes met en évidences des relations hiérarchiques qui permettent de comprendre les relations entre les différents régulateurs et avec les hormones

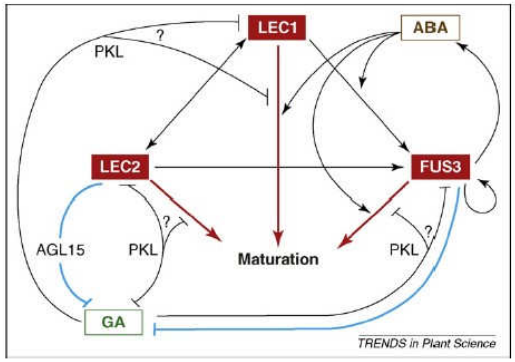


Figure 2. Regulation of the maturation phase by LEC transcription factors (TFs). LEC TFs display complex regulatory interrelationships during embryo development. The three LEC TFs induce maturation characteristics and activate seed protein genes (red arrows). LEC1 activates both *LEC2* and *FUS3* [24,38], whereas LEC2 activates *LEC1* and *FUS3* [26]. LEC TFs also interact with ABA and GA. The observations that ABA enhances LEC1- and FUS3-mediated expression of seed protein genes [24,25] and that FUS3 causes an increase in ABA levels [48] suggest a positive feedback regulatory loop. Both FUS3 and LEC2 negatively affect enzymes that catabolize bioactive GA [50]. FUS3 represses the GA biosynthesis genes *GA3ox1* [48] and *GA3ox2* [50]. *lec2* mutant embryos have increased *GA3ox2* RNA levels, although a causal relationship has not been demonstrated [50]. LEC2 directly induces *AGL15* [36], which activates *GA2ox6*, a gene encoding a GA-inactivating enzyme [51]. GA has also been implicated in the repression of *LEC* genes in seedlings. The *LEC1* overexpression phenotype of the *lec1-tnp* allele is enhanced by a GA inhibitor [52]. PKL represses LEC activity in seedlings [53,54]. *pkl* mutants ectopically express *LEC* genes, and the *LEC* overexpression phenotype is enhanced by GA synthesis inhibitors. It is not known whether GA acts in PKL-mediated repression of maturation processes upstream or downstream of the LEC TFs (indicated by question marks). Blue lines represent regulatory relationships with mechanistic support and black lines represent relationships for which molecular mechanisms have not been described.

LECs go crazy in embryo development. Siobhan A. Braybrook and John J. Harada. Trends in Plant Science 13, 624-630 (2008)

En résumé:

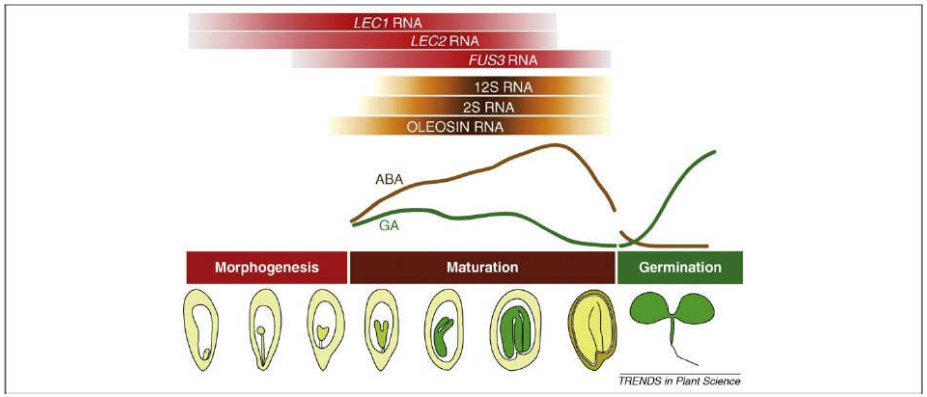


Figure 1. Embryo development in seed plants. Embryo development consists of two phases, morphogenesis and maturation. During the morphogenesis phase, different morphological domains of the embryo are specified and the embryonic tissue and organ systems are formed. During the maturation phase, the embryo expands by accumulating storage macromolecules (represented by brown/orange bars at the top of the figure), including 2S and 12S storage proteins, fatty acids and the major oil body protein oleosin, and starch. The embryo also acquires the ability to withstand desiccation during the maturation phase. LEC TFs are master regulators of embryo development whose RNAs (represented by red/pink bars at the top of the figure) primarily accumulate during embryogenesis in both the morphogenesis and maturation phases of development. Maturation-phase seeds also possess a high ratio of ABA to GA that influences physiological processes. At the end of maturation, embryos are arrested developmentally and metabolically quiescent. Upon favorable environmental conditions, the embryo will recommence growth and seeds will germinate, as marked by radicle emergence. The ratio of ABA to GA becomes low during germination. The break in lines depicting ABA and GA levels represents the metabolically quiescent mature seed. GA and ABA levels represented in this figure are from published studies of *Brassicaceae* plants [39–41]. To our knowledge, measurements of GA and ABA levels during the morphogenesis phase of seed development have not been published.

LECs go crazy in embryo development. Siobhan A. Braybrook and John J. Harada. Trends in Plant Science 13, 624-630 (2008)

3. Dormance et germination

- **Définition de dormance**: incapacité pour la graine de germer après la phase de maturation, malgré la réhydratation
- **Fonction:**
  - optimiser le moment de la germination: printemps, p.e.
  - favoriser germination dans de bonnes conditions: milieu approprié, à la surface du sol
  - étaler la germination dans l'espace et le temps
    (degré de dormance variable d'une graine à l'autre)

http://www.seedservices.sgs.com/

Le choix dormance/germination repose sur l'intégration de nombreux facteurs internes et externes

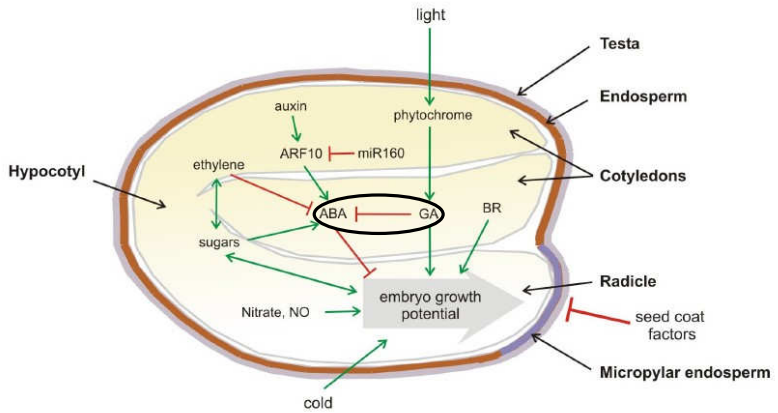


Figure 1. Schematic presentation of processes controlling seed dormancy and germination in an Arabidopsis seed. The Arabidopsis seed is characterized by the embryo with two cotyledons and a single cell layer endosperm. Germination promoting (green arrows) and inhibiting factors (red arrows) are indicated.

Remarque:
- dormance primaire: mise en place **pendant** l'embryogenèse
- dormance secondaire (induite) mise en place **après** l'embryogenèse

Gènes/ mutations affectant la dormance

- Mutants réduisant ou éliminant la dormance (favorisant la germination)

- Mutants augmentant la dormance (réduisant le potentiel de germination)

→ dormance peut encore être levée par traitement physique ou chimique

NB Quelques-uns de ces gènes ont un **effet maternel**: expression chez la plante-mère, pas chez l'embryon

p.e. Gène ***DAG1***

Molecular Aspects of Seed Dormancy. Ruth Finkelstein, Wendy Reeves, Tohru Ariizumi, and Camille Steber. Annu. Rev. Plant Biol. 2008. 59:387–415

