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Abstract Plant development is governed by a wide variety of genetic and epigenetic events that regulate cell fate. Flower to seed developmental transition varies greatly between plants and is of importance in research programs because of its relevance for crop production and human diet. In this chapter, we review the latest research on epigenetics regulation of flower, fruit, and seed development in crop plants. We use tomato (*Solanum lycopersicum* L.) as our reference crop model while referring to *Arabidopsis thaliana* for in-depth

studies and look into additional crop model plants such as maize (*Zea mays*), wheat (*Triticum* spp.), and rice (*Oryza sativa*) in order to cover a wide range of flower and fruit/seed types. Tomato is an interesting biological model thanks to its fleshy fruit. Tomato has the second natural epimutation reported, the *Colorless non-ripening* (*Cnr*), as well as newly reported studies on the paramutation SLTAB2, the role of the demethylase DML2 in fruit ripening, and the identification of two long noncoding RNAs (lncRNAs) involved in the ripening process. Altogether, these works make tomato an interesting and important epigenetic model for plants. A variety of epigenetic-based regulations are involved in each stage of the tomato fruit set, development, and ripening. Four epigenetic mechanisms are proved to be involved in flower, fruit, and developmental processes: histone modifications, DNA (de)methylation, small RNA posttranscriptional *locus* regulation, and lncRNA-associated regulatory pathways. Epigenetic mechanisms are involved at all stages of reproductive organs development, from the flower to the mature seed.

Keywords

(separated by '-')

Tomato - Small RNAs - DNA methylation - Epimutation

Epigenetics in Plant Reproductive Development: An Overview from Flowers to Seeds

Antoine L.F. Gady, Cristiane S. Alves, and Fabio T.S. Nogueira

Abstract Plant development is governed by a wide variety of genetic and epigenetic events that regulate cell fate. Flower to seed developmental transition varies greatly between plants and is of importance in research programs because of its relevance for crop production and human diet. In this chapter, we review the latest research on epigenetics regulation of flower, fruit, and seed development in crop plants. We use tomato (*Solanum lycopersicum* L.) as our reference crop model while referring to *Arabidopsis thaliana* for in-depth studies and look into additional crop model plants such as maize (*Zea mays*), wheat (*Triticum* spp.), and rice (*Oryza sativa*) in order to cover a wide range of flower and fruit/seed types. Tomato is an interesting biological model thanks to its fleshy fruit. Tomato has the second natural epimutation reported, the *Colorless non-ripening* (*Cnr*), as well as newly reported studies on the paramutation SLTAB2, the role of the demethylase DML2 in fruit ripening, and the identification of two long noncoding RNAs (lncRNAs) involved in the ripening process. Altogether, these works make tomato an interesting and important epigenetic model for plants. A variety of epigenetic-based regulations are involved in each stage of the tomato fruit set, development, and ripening. Four epigenetic mechanisms are proved to be involved in flower, fruit, and developmental processes: histone modifications, DNA (de)methylation, small RNA posttranscriptional *locus* regulation, and lncRNA-associated regulatory pathways. Epigenetic mechanisms are involved at all stages of reproductive organs development, from the flower to the mature seed.

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Contents

27 1 Introduction 00
 28 2 Flowering and Pollen Development 00
 29 3 Flower and Fruit Development 00
 30 3.1 Histone Acetylation Mediated Regulation 00
 31 3.2 DNA Methylation-Mediated Regulation 00
 32 3.3 MiRNA Mediated Regulation 00
 33 4 Fruit Ripening 00
 34 4.1 DNA Methylation-Mediated Regulation 00
 35 4.2 MiRNA Mediated Regulation 00
 36 4.3 LncRNA Mediated Regulation 00
 37 5 Seed Development 00
 38 5.1 Seed Dormancy 00
 39 5.2 Embryo–Endosperm Interaction 00
 40 5.3 Genomic Imprinting 00
 41 6 Conclusions and Future Prospects 00
 42 References 00

43 1 Introduction

44 Plasticity plays a fundamental role in adaptation and resilience of crops allowing
 45 cultivars to adjust and grow in different environments. Plasticity is responsible for
 46 homeostasis maintenance when environmental conditions change, allowing gene
 47 expression to adapt to biotic or abiotic stress or variations like altitude, soil type,
 48 seasonal, day length, rain, ambient temperature, disease, plant–plant competition,
 49 herbivory, among others (Latzel et al. 2012). To our current knowledge, epigenetic [AU1]
 50 mechanisms are responsible for conferring genetic plasticity to crops by DNA
 51 methylation, histone modification, noncoding RNAs, and chromatin modulation.
 52 These variations can regulate genome expression leading to new phenotypes in
 53 response to environmental changes.

54 Eukaryotic chromatin is a nucleoprotein complex, where the DNA is packed and
 55 condensed enfolded histone proteins. Even being tightly condensed, the chromatin
 56 needs to be dynamic in order to allow basic functions like transcription, replication,
 57 and DNA repair to occur. For the compaction to happen, 147 base pairs of DNA are
 58 wrapped into an octamer of core histones, containing two of each H2A, H2B, H3,
 59 and H4, linked by H1 at the entry/exit point. Histones play important roles in [AU2]
 60 metabolic chromatin functions such as chromatin integrity maintenance, DNA
 61 recombination, and process of DNA replication in several organisms. In fact,
 62 these proteins date back to the dawn of eukaryotic evolution, spanning protozoans,
 63 fungi, animals, and plants. Prokaryal and archaeal species are the earliest genomes
 64 known to have evolved histone-like proteins (Grove 2011; Sandman and Reeve
 65 2006). In the last 15 years, histones became the focus of research interests thanks to
 66 the light shed on the histone code and its implications. This code is a resultant from
 67 covalent posttranslational modifications (PTM)—methylation, acetylation, phos-
 68 phorylation, ubiquitination, and poly-ADP ribosylation—that takes place at the C-

or N-terminal tail of histones and histone variants. There are five histones families—H1, H2A, H2B, H3, and H4—which are subject to PTM. Euchromatin is usually constituted by transcribed *loci* and heterochromatin is enriched with TEs (transposable elements) and is typically transcriptionally silenced through vegetative phases by DNA methylation and histone modifications (Bernatavichute et al. 2008; Law and Jacobsen 2010).

Contrarily to animals, in which cytosine DNA methylation in the CG context is predominant, in plant kingdom cytosine DNA methylation can occur in three contexts: CG, CHG, and CHH (where H=A, T or C) (Law and Jacobsen 2010). Plant DNA methyltransferases are distributed in four families: (1) MET1: similar to DNMT1, which is a CG maintenance DNA methyltransferase; (2) CMT: a plant-specific chromomethylase that lacks the N-terminal extension, and it is a CNG DNA methyltransferase; (3) DRM: a plant de novo DNA methylases of non-CG DNA sequences, which have an unusual arrangement of some conserved catalytic motifs; and (4) DNMT2: highly conserved but enigmatic methyltransferase (Vanyushin and Ashapkin 2009). Recent studies in *Solanum lycopersicum* (tomato) genome reveal the presence of nine genes encoding DNA methyltransferases, comprising the four DNA methyltransferase families: *MET1*, *SIDNMT2*, *SICMT2*, *SICMT3*, *SICMT4*, *SIDRM5*, *SIDRM6*, *SIDRM7*, *SIDRM8* (Kumar et al. 2016).

The DNA methylation marks are reversible, which confer plasticity by facilitating the modulation of RNA transcription from genomic regions or specific *loci*. Alteration in DNA methylation can have meaningful outcomes on chromatin structure, with increases in 5mC marks connected with the arrangement of nucleosomes in plant genomes (Chodavarapu et al. 2010). Based on bisulfite sequencing—where unmodified cytosines are converted to uracil (sequenced as T) and 5mC remains sequenced as C, researchers generated methylome maps for tomato (Zhong et al. 2013), *Arabidopsis* (Zhang et al. 2006), and rice (*Oryza*; Li et al. 2012). Tissue-specific methylomes can provide evidence of epigenetic plasticity by analyzing the changing patterns in gene regulation. Euchromatin is associated with hypomethylated DNA in actively transcribed regions, while heterochromatin contains silenced genes, which were frequently hypermethylated (May 2010).

Noncoding RNAs, RNA molecules that do not code for proteins, have important roles in the regulation of plant reproductive organ development. Small interfering RNAs (siRNA) function as part of the RNA-directed DNA methylation (RdDM) machinery where they direct the RdDM complex to specific DNA locations to be methylated. MicroRNAs (miRNA) provoke transcriptional gene silencing by recruiting the RISC complex to specific target mRNAs by sequence complementarity. Finally, long noncoding RNAs (lncRNA) are transcripts over 200 bp that can act in a multitude of ways to regulate gene expression; depending on their genome location, they can act as *cis*- or *trans*-regulatory elements (Chekanova 2015; Liu et al. 2015b). The implication of ncRNAs in the development of both flower and fruits has been repeatedly proven and have unraveled a vast network of RNA molecules acting as fine regulators of gene expression during plant organ development.

113 In this chapter, we will highlight the main findings on epigenetic regulation
114 affecting flower, fruit, and seed development. We will discuss the latest findings
115 regarding epigenetic regulations of tomato flower and fruit development that might
116 help to improve the future of tomato breeding.

117 2 Flowering and Pollen Development

118 Perennial and overwintering annuals crops can respond to the length and severity of
119 the winter thanks to their plasticity, securing that individual plants are adjusted and
120 reaching reproductive maturity. In some cases, this environmental cue causes
121 changes that are mitotically stable throughout the rest of development. To date,
122 the best-characterized example of such epigenetic memory in plants is the vernal-
123 ization process, namely the acceleration of flowering as a result of exposure to cold
124 temperatures in winter. Flowering program is promoted by the perception of the
125 vernalization signals, including chromatin-based mechanisms, as the winter passes
126 (reviewed in Bloomfield et al. 2014).

127 *FLOWERING LOCUS C (FLC)* is a major floral repressor that suppresses
128 flowering during exposure to prolonged periods of low temperature. An additive
129 effect of histone modifications at the *FLC* locus takes place in vernalization-
130 sensitive species when the length of winter daytime is noted as temperature degrees
131 below a threshold (Sheldon et al. 2009). Depending on the species, the vernalization
132 signaling can happen at different developmental stages, registered by epigenetic
133 switches maintained during in vitro vegetative propagation and deleted through
134 sexual reproduction (Song et al. 2012). When the winter ends, histone
135 remodification gradually occurs allowing the derepression of *FLC locus* (Song
136 et al. 2012). In response to cold treatment, 5mC (5-methyl cytosine) is significantly
137 reduced both in winter and spring *B. napus* following a gradual DNA
138 re-methylation to pretreatment levels in spring *B. napus*, but only up to 70% in
139 winter *B. napus* (Guzy-Wrobelska et al. 2013). It seems that this primary mecha-
140 nism of epigenetic regulation is conserved across plant species, as similar results
141 from cold treatment also change 5mC patterning in different crops like cotton,
142 maize, rice, and wheat (Steward et al. 2002; Sherman and Talbert 2002; Pan et al.
143 2011; Fan et al. 2013). Active repression of *FLC* or *FLC*-like *loci* is required as a
144 standard mechanism until a target threshold is sensed (Sheldon et al. 2009; Xiao
145 et al. 2013). *FLC* is repressed in *Arabidopsis* by decreasing H3 acetylation and
146 demethylation of H3K9 through *REDUCED VERNALIZATION1/VERNALIZA-*
147 *TION INSENSITIVE 3 (VRN1/VIN3)* (Bastow et al. 2004; Sung and Amasino 2004).
148 Pollen development undergoes a transcriptional and translational
149 reprogramming to promote the production of male gametes from somatic lineages
150 (Calarco et al. 2012). Small RNAs (sRNAs), important components of the plant
151 epigenetic machinery, play an essential role in the pollen reprogramming process,
152 altering the transcriptional and translational dynamics characterizing the individual
153 developmental stages (Borges et al. 2011). Pollen is notably sensitive to elevated

temperatures, and little is known about the mechanisms underlying this stress response. Recently, it has been shown in heat-stressed tomatoes that the accumulation of pollen miRNAs, tRNAs, and snoRNAs is affected by heat stress in distinct pollen developmental stages, especially in post-meiotic and mature stages of male gametophyte development (Bokszczanin et al. 2015). Though still speculative, some of these sRNAs might have important roles in the epigenetic regulation of pollen development in response to environmental cues.

3 Flower and Fruit Development

The final size and shape of the fruit is the result of a multitude of developmental events that go back as far as the floral initiation. The differentiation of the inflorescence meristem into a floral meristem marks the first stage of floral initiation. The size of the floral meristem depends on the number of cells it is composed of, and therefore, it is a parameter that influences the final fruit size (van der Knaap et al. 2014; Xu et al. 2015). Five days after floral initiation, the gynoecium starts to grow. The way the gynoecium will develop pre- and post-anthesis will determine the shape and size of the final fruit. See van der Knaap et al. (2014) for a complete review of the genes influencing tomato fruit weight and shape.

3.1 Histone Acetylation Mediated Regulation

Flower architecture is molecularly determined by the reference ABC model (Bowman et al. 1991). In this model, specific combinations of gene expression and protein interactions determine the geographical limits of each flower whorl (sepals, petals, stamens, and carpel). As demonstrated in *Arabidopsis*, the A class gene *APETALA2* (*AP2*) regulates target gene expression as part of complex it forms together with *TOPELESS* (*TPL*) and *HISTONE DEACETYLASE19* (*HDA19*). That transcription repressing complex negatively regulates *AGAMOUS* (*AG*), a C class gene, and *SEPALATA3* (*SEP3*), an E class gene from the ABC flower architecture model. The gene repression is mediated by deacetylation of H4K16 in regulatory regions of both *AG* and *SEP3* (Krogan et al. 2012). Expression studies in *Arabidopsis* tissues highlighted additional HDACs expressed in reproductive tissues: *HDA5*, *HDA6*, *HDA7*, *HDA9*, *HDA15*, and *HDA18*, but their function in either fruit or flower development remains unidentified. Only for *HDA6* do we know that it is involved in the regulation of flowering time. *HDA6* directly interacts with *FLOWERING LOCUS D* (*FLD*), a histone H3K4 demethylase). The complex removes acetyl and methyl groups from Histone 3 at loci of three repressors of flowering: *FLD*, *MADS AFFECTING FLOWERING 4* (*MAF4*), and *MAF5*, thus repressing their expression (Yu et al. 2011). From that set of HDACs, *HDA6* is known to be involved in RNA-directed DNA methylation (RdDM), a plant-specific

191 mechanism to regulate chromatin silencing of developmental genes as well as
192 transposable and repetitive elements.

193 In plants, the major small-interfering RNA (siRNA)-mediated epigenetic path-
194 way is RNA-directed DNA methylation (RdDM). RdDM is a complex epigenetic
195 machinery that involves a large number of players whose activity can be broken
196 down into a few steps (Matzke and Mosher 2014). Mainly two types of transcripts
197 are involved in the RdDM machinery: Pol IV and Pol V transcripts. Pol IV
198 transcribes long noncoding RNAs (lncRNAs) that are subsequently converted
199 into double-stranded RNAs (dsRNAs) by *RDR2* (Haag et al. 2012). These dsRNAs
200 are processed by *DICER-like3* (*DCL3*) into siRNAs. The siRNAs are exported to
201 the cytoplasm where they are loaded into *AGO4* and reimported into the nucleus.
202 The role of the siRNAs is to guide, by specific base pairing, *AGO4* toward nascent
203 scaffold transcripts of Pol V. The formation of this siRNA, *AGO4*, Pol V-derived
204 lncRNA scaffold ultimately recruits histone deacetylases (HDACs) and DNA
205 methyltransferases that through histone deacetylation and DNA methylation silence
206 the genomic loci transcribed by Pol V. Promoters silenced by RdDM are charac-
207 terized by histone deacetylation, which in *Arabidopsis* is acted by the RPD3-type
208 histone deacetylase *AtHDA6*, for which the tomato homologue is *SIHDA3*. *AtHDA6*
209 activity results in the deacetylation of histone H3 lysines 9 and 14 which leads to
210 gene expression downregulation (Aufsatz et al. 2007). Functional *AtHDA6* is
211 required to control siRNA-dependent heterochromatin and that deacetylation is a
212 prerequisite for subsequent methylation by HMTs (Aufsatz et al. 2007). Complete
213 loss-of-function mutants for *AtHDA6* exhibit reactivation of RdDM-silenced pro-
214 moters, despite the continuous presence of the RNA-silencing signal. Moreover,
215 cytosine methylation is reduced, highlighting a function for *AtHDA6* in methylation
216 maintenance. This function of *AtHDA6* might be mediated by the physical associ-
217 ation with DMTs, *MET1*, and *CMT3* (Aufsatz et al. 2002).