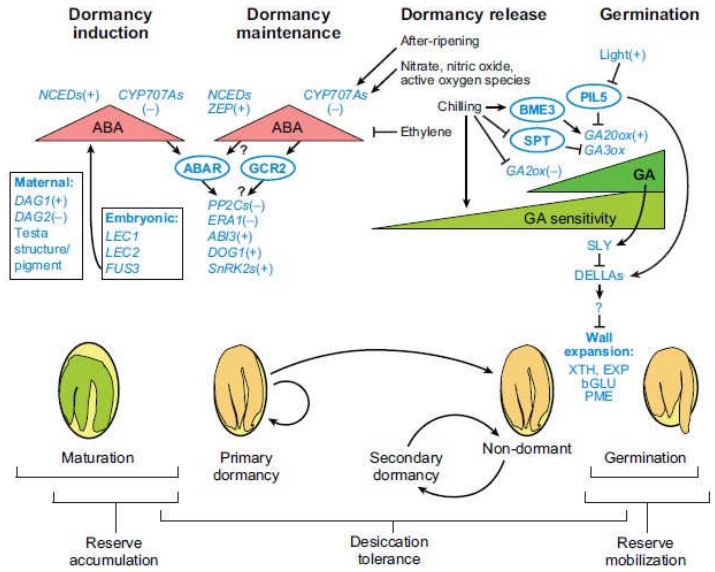


Figure 1

Time course of dormancy induction and release, including partial summary of regulatory factors. Width of abscisic acid (ABA) and gibberellin (GA) symbols represents relative hormone levels due to action of indicated biosynthetic and catabolic loci. Induction depends on combination of ABA-independent maternal and embryonic factors and ABA-dependent signaling. Release is promoted by many environmental factors, largely integrated through changes in ABA:GA signaling balance, eventually resulting in wall expansion to permit radicle emergence. Positive regulation is indicated by (+) and arrows, negative regulation by (–) and bars. NCED, 9-*cis*-epoxycarotenoid dioxygenase; CYP707A,

Exemples de gènes exprimés dans l'embryon dont la mutation réduit ou élimine la dormance

- Gènes ***LEC1, LEC2, FUS3*** **et** ***ABI3 (ABA Insensitive)***:

  pas de maturation de la graine

  → germination est le processus de développement « par défaut »
- Gènes de synthèse et de réponse à l'ABA
  - ABA: mise en place et maintien de la dormance
  - Mutants abi, p.e. (réponse à l'ABA): germent en présence d'ABA
- Gènes contrôlant le développement des téguments
- ***DELLA***: inhibe la germination (effet au niveau de la paroi)

  GA: stimule dégradation de la protéine DELLA

  (alors que l'ABA stabilise DELLA)

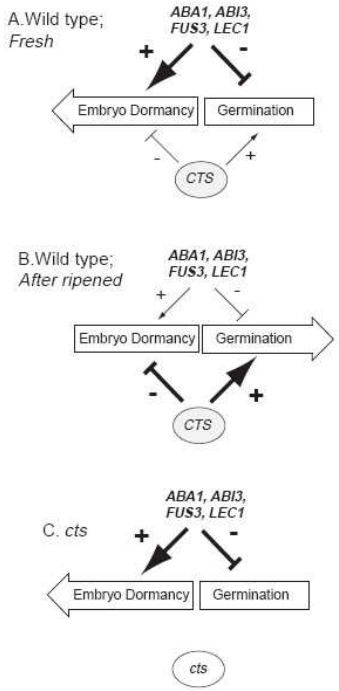
Exemples de gènes exprimés dans l'embryon dont la mutation augmente la dormance