218 The roles of histone acetylases and deacetylases in tomato flower or fruit
219 development are poorly understood. Using the sequencing data generated by the
220 international tomato genome sequencing consortium (Tomato Genome C 2012),
221 Cigliano et al. (2013) identified in silico potentially all the histone modifiers of the [AU4](#)
222 tomato genome. Next they used the RNAseq data from that same source to look at
223 the expression profile of each histone modifiers in 10 sample tissues. The histone
224 acetylases *SIHAG18* and *SIHAG6* both presented peaks of expression in the flower
225 samples, possibly indicating a role in reproductive development. A recent study
226 (Zhao et al. 2014) identified 15 tomato histone deacetylases. *AtHDA6* tomato
227 homologue, *SIHDA3*, was expressed in all tissues with a maximum expression at
228 the flower stage. *SIHDA1*, the homologue to *AtHDA19*, was as well highly
229 expressed in flowers and then repressed for all fruit samples except for a peak at
230 the ripe fruit stage. In addition, yeast two-hybrid assays showed that *SIHDA1*,
231 *SIHDA3*, and *SIHDA4* interact with MADS-box transcription factors TOMATO
232 AGAMOUS1 (*TAG1*) and TOMATO MADS-BOX29 (*TM29*) (Zhao et al. 2014).
233 *TAG1* is a transcription factor necessary to express ethylene dependent and inde-
234 pendent ripening genes (Klee and Giovannoni 2011). *TM29* is a homologue to

SEPALATA which when silenced provokes the formation of parthenocarpic fruits and aberrant flowers (Ampomah-Dwamena et al. 2002). 235
236

3.2 DNA Methylation-Mediated Regulation 237

The *clark kent* hypermethylated epialleles of the *SUPERMAN* (*SUP*) gene are a clear illustration that DNA methylation can affect flower development through the regulation of gene expression level. The *sup-5 Arabidopsis* mutant which contains a nearly complete deletion of the *SUP* gene produces flowers with an increased number of stamens and carpels: 12 stamens compared to six and three carpels against two in wild-type *Arabidopsis* flowers. The stronger *clk3* epiallele contains an average of eight stamens and three carpels. The *clk* alleles have the exact *SUP* sequence of the wild-type accession but are extensively methylated from the start of transcription and covering most of the gene region (Jacobsen and Meyerowitz 1997). Antisense *MET1* and *ddm1* and *ddm2* mutant plants are hypomethylated but contain methylation-rich regions such as the *SUP* locus that is consistently hypermethylated in the three DNA methylases' mutant or antisense backgrounds (Jacobsen et al. 2000). In addition, *agamous* mutant-like flower phenotypes were identified in the *MET1*-antisense plants. Reduced levels of *AGAMOUS* mRNA were observed in the transgenic plants. An increase in methylation of the *AG* locus was measured by bisulfite genomic sequencing. Interestingly, hypermethylation of the *AG* locus only occurred in lines with a hypermethylated *SUP* locus, thus suggesting that hypermethylation of *SUP* is necessary to the hypermethylation of *AG*. 238
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To understand the extent of the epigenetic regulation of flower development by DNA methylation, Yang et al. (2015) used high-throughput sequencing of DNA fragments obtained by *Msp*JI digestion to obtain a whole genome profile of DNA methylation patterns. They divided the *Arabidopsis* flower development in three key stages: (1) floral meristem from *ap1 cal* double mutants, (2) wild-type early flowers at stages 1–9, and (3) wild-type late flowers at stages 10–12. In plants, DNA methylation occurs on the Cytosine nucleotide in three distinct sequence contexts: *MET1* transfers a methyl residue to CG sites, *CMT3* to CHG sites, and *DRMs* add methyl groups to CHH sites where H stands for A, T, or C nucleotides. The number of methylated cytosine increased by 8% between floral meristem and early flower stage and then decreased by 0.55% from the early to late flower stages. The initial stages of flower development are marked by numerous de novo methylation: 80,056 new methylation events reported. The study identified 3067 genes out of 24,035 that are co-differential: genes with significant variations in both methylation and gene expression. In the transition from floral meristem to early flower stage, 1048 genes were co-differential at ^mCG, 601 at ^mCHG, and 509 ^mCHH-containing genes. A total of 909 genes were co-differential in the early to late flower transition. Among them are important flower development regulators such as *SEP1*, *LEUNIG* (*LUG*), and *SEEDSTICK*. Moreover, 33 genes associated with flower development, 257
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276 21 genes involved with pollen development, 201 transcriptional regulators,
277 29 genes linked to chromatin organization, and 56 to signal transduction have
278 differential gene expression profile along flower development that is linked to
279 DNA methylation variation. While the number of methylated cytosine increased
280 during the meristem to early flower transition in all sequence contexts, only ^mCGs'
281 number increased in the early to late flower shift, thus hinting at a role for MET1
282 methylations in a developmental phase characterized by organ growth. Considering
283 the three studied developmental stages, over 1000 genes for each CHG and CHH
284 methylation were both transcriptionally and epigenetically differentially regulated,
285 suggesting an important role for these methylation types into reproductive organ
286 development. Finally, Yang et al. (2015) also show that DNA methylation does not
287 only regulate protein coding gene expression but as well that other epigenetic
288 players are regulated in this manner: transposable elements, miRNAs, and noncod-
289 ing RNA also had variation in the methylation status along flower development.
290 This data thus hints toward the idea that the activity of additional epigenetic players
291 is regulated by their methylation state.

292 3.3 *MiRNA Mediated Regulation*

293 MicroRNAs, small 21 nucleotides RNA molecules, regulate gene expression by
294 specifically binding to mRNA with near-perfect sequence complementarity and
295 thereof provoking their degradation. In *Arabidopsis thaliana*, at least eight miRNA
296 families are responsible for the regulation of transcription factors involved in flower
297 development. The miR164, miR169, and miR172 families are participating in
298 setting boundaries between floral organs. The miR164 family regulates transcrip-
299 tion factor of the NAC-domain family such as *CUP-SHAPED COTYLEDONS*
300 (*CUC1* and *CUC2*) in *Arabidopsis*. Alteration to *CUC1* and *CUC2* expression
301 results in modified sepal boundaries leading to fused sepals and fewer petals
302 (Baker et al. 2005; Laufs et al. 2004). In addition, miR164 could play a role in
303 carpel development (Baker et al. 2005; Sieber et al. 2007). Through the regulation
304 of NF-YA transcription factors, the miR169 family limits the expression of the
305 C-class family of genes to the inner two whorls of petunia and antirrhinum flowers.
306 MiR172 on the other hand restricts the expression of *AP2* to the two outer whorls of
307 the floral meristem (Chen 2004; Wollmann et al. 2010; Zhao et al. 2007). In
308 addition to its role in flower development in *Arabidopsis*, miR172 was shown to
309 be actively regulating flower formation in both rice and barley (Lee and An 2012;
310 Nair et al. 2010; Zhu et al. 2009). MiR159 targets GAMYB-like genes such as
311 *LEAFY*, *MYB33*, or *MYB65* and thus regulates flowering time but as well anther
312 formation during flower development. Overexpression of miR159 downregulates
313 *MYB33* and results in male sterility. Similarly, to what is observed in *Arabidopsis*,
314 in rice the GAMYB gene expression is restricted to anthers. Resembling the effect
315 of miR159, the overexpression of miR319 as well leads to defects in stamen
316 development and male sterility, but the phenotype is the consequence of the

mis-regulation of a set of TCP transcription factor genes (Palatnik et al. 2007; 317 Schommer et al. 2012). Plants with reduced levels of miR159 and miR319 show 318 similar floral phenotypes as *arf6/arf8* double mutants. Auxin Response Factors 319 6 and 8 through the regulation of Auxin level regulate the extent of cytokinin 320 activity in the developing floral meristem. *ARF6* and *ARF8* are regulated by 321 miR167 (Rubio-Somoza and Weigel 2011), and miR167 is upregulated by either 322 *TCP4* or *MYB33*, the targets of miR159 and miR319, thus forming a complex floral 323 development regulatory network. Another regulator of ARF genes is miR160 which 324 targets *ARF10*, *ARF16*, and *ARF17* genes. Downregulation of miR160 in transgenic 325 *Arabidopsis* plants increases its target expression. These plants have defects in 326 fertility and in floral organ formation and floral organs appeared inside siliques (Liu 327 et al. 2010). MiR165 and miR166 regulate HD-ZIP III genes *ATHB15*, *ATHB8*, 328 *REVOLUTA*, *PHABULOSA*, and *PHAVOLUTA*. Downregulation of these genes 329 through overexpression of miR165 results in plants with carpel developmental 330 defects, enlarged apical meristem, and short sterile carpels (Kim et al. 2005). The 331 involvement of miRNAs in *Arabidopsis* reproductive development from juvenile to 332 the flower producing plant phases is reviewed in Hong and Jackson (2015). 333

The expression of *SIARF6* and *SIARF8* is also regulated by miR167 in tomato 334 plants. Plants overexpressing AtmiR167a produce female sterile flowers with 335 shortened sepals, stamens, and style which is a consequence of the *SIARF6* and 336 *SIARF8* low expression levels in developing flowers (Liu et al. 2014). MiR160 is 337 abundant in tomato ovaries. In tomato, miR160 preferentially targets ARF10A and 338 to a lesser extent *SIARF10B* and *SIARF17* (Damodharan et al. 2016). Therefore, the 339 use of a target mimic to sequester miR160 and inhibit its natural function pro- 340 vokes an increase in *SLARF10A* accumulation in tomato ovaries which result in 341 perturbed ovary patterning: an excessive elongation of its proximal end and thin- 342 ning of the placenta. Consequently, postfertilization, the fruit is pear shaped. This 343 fruit shape phenotype is the result of a mis-distribution of auxin in the early stages 344 of ovary development regulated by SlmiR160 (Damodharan et al. 2016). In rice, 345 Huang et al. (2016) showed that OsmiR160 regulates *OsARF18* and thereby auxin 346 signaling. mOsARF18 transgenic plants express a modified allele of *OsARF18* that 347 is not recognized and thus not regulated by OsmiR160. These transgenic rice plants 348 had overall growth and development defects such as dwarfism, rolled leaves, small 349 seeds, and abnormal flowers. mOsARF18 plants were impaired in reproductive 350 organ development: the lemma and palea did not contain flowers and stamens 351 remained attached to developing seeds when fertilization did occur, suggesting 352 abnormal senescence of stamens, reminiscent of the senescence phenotypes 353 observed in tomato (Damodharan et al. 2016). 354

SlmiR396 targets 8 out of the 13 tomato Growth Regulating Factors (GRF): 355 *SIGRF1*, *SIGRF2*, *SIGRF3*, *SIGRF4*, *SIGRF5*, *SIGRF7*, *SIGRF8*, and *SIGRF12*. 356 GRFs are a class of transcription factors expressed in most developing organs. 357 GRFs regulate cell number, and their overexpression in *Arabidopsis* results in 358 enlarged organs (Cao et al. 2016). On the other hand, overexpression of miR396 359 in *Arabidopsis* plants developed flowers with a single carpel; in rice a similar 360 approach led to similar results: altered floral organ morphology (Cao et al. 2016). 361

362 Strong downregulation of miR396a and miR396b with STTM396a/396a-88 in
363 tomato produced plants with increased cell number and cell size in both flowers
364 and fruits leading to larger sepals and larger fruits (Cao et al. 2016). Fruits from
365 STTM396a/396a-88 transgenic lines were 39 and 45% larger than control fruits.
366 This might prove to be a new way to improve yield. Overexpression of miR172 was
367 shown as well to increase fruit size in tomato (Yao et al. 2016). MiR172
368 overexpressing tomato plants contain numerous flower defects such as sepal to
369 petal transformation, poorly developed stamen that produce sterile pollen, and the
370 development of seedless parthenocarpic fruits with ectopic ovaries inside the fruits
371 and occasionally fruit in fruit phenotypes. Similar phenotypes were also observed
372 by our group in Micro-Tom tomato plants overexpressing miR156 (Silva et al.
373 2014). The altered fruit morphology, fruit-like structures emerging from the main
374 fruit, was correlated with accumulation of miR156 in meristematic tissues such as
375 placenta and ovules of developing ovaries and immature fruits. miR156
376 overexpression plants (miR156-OE) had flower buds with extra whorls and
377 meristem-like structures that developed into ectopic structures instead of ovaries
378 and ovules. The overexpression of miR156 prolonged the phase of floral meristem
379 proliferation, and when organs finally formed, they produced flowers with addi-
380 tional partly fused carpels that likely account for the appearance of the miR156-OE
381 fruits (Silva et al. 2014). We have identified five miR156-targeted SQUAMOSA
382 promoter binding protein like (*SPL/SBP-box*) genes that are differentially
383 expressed in pre- and post-anthesis ovaries. Our data show that the *LeT6/TKn2*
384 and *GOBLET (GOB)* are repressed by SI-SBPs to control meristem maintenance
385 and cell proliferation at the onset of flower organs' initiation and differentiation,
386 thereby controlling proper carpel and ovule development. In addition, we showed
387 that *MACROCALLIX (MC)*, *FRUITFUL1 (FUL1)*, and *FALSIFLORA (FA)* may act
388 under the control of the miR156/SI-SBP node to regulate floral meristem identity
389 and specification of organ whorls, but while *GOB* is controlled by the miR156/SI-
390 SBP node through miR164, it remains unknown how miR156/SI-SBP controls *MC*,
391 *FUL1*, and *FA*.

392 The epigenetic factors controlling the expression profile of genes involved in
393 both flower and fruit development as reviewed in this chapter are all schematically
394 overviewed in the Fig. 1.

395 4 Fruit Ripening

396 The ripening development process is a unique feature to plants bearing fleshy fruits.
397 Its function is to help the dissemination of the plants seeds through animal con-
398 sumption. Therefore, the fruit undergoes important changes in, for example, color:
399 making them more visible, in metabolite composition, such as production of sugars
400 and volatiles. In tomato, this transition from a mature green to a ripe red fruit is
401 induced at the breaker stage by a concomitant burst in ethylene production and by a
402 sharp increase in differential expression of transcription factors. Our understanding

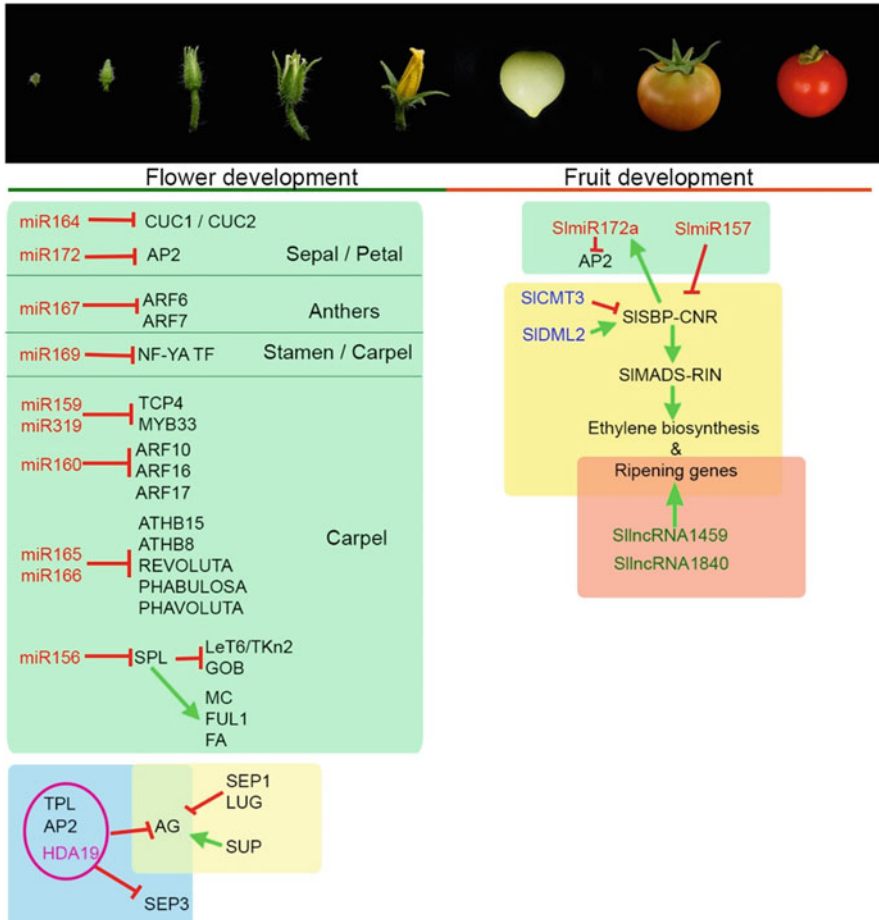


Fig. 1 Epigenetic regulation of genes governing flower and fruit development. Flower development, on the left, is monitored by a combination of histone acetylation, DNA methylation, and miRNA posttranscriptional gene silencing. The green backgrounds encapsulate regulations monitored by miRNAs. The list of miRNA directed regulations is divided according to organ location specificity. On the yellow backgrounds are genes whose expression is dependent on DNA methylation status: *SEPALATA1* (*SEP1*), *LEUNIG* (*LUG*), and *SUPERMAN* (*SUP*), all of which regulate *AGAMOUS* (*AG*). On the blue background are represented flower development genes regulated by *HISTONE DEACETYLASE 19* (*HDA19*). Regulation of the transition from green to maturing fruit is regulated by the pivotal gene *SLSBP-CNR* which is controlled (1) by promoter methylation status thanks to *SICMT3* and *SIDML2* and (2) by *SlmiR157* (green background). lncRNA1459 and lncRNA1840 that are involved in the regulation of fruit ripening genes are shown on the red background

403 of the role of epigenetic players in fruit ripening regulation is a recent addition to
404 this complex development process.