- Gènes de synthèse et réponse à: GA, éthylène, brassinostéroïdes

  → antagonisme avec ABA
- Gènes pour les phytochromes: perception de la lumière
- **Gène *CTS*** (*Comatose*):
  - produit: transporteur membranaire (de type ABC: ATP Binding Cassette)
  - Fonction?
    - mobilisation des réserves lipidiques
    - autre…
  - Expression: augmente après l'imbibition (fin de la phase II)

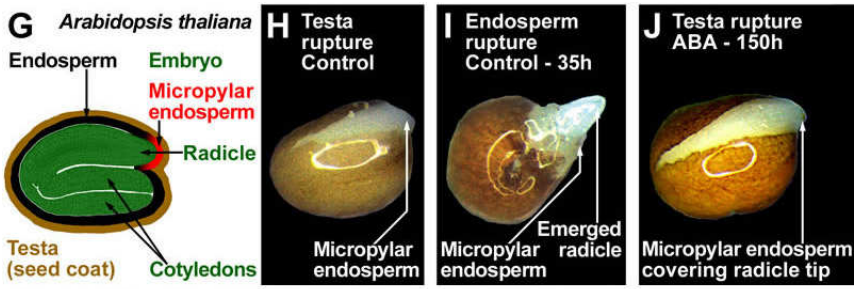
- **Mutant cts**:

  pas de réponse aux conditions de levée de dormance (froid, GA)
- ***CTS*** :
  - effet **antagoniste** aux gènes *LEC1, LEC2, FUS3 et ABI3*
  - avec GA, favorise expression des gènes de germination
- *CTS* agit comme un « commutateur » entre deux programmes de développement opposés: dormance (accumulation de réserves) et germination (utilisation des réserves)



Germination: ensemble des événements

- du début de la réhydratation de l'embryon en latence (imbibition)
- jusqu'au début d'élongation de l'axe embryonnaire (émergence de la radicule)



Müller et al. (2006) - © Plant Cell Physiology, Oxford University Press, http://pcp.oxfordjournals.org

Remarque: A quoi est due la perte progressive du pouvoir germinatif?

→ dommages aux chromosomes et aux membranes (oxydation)

La germination est un processus **triphasique**

- **Phase I:** - imbibition passive (p.e. affinité des mucilages pour l'eau)
  - réparation des dommages survenus pendant la phase de dormance
- **Phase II:** « attente », possibilité de dormance secondaire
- **Phase III:** - imbibition active (transporteurs)
  - début de la croissance végétative

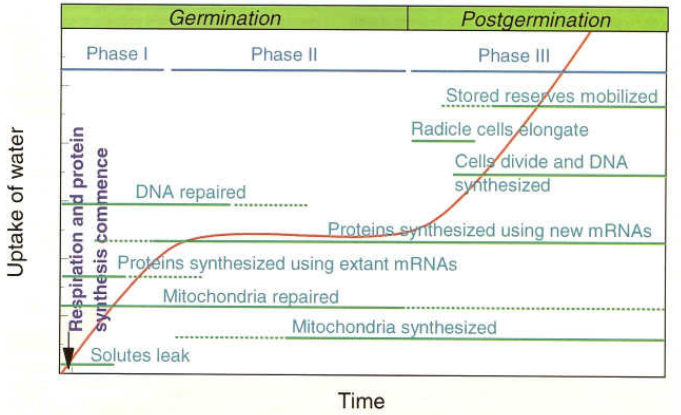
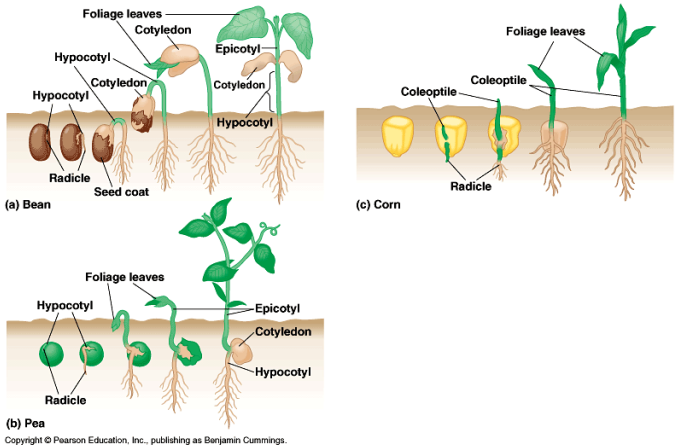


Figure 1. Time Course of Major Events Associated with Germination and Subsequent Postgerminative Growth.