405 **4.1 DNA Methylation-Mediated Regulation**

406 The regulation of the fruit transition from green to ripe is epigenetically regulated
407 by DNA methylation of cytosine nucleotides. Progress in understanding the molec-
408 ular regulations of the ripening process in tomato have been achieved by the study
409 of ripening mutants such as ripening inhibitor (*Rin*) and Colourless non-ripening
410 (*Cnr*). The *Cnr* mutation was mapped to a *SISBP3*-like gene (Manning et al. 2006),
411 and since the nucleotide sequence of the *SISBP-CNR* mutant was identical to the
412 one of the wild-type plant, the authors hypothesized that it could be an epimutation.
413 Bisulfite sequencing of 2.4 kb upstream of the *SISBP-CNR* start site revealed a
414 286 bp region rich in cytosine methylation. Further VIGS experiment confirmed the
415 role of *SISBP-CNR* as regulator of fruit ripening. The *Rin* mutation mapping and
416 sequencing characterized the *Rin* locus as a MADS box transcription factor gene
417 whose expression in the *Rin* background restored ripening (Vrebalov et al. 2002).
418 *SIMADS-RIN* acts upstream of ethylene in the ripening regulatory chain. *SIMADS-*
419 *RIN* regulates, through interaction with their promoters, the expression of numerous
420 genes involved in various ripening-related pathways such as ethylene biosynthesis,
421 perception, and response; cell wall metabolism; and carotenoid biosynthesis
422 (Martel et al. 2011). In addition, the binding of *SIMADS-RIN* to its target promoters
423 cannot occur in the hypermethylated *Cnr* mutant (Martel et al. 2011); thus, tran-
424 scription activation by *SIMADS-RIN* is impaired by methylation of these promoters
425 and can only occur in plants with proper *SISBP-CNR* activity. Inhibition of
426 5-cytosine DNA methyltransferases in green immature fruits leads to early fruit
427 ripening, before seeds are mature. Whole genome methylome showed that, in genic
428 regions, differentially methylated regions were located on the 5'-end of genes,
429 therefore likely to be associated with promoter regulatory regions (Zhong et al.
430 2013). While whole genome methylome showed that a substantial epigenome
431 reprogramming is occurring during fruit development, it also identified 292 genes
432 that are regulated by the *SIRIN* MADS box transcription factor. These genes, in the
433 hypermethylated *Cnr* background, have methylation in their promoter region that
434 prevent binding and activation by *SIRIN*. This work confirmed 16 previously
435 identified RIN targets. RIN-regulated genes are demethylated in the control plants,
436 thus allowing normal production of ethylene, volatiles, sugar metabolites, caroten-
437 oids, and fruit softening. Genome methylation is thus the third key determining
438 factor to the transition to ripening in fleshy fruit plants in addition to ethylene
439 hormonal control and fruit-specific transcription factors (Zhong et al. 2013).

440 To identify which DNA methyltransferase is responsible for the methylation of
441 the promoter of the *Cnr* locus Chen et al. (2015b) used the VIGS system to silence a
442 range of DNA methyltransferases in *Cnr* fruits. *Cnr* fruits with silenced *SIDRM7*,
443 *SIMET1*, *SICMT2*, and *SICMT3* ripened to various degrees. *SICMT3*-silenced fruits

ripened almost completely. The expression of *SICNR* and of the ripening genes *SIRIN*, *SIAP2a*, *SITAGL1*, of the ethylene biosynthesis, and signaling pathway were all upregulated in the red sections of the VIGS-*SICMT3* fruits. Bisulfite sequencing determined that 8 out of 18 methylated cytosine in the *Cnr* promoter were demethylated in the VIGS-*SICMT3*-silenced fruits. This reduction in methylation was sufficient to alter the expression level of the *CNR* gene. In addition, the bisulfite sequencing data showed that hypomethylation in *SICMT3*-silenced tissues also occurred at the promoter sites of *SIRIN* and *RIN*-targeted promoters directing the expression of ripening genes. Thus, *SICMT3* is essential to the maintenance of the stable *Cnr* epiallele. In wild-type plants, at the time when the fruit is ready to switch into its ripening phase, the promoters of genes discussed above have to be demethylated. The tomato genome contains four DEMETER-like DNA demethylases (DMLs), but only *SIDML2* is expressed from the onset of fruit ripening and further on until the fruit is fully ripe (Liu et al. 2015a). *SIDML2* RNAi silencing lines showed delayed initiation of ripening, from 10 to 20 days, and the ripening was never fully completed in fruits of these lines. Thus, active demethylation is a prerequisite to tomato fruit ripening. *SIDML2* is the only demethylase expressed at the developmental stage corresponding to demethylation of ripening genes characterized in *Rin* and *Cnr* mutants such as *SIPSY1* (Liu et al. 2015a).

4.2 MiRNA Mediated Regulation

Deep sequencing of tomato short RNAs and comparative genomics have contributed to the identification of hundreds of miRNA expressed in tomato tissues (Din and Barozai 2014; Karlova et al. 2013; Moxon et al. 2008; Zuo et al. 2012). These large-scale projects showed that miRNAs are involved in most aspects of plant and fruit development and along all stages of fruit growth and ripening. But few of the predicted miRNA/target gene interaction have been experimentally validated. Moxon et al. (2008) predicted that miR157 and miR156 play a role during fruit ripening through an interaction with *SISBP-CNR*. This prediction was experimentally validated, and it was demonstrated that SlmiR157 regulates the expression of *SISBP-CNR* and thereby participates in the fine-tuning of the ripening process (Chen et al. 2015a). SlmiR156 on the other hand does not take part in ripening regulation, but on top of its role in fruit development (Silva et al. 2014), it has a function in fruit softening (Chen et al. 2015a). While *SISBP-CNR* is to some extent regulated by SlmiR157, *SIRIN* on the other hand controls the expression of multiple miRNAs (Gao et al. 2015). Out of 33 identified miRNA families in the *rin* mutant, 14 of them were differentially regulated in ripening fruits of the mutant plants. *SIRIN* CARG-box binding sites were identified in four out of ten looked into promoters of SlmiRNA precursors. A ChIP-qPCR assay experimentally proved that indeed *SIRIN* binds to the promoter region of miR172a.

484 **4.3 LncRNA Mediated Regulation**

485 Besides small RNAs, long noncoding RNAs (lncRNAs) are also important for
486 epigenetic regulation in plants. LncRNAs are defined as RNA molecules over
487 200 bp that do not contain an open reading frame coding for a polypeptide longer
488 than 100 amino acids. Around 40,000 lncRNAs were identified in *Arabidopsis* (Liu
489 et al. 2012; Wang et al. 2014). lncRNAs are transcribed by Pol II, Pol IV, and Pol V
490 polymerases. Pol II lncRNA transcripts have a 5'-cap and a 3' polyadenylated tail,
491 similarly to mRNA. Long noncoding RNAs function as epigenetic regulators
492 through various mechanisms. Functions as target mimics of miRNA were identified
493 in *Arabidopsis* and Rice (Franco-Zorrilla et al. 2007; Wu et al. 2013); in this role,
494 the lncRNA is a decoy that capture specific miRNA in place of the miRNA target
495 gene, thus regulating the miRNA activity. Additional functions include posttran-
496 scriptional modification of transcription factors, regulation of mRNA alternative
497 splicing, regulation of the Pol II transcription machinery, and working as enhancers
498 or super-enhancers of mRNA transcription, and lncRNAs have a central role in the
499 plant-specific RdDM epigenetic machinery [lncRNA roles in plants are reviewed in
500 Chekanova (2015) and Liu et al. (2015b)]. Pol IV and Pol V transcripts are long
501 noncoding RNAs (lncRNAs) essential to the RdDM machinery: Pol IV transcripts
502 are transformed into double-stranded RNA (dsRNA) by RDR2 and broken down
503 into siRNA by DCL3. They direct, through sequence complementarity to Pol V
504 lncRNA, the DNA and histone epigenetic modifiers to their target genome location.
505 Therefore, both types of lncRNA are involved in the direction of the RdDM
506 complex to the genome's target sites. A G to C SNP mutation in the rice lncRNA,
507 long-day-specific male fertility-associated RNA (LDMAR), was sufficient to affect
508 the RNA secondary structure which in turn led to increased methylation in the
509 promoter of LDMAR. Promoter methylation decreased the accumulation of
510 LDMAR levels in anthers under long day condition, thus leading to male sterility
511 (Ding et al. 2012). lncRNAs, like mRNAs, are differentially expressed depending
512 on location and developmental stages, thus when aiming at systematic identification
513 of expressed transcripts, it is important to carefully select the tissues under exam-
514 ination. From RNAseq data generated from rice anthers, pistils, seeds, and shoots,
515 2224 lncRNAs were identified (Zhang et al. 2014). Among them, the authors
516 identified several lncRNAs acting as target mimics for miR160 and miR164. In
517 addition, the T-DNA insertion mutant of lncRNA XLOC_057324 had earlier
518 flowering and low seed set phenotypes, pointing to a role for that lncRNA in the
519 formation of rice panicle and flower fertility. In the rice and maize cereals,
520 lncRNAs are very probably contributing to agronomic traits because the combina-
521 tion of results from Genome Wide Association Studies (GWAS) with the position
522 of about 29,000 lncRNAs in rice and maize showed that 234 SNPs associated with
523 34 morphological, developmental, and agronomical traits were mapping to lncRNA
524 genomic positions (Wang et al. 2015a). In tomato, Wang et al. (2015b) generated
525 RNAseq datasets from control and TYLC virus-infected leaves to identify lncRNAs
526 involved with the defense of tomato against TYLCV. They predicted 1565