The time for events to be completed varies from several hours to many weeks, depending on the plant species and the germination conditions.

Deux types de germination

- **Hypogée:**
  - peu ou pas d'allongement de l'hypocotyle
  - cotylédons sur ou dans le sol → organes d'absorption
- **Epigée:**
  - allongement important de l'hypocotyle
  - cotylédons au-dessus du sol → photosynthèse



En résumé: la dormance et de la germination sont contrôlées par des interactions entre facteurs hormonaux, génétiques et de l'environnement

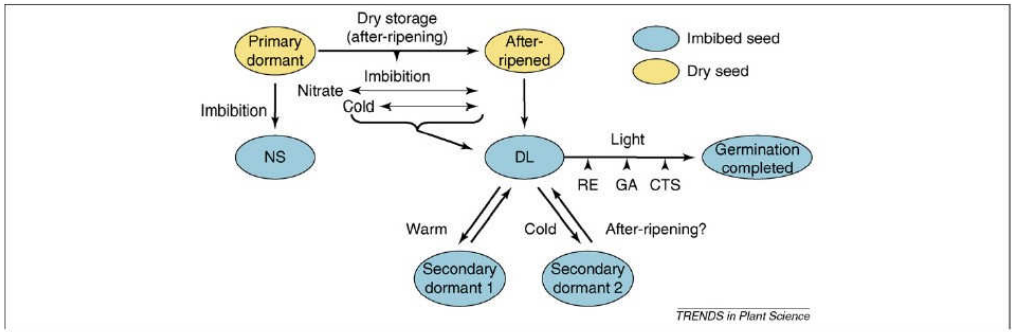


Figure 1. Dormancy cycling and physiological status in the progression to germination completion of *Arabidopsis* (accession Cvi) seeds. *Arabidopsis* seeds are shed with non-deep physiological dormancy [35], which is imposed by the tissues surrounding the embryo (the endosperm and testa). A range of environmental signals will relieve this dormancy [8,13]; those signals include: dry storage (after-ripening, AR), temperature (cold), light and nitrate. However, to break dormancy, these signals must be experienced in the correct order. The newly mature primary dormant seed is initially not sensitive (NS) and first requires some AR to develop sensitivity to nitrate and later to cold. Sensitivity to light is acquired after prolonged AR, AR followed by cold, or AR followed by nitrate. In this dormant light-requiring (DL) state, light is required to terminate dormancy and initiate the completion of germination. If the imbibed DL seed is not exposed to light, it will become secondary dormant. Secondary dormancy can be broken by cold, but will return if seeds are not exposed to light. Up to a point before germination completion, the imbibed seeds can reinitiate (RE) the late embryogenesis pathway, either from *de novo* transcription [36] or translation of stored mRNAs [11]. After this stage, seeds in the presence of light have the capacity to establish the GA biosynthetic pathway leading to the destabilization of DELLA proteins that repress germination (see Ref. [6] and references therein) and the expression of GA-responsive genes to activate germination completion and radicle protrusion (GA), for example, those involved in cell-cycle re-entry [5,9,10]. Prior to radicle protrusion, *CTS* expression is required for the expression of specific gene sets associated with seedling establishment and survival [6]. In this last step, *CTS*, a single-copy gene encoding a full-length ATP-binding cassette transporter, is required for the import of several biologically important molecules into the peroxisome, including very-long-chain fatty acids associated with breakdown of seed-storage lipids.