lncRNAs as potentially involved in TYLCV infection. Results from differential expression analysis were confirmed by qRT-PCR, and the implication of one lncRNA into the response to TYLCV virus infection was confirmed using a VIGS approach (Zhu et al. 2015), focused on the fruit ripening process. They compared lncRNAs identified in a RNAseq dataset from breaker stage (the transition from green to ripe tomato fruit) in Aisla Craig control plant to a RNAseq dataset from the same ripening stage but from the *ripening inhibitor (rin)* mutant. A total of 3679 lncRNAs were identified in these samples from which 677 were differentially expressed between the two conditions. The involvement of lncRNAs in the ripening transition process was validated for two lncRNAs (SlincRNA 1459 and SlincRNA1840) using the VIGS method to silence the target lncRNAs. VIGS plants for both lncRNAs showed delayed ripening compared to the control, thus validating the function of lncRNA in the ripening process. The mechanisms by which these lncRNAs operate to produce the observed phenotype remain to be deciphered. Using publicly available RNAseq datasets, Wang et al. (2016) looked into the evolution of lncRNAs by analyzing noncoding transcripts in both cultivated *Solanum lycopersicum* and the wild accessions *Solanum pimpinellifolium* and *Solanum pennellii*. The authors identified 413 lncRNAs from *S. lycopersicum* Heinz1706 and confirmed by qRT-PCR that the transcripts they identified match the expression profile of the RNAseq data. The datasets from Heinz1706 used in this study were generated by the tomato genome sequencing consortium. They cover a vast range of tomato tissues: from roots to fruits. Wang et al. (2016) analyzed the expression profile of 413 lncRNAs in developing flowers and fruits and confirmed with qRT-PCR that some lncRNAs are differentially regulated throughout fruit development, thus leading to the hypothesis that lncRNAs are involved in this process.

5 Seed Development

552

Seeds are the keystone of human development. Plants evolved several strategies and a wide range of adaptations to preserve successful germination of its genetic content and to conquer several different environments. Angiosperm seed development initiates with the double fertilization of the megagametophyte, where the pollen tube delivers two haploid sperm cells to the embryo sac. One sperm cell fuses with the haploid egg to generate a diploid embryo, and the other sperm cell fuses with the diploid central cell to form the triploid endosperm. The resulting embryo and endosperm are genetically identical except for their ploidy level with the endosperm having two maternal doses of the genome and one male dosage (reviewed in Bai and Settle 2014). The fertilized egg and central cell go on to form the embryo and the endosperm, respectively, by multiplying and expanding through several cell cycles. Core cell cycle factors play important roles in the regulation of the cell division cycle during seed development and its coordination with cell differentiation and maturation. Diverse aspects of the seed development such as seed dormancy and embryo and endosperm development involve epigenetic

568 mechanisms (Kohler and Makarevich 2006; Wollmann and Berger 2012). Although
569 seed development is regulated through physiological and transcriptional regulation,
570 in this chapter we are going to focus only on the epigenetic aspects of seed
571 development.

572 5.1 Seed Dormancy

573 Seed dormancy is a process that allows germination delay until a favorable envi-
574 ronment arrives. Pieces of evidence for an epigenetic regulation of gene expression
575 in controlling dormancy and germination in cereal seeds have emerged only
576 recently. Genes associated with histone and chromatin structure are overrepre-
577 sented among *loci* transcriptionally induced at the whole-seed level during germi-
578 nation of non-dormant barley seeds, specifically during the phase of late
579 germination (An and Lin 2011). Moreover, the SET family—transcription factors AU5
580 that play role in histone methylation—are consistently expressed in the embryos
581 during germination of non-dormant rice seeds (Malagnac et al. 2002; Xiao et al.
582 2003; Howell et al. 2008). Recent study analyzing the whole wheat seed transcrip- AU6
583 tion showed several genes activated during imbibition of after-ripened samples that
584 were enriched in the chromatin assembly gene ontology (Gao et al. 2012). Such
585 genes include those encoding for histone proteins such as H4, HTA11, HTA12,
586 HTB11, HTB9, and FASCIATA 1 (FAS1), a histone-binding protein, which are
587 important for nucleosome and chromatin formation, and thus gene expression
588 regulation. Furthermore, orthologues of DNA methylation-related genes, including
589 *CHROMOMETHYLASE 3 (CMT3)* and *METHYLTRANSFERASE 1 (MET1)*,
590 exhibit transcriptional induction in embedded, after-ripened dormant seeds. Such
591 findings suggest an epigenetic role in regulating gene expression and modulating
592 after-ripening-induced developmental switch of wheat seeds from dormant to
593 non-dormant state (Fig. 2) (Gao and Ayele 2014). Additional studies are required AU7
594 to identify more dormancy-related epigenetic regulators and define how epigenetic
595 mechanisms are involved in the control of wheat seed dormancy and germination.

596 In *Arabidopsis thaliana*, seed dormancy-specific genes include the *DELAY OF*
597 *GERMINATION (DOG)* family. *DOG1* is expressed in seeds during the maturation
598 stage; the transcript accumulates during seed maturation stage with peaks around
599 14–16 days after pollination (DAP) (Bentsink et al. 2006) and then is
600 downregulated around 20% in freshly harvested seeds, vanishing during imbibition.
601 Loss of function of *DOG1* results in the absence of dormancy (Bentsink et al. 2006).
602 Wheat transcription factor Histone Binding Protein-1b (HBP-1b) displays the
603 highest similarity with *Arabidopsis* *DOG1* (Bentsink et al. 2006). The leucine
604 zipper class transcription factor HBP-1b binds to the H3 hexamer motif ACGTCA
605 in the promoter regions of wheat histone H3 loci (Mikami et al. 1989). This motif is
606 required for transcription of wheat *H3 histone locus* (Nakayama et al. 1989).

607 A suitable candidate for a seed dormancy-imposing gene is *HUB1 (histone*
608 *mono-ubiquitination1)* since *hub1* seeds exhibit reduced dormancy (Liu et al.

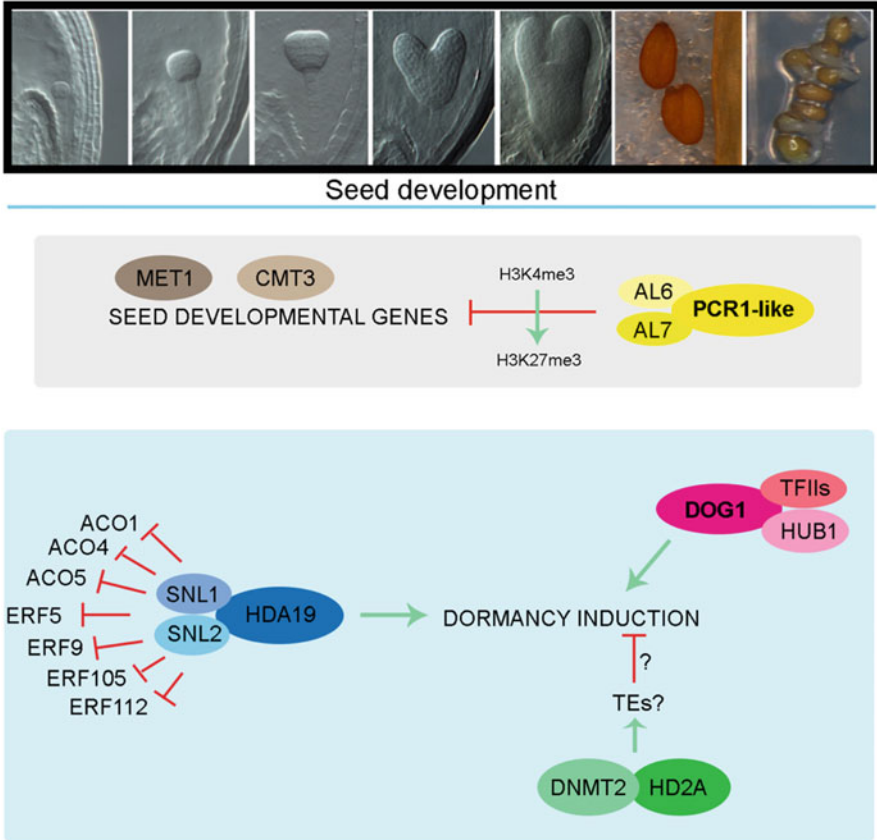


Fig. 2 Epigenetics of *Arabidopsis thaliana* seed development. Embryo developmental stages shown as octant stage, globular stage, early-heart stage, heart stage, torpedo stage, mature dry seed, and imbibed seeds, respectively. *CHROMOMETHYLASE 3* (*CMT3*) and *METHYLTRANSFERASE 1* (*MET1*) regulate developmental seeds genes by methylating DNA during embryogenesis. *AL* (*Alfin1-like*) proteins *AL6* and *AL7* interact with the Polycomb repressive complex 1 (PRC1)-like complex repressing seed developmental genes by switching from active H3K4me3 to inactive H3K27me3 marks of seed genes during seed germination. *DELAY OF GERMINATION1* (*DOG1*) targets *HUB1* (histone mono-ubiquitination 1) and *TFIIS* (transcription elongator factor IIS) controlling seed dormancy. *SIN3-LIKE1* (*SNL1*) and *SNL2* interact with *HDA19* (histone deacetylase19) positively regulating seed dormancy, targeting *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE 1* (*ACO1*), *ACO4*, and *ACO5* and ethylene responsive genes, such as *ETHYLENE RESPONSE FACTOR 9* (*ERF9*), *ERF105*, and *ERF112*, triggering seed dormancy by suppressing the ethylene pathway, affecting positively the seed germination. *HISTONE DEACETYLASE2A* (*HD2A*) and *DNA METHYLTRANSFERASE2* (*DNMT2*) are possibly working together in the germination process affecting early embryogenesis stages

609 2007). *ABA INSENSITIVE 4 (ABI4)*, *DOG1*, *NINE-CIS-EPOXYCAROTENOID*
610 *DIOXYGENASE 9 (NCED9)*, and other genes have been identified as possible
611 targets of *HUB1* (Liu et al. 2007). *TFIIS* (transcription elongator factor IIS) and
612 *HUB1*, two positive regulators of transcription, are induced during the same stages
613 of seed maturation (~18–19 DAP). There is a significant overlap of differentially
614 expressed genes in *tfiis* and *hub1* mutants. This finding suggests that *TFIIS* and
615 *HUB1* might share common targets. One of the genes commonly downregulated in
616 both two mutants is *DOG1* (Liu et al. 2011). Therefore, chromatin remodeling and
617 transcriptional elongation might activate *DOG1* through a primary mechanism for
618 seed dormancy. A recent analysis of the *tfiis* mutant, in which seed dormancy is
619 decreased but returned to the wild-type level by an extra copy of *DOG1*, supports
620 the hypothesis that seed dormancy is controlled by the efficiency of transcription
621 elongation of *DOG1* (Fig. 2) (Mortensen and Grasser 2014). More analyses of the
622 specific targets of epigenetic modification and transcriptional elongation will be
623 necessary to shed more light on seed dormancy regulation through these processes.

624 While activation of dormancy *loci* through transcription elongation seems to be
625 critical for dormancy induction, continuous repression of seed germination-
626 associated genes is perhaps an essential part of dormancy maintenance. There is
627 evidence that histone deacetylation is imperative for repression of loci positively
628 affecting seed germination. In mammals and yeast, histone deacetylase (HDAC)
629 interacts with SWI-INDEPENDENT3 (SIN3), an amphipathic helix-repeat protein,
630 to remove acetyl groups from lysine in the histone tails, creating a transcriptionally
631 inactive state of the chromatin (Lai et al. 2001; Grzenda et al. 2009). In AUB
632 *Arabidopsis*, SIN3-LIKE1 (SNL1) physically interacts with HDA19, an
633 *Arabidopsis* HDAC orthologue, both in vitro and in planta (Wang et al. 2013).
634 The *Arabidopsis* genome contains also *SNL2*, which is partially redundant to *SNL1*.
635 Seeds from *snl1/snl2* double mutant exhibit decreased dormancy. A decreased
636 dormancy phenotype is also observed in *hda19* mutant seeds (Wang et al. 2013).
637 These data imply that *SNLs* and *HDA19* are seed dormancy positive regulators. It
638 seems that suitable repression of SNL-HDA19 complex targets, which are most
639 likely germination-inducing *loci*, through histone deacetylation, is essential for
640 typical seed dormancy. Acetylation of H3K9/18 and H3K14 is increased in the
641 *snl1/snl2* double mutant (Wang et al. 2013), corroborating that in wild-type seeds,
642 the SIN3-HDAC complex deacetylates histones and therefore adds repressive
643 marks on the chromatin (Richon and O'Brien 2002). Global gene expression
644 analysis of *snl1/snl2* double mutant and wild-type seeds identified possible targets
645 for *SNL-HDA19* such as *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXI-*
646 *DASE 1 (ACO1)*, *ACO4*, and *ACO5* and ethylene responsive genes, such as *ETH-*
647 *YLENE RESPONSE FACTOR 9 (ERF9)*, *ERF105*, and *ERF112* (Wang et al. 2013).
648 Quantitative PCR combined with chromatin immunoprecipitation employing
649 H3K9/18 acetylation-specific antibodies confirmed that *ACOs* and *ERFs* genes
650 were indeed hyper-acetylated in the mutant, mostly in the promoter region,
651 although hyperacetylation was also found in coding regions (Wang et al. 2013).
652 These results indicate that *SNL-HDA19* triggers seed dormancy by suppressing the

ethylene pathway, affecting positively the seed germination in *Arabidopsis* 653
(Chiwocha et al. 2005; Arc et al. 2013) (Fig. 2). 654 AU9

Another family of plant histone deacetylase, the HD2s (HD2A, HD2B, HD2C, 655
HD2D), are probably part of the seed dormancy-regulated pathways. HD2A can 656
mediate transcriptional repression (Ueno et al. 2007) and is correlated with early 657
stages of somatic embryo development (Zhou et al. 2005). HD2s and DNMT2 658
(an intriguing enzyme that holds a DNA methylation motif, but methylates specific 659
tRNAs instead) proteins interact in *Arabidopsis* nucleus; then one of the hypothesis 660
is that they are working together in epigenetic pathways, playing role in plant 661
development (Song et al. 2010). Unpublished data from our group on *Arabidopsis* 662
hd2a/dnmt2 double mutants show a consistent difference at the germination and 663
seedling growing rates in the first 48 h compared to wild-type seeds, where double 664
mutant seeds germinate earlier and seedlings grow faster than the wild type. It 665
seems that HD2A and DNMT2 work together, most likely as a complex (Song et al. 666
2010), in the germination process affecting male gametogenesis and/or early 667
embryogenesis stages (Alves 2015). This conclusion is supported by data on 668
mice, suggesting that DNMT2 is required for male gametogenesis (Kiani et al. 669
2013). Furthermore, HD2A is already known to be a seed germination and fine-tune 670
growing regulator in *Arabidopsis* (Zhou et al. 2004; Colville et al. 2011). Further 671
studies are necessary to unravel the epigenetic mechanisms by which DNMT2- 672
HD2A complex regulates seed germination and early seedling development. 673

Histone modification may be partially inherited through cell division and epige- 674
netic reprogramming should take place at fertilization, but these processes remain 675
far from being completely understood. DNMT2 targets transcripts other than 676
tRNAs (Alves 2015), and it could work as a cofactor together with HD2A to 677
remove histone acetylation and, as a result, some *loci* that should be repressed 678
during seed germination may be activated or partially activated in the *hd2a/dnmt2* 679
Arabidopsis double mutant. Moreover, methylome profiling of pollen indicates 680
dynamic DNA methylation changes during male gametogenesis, but information 681
regarding methylation enzymes acting at this stage is unknown. Non-CG methyl- 682
ation increases in pollen vegetative cells on transposable element (TE)-rich 683
regions, probably to regulate these elements (Borges et al. 2012). Normally, LTR 684 AU10
(long terminal repeats) retrotransposons are regulated by RdDM pathway. Low 685
levels of siRNAs associated with LTR retrotransposons were found in the pollen 686
vegetative cell (Slotkin et al. 2009). However, 21-nucleotide (nt) siRNAs are found 687
at this stage and not 24-nt siRNAs. The current model is that noncanonical RdDM 688
pathways take place at this stage to control these elements. 689

5.2 Embryo–Endosperm Interaction 690

In *Arabidopsis* endosperm, genes and TEs are regulated by both DNA and histone 691
(H3K37me3) methylation (Schmidt et al. 2013), with substantial variation between 692
endosperm and embryo tissues (Gehring et al. 2009). In rice, endosperm 693

694 hypomethylation occurs in all sequence contexts (CG and non-CG), although CG
695 methylation is not similar to CHG and CHH, which are hypomethylated similarly
696 across the genome (Zemach et al. 2010). For seed viability, MET1 and CMT3
697 activity is required during *Arabidopsis* embryogenesis (Xiao et al. 2006), leading to
698 preferential maternal hypomethylation in the endosperm, while paternal methylated
699 alleles are maintained. However, the function of the remaining methylated *loci* is
700 largely unknown (Zhang and Xue 2013).

AU11

701 Epigenetic regulation is crucial for leading tissue differentiation into distinct
702 *primordium* cell lineages and driving inheritance of each transcriptional
703 programmed through mitosis at early stages of embryo development (Bantignies
704 and Cavalli 2006). In the egg cell, MET1 is expressed following gametogenesis, yet
705 the developing embryo, endosperm, and seed coat also contain its transcripts
706 (Schmidt et al. 2013). The jmjC domain-containing histone demethylase from
707 *BcJMj30* in *Brassica rapa* is associated with pollen development and fertilization
708 (Li et al. 2012). Although *Helianthus LEAFY COTYLEDON1-LIKE (HaLIL)* is
709 involved in early stages of zygotic and somatic embryogenesis, with multiplexed
710 transcriptional regulation by DNA methylation, TFs (Transcription Factors), auxin,
711 and ABA (Salvini et al. 2012).

712 The *MATERNALLY EXPRESSED LOCUS 1 (MEG1)* in maize is expressed only
713 in the basal nutrient transfer region of the endosperm (Gutiérrez-Marcos et al.
714 2004), where the genomic imprinting (an epigenetic event that silence one allele
715 from one of the parents; see next section) of *MEG1* supports nutrient transfer from
716 endosperm to the newly developing embryo (Costa et al. 2012). AL (Alfin1-like)
717 proteins are PHD-containing proteins, and there are seven AL proteins in
718 *Arabidopsis*, identified by AL1–AL7 (Lee et al. 2009; Molitor et al. 2014). The
719 AL proteins are named following their homologue Alfin1 in alfalfa, which partic-
720 ipates in salt tolerance (Winicov 2000). Functional studies have uncovered that
721 AL6 and AL7 interact with the Polycomb repressive complex 1 (PRC1)-like
722 complex (a complex that contains Polycomb group-like/PcG-like proteins respon-
723 sible for the methylation of Lys 27 of histone H3 (Deleris et al. 2012), in
724 *Arabidopsis* to repress seed developmental genes by switching from active
725 H3K4me3 to inactive H3K27me3 marks of seed genes during seed germination
726 and early seedling growth. Also, this function depends on the interaction between
727 AL6/AL7 and H3K4me3 (Molitor et al. 2014). A delay in seed germination under
728 osmotic treatments but not under normal conditions is manifested in AL6 and AL7
729 double mutants, in agreement with the function of their homologue Alfin1 (Molitor
730 et al. 2014; Winicov 2000). However, the single mutants of *al6* or *al7* show a
731 normal phenotype under any conditions, indicating that AL6 and AL7 act redun-
732 dantly in seed germination (Molitor et al. 2014).

AU12

5.3 Genomic Imprinting

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Imprinting is an epigenetic phenomenon by which one of the alleles is silenced through methylation and histone modification mechanisms (Raissig et al. 2011). Imprinted alleles can be inherited maternally or paternally, and the imprinting takes place at the germline and is maintained through mitosis in somatic cells. It is an important mechanism to ensure the correct information transmission to the offspring. Imprinting also guarantees that TEs stay epigenetically silenced during reprogramming of plant gametogenesis, facilitating seed germination events (Wollmann and Berger 2012). Exposure to pathogens can initiate differential 5mC (5-methylcytosine) patterning, activating *NON-EXPRESSOR OF PR GENE* (*NPR1*), a defense regulatory gene (Downen et al. 2012; Luna and Ton 2012). The transgenerational genomic imprinting of *NPR1* is probably due to posttranslational histone modifications and expression of RNA Polymerase V acting along with siRNAs to recruit methylation machinery (Luna and Ton 2012; You et al. 2013). In developing embryo genomic imprinting, sRNAs produced maternally in plant reproductive tissue can be mobile and may target specific genes, providing the first evidence for a link between genomic imprinting and RNA silencing in plants (Gutierrez-Marcos et al. 2012; Mosher et al. 2009). A variety of microRNAs, including at least four associated with nutrient homeostasis (miR169, miR395, miR398, and miR399), are indeed mobile and graft transmissible and detected in the phloem (Marin-Gonzalez and Suarez-Lopez 2012). These findings reinforced that maternally produced small RNAs (miRNAs or siRNAs) may be present in the next generation. sRNA-based regulation found in fertilization can take place during seed maturation and possibly during seed dormancy as well (Mosher et al. 2009). It is possible that the female sRNAs may influence gene regulation during germination to assist seedling establishment. This female-specific genomic imprinting mechanism may have evolved from the advantage fitness from the maternal niche germinated offspring (Gorecki et al. 2012). Throughout plant embryogenesis, hypomethylation is less prominent when compared to mammalian systems, with a higher proportion of parental DNA methylation events carried to the following generation (Reinders et al. 2009). An example is the different methylated profile in the *Arabidopsis PHE1* (*PHERES1*), in which the male *PHE1* allele is methylated, and the female allele is hypomethylated (Kohler and Makarevich 2006; Makarevich et al. 2008).

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A few years back, plant imprinting was believed to occur only in the triploid endosperm, so, gymnosperms were presumed to lack imprinting mechanisms (Garnier et al. 2008). Nonetheless, more recently, it has been shown that genomic imprinting can occur in angiosperm and gymnosperm embryos (Scholten 2010). The exposure to different temperatures during embryo development can store epigenetic memory during embryogenesis, fixing epigenetic marks before seed maturation, leading to modified germination time and seedling development in the gymnosperm Norway spruce (Yakovlev et al. 2010). The epigenetic memory in long-lived plant species may confer adaptive plasticity to environmental drift in a

776 single generation, with significant consequences for perennial and clonally propa-
777 gated crops (Bloomfield et al. 2014).

778 6 Conclusions and Future Prospects

779 The epigenetic mechanism most amenable to plant breeding programs is gene
780 expression regulation through DNA methylation. Epigenetic variability in crop
781 plants can be either induced through chemical treatment using methyltransferase
782 inhibitors or induced by exposure to specific stressful growing environments.
783 Individual plants showing desired phenotypes as well as stable methylation profiles
784 can be selected to be part of selection programs (Rodriguez Lopez and Wilkinson
785 2015). Thus, fixed epimutations as described for the *Cnr* mutant are not the only
786 source of epigenetic diversity. Using a population of isogenic *Arabidopsis* lines that
787 segregate for differentially methylated regions, Cortijo et al. (2014) showed that
788 two agronomical traits, flowering time and primary root length, are controlled by
789 epigenetic quantitative trait loci. Thus, forward epigenetic approaches could be
790 actually implemented as part of crop breeding for the improvement of significant
791 traits.

792 The *MutS* HOMOLOGUE1 (*MSH1*) gene is known in *Arabidopsis* to influence
793 plant growth behavior. *msh1* mutant plants have affected vigor and development
794 reprogramming linked to altered genome methylation. The increased plant vigor is
795 characterized by rapid growth and earlier flowering, and greater aboveground
796 biomass was also identified in tomato plants silenced for the *MSH1* gene (Yang
797 et al. 2015). In tomato, the most relevant phenotype in a plant breeding perspective
798 was the increased flower and fruit set that resulted in increased yield. *MSH1* RNAi
799 plants were crossed with the wild-type Rutgers parent and $-/-$ plants not carrying
800 the RNAi transgene were selected as epi-lines. The enhanced hybrid vigor and fruit
801 yield was increased until the epiF4 generation. Such heritable increased methyl-
802 ation in *msh1* plants was observed in multiple plant species (*Arabidopsis*, tomato,
803 sorghum). This work, together with the studies demonstrating the role of methyl-
804 ation status of ripening genes, proves the relevance of considering the methylome
805 as part of breeding programs.

806 Methylations and miRNAs are both involved in the regulation of the tomato fruit
807 ripening process. Another group of epigenetic regulators involved in relevant traits
808 is the lncRNAs. Zhu et al. (2015) showed that lncRNAs are another layer of
809 regulation to the ripening process. In addition, lncRNAs are involved in tomato
810 defense against pathogens such as TYLCV (Wang et al. 2015b) and *phytophthora*
811 *infestans* (Cui et al. 2016). Together, the works on the identification of lncRNAs
812 implicated in plant quality traits and disease resistance traits have permitted the
813 identification of thousands of lncRNAs in a large array of tissues, developmental
814 stages, and disease treatments. lncRNAs, as genes, are being mapped to the genome
815 sequence and, therefore like genes, can and should be looked at in association
816 studies, forward and reverse genetic approaches. Discovery of tomato lncRNAs is

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at its early stage and much of their functions remains to be identified. One particular type of lncRNA is of interest for future fundamental and applied studies: lncRNAs coding for micropeptides (miPEPs). In *Medicago truncatula* and *Arabidopsis thaliana*, miPEPs coded by pri-miRNA of miR171b and miR165a are involved in root development through positive feedback regulation of their own pri-miRNAs (Laressergues et al. 2015). Treatment with 0.1 μM of miPEP172c increased soybean root nodulation through the stimulation of miR172c and thus AP2 downregulation (Couzigou et al. 2016). This illustrates the potential of this novel type of epigenetic regulator to regulate agronomical traits and to be able to regulate gene networks through a simple treatment with a synthetic micropeptide.

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