

Chapter 15

Epigenetics in Forest Trees: Keep Calm and Carry On



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Abstract Trees are sessile and long-lived organism so they have to rapidly adapt to dynamic and unfavorable environments (drought, soil salinity, heat, ...) for ensure their survival. Acclimation is mostly related to epigenetic regulation mechanisms that act responding to environmental stimuli and thus regulating gene expression during leaf development, floral transition, bud dormancy, and climate change induced abiotic stress response. Also, environmental stresses have been related to the transgenerational inheritance of epigenetic marks, called epigenetic memory. Epigenetic variation complements natural genetic variation as a source of phenotypic and functional diversity in plants, resulting in a phenotypic plasticity including also traits of transgenerational inheritance. This chapter provide an overview about how epigenetic mechanisms act, the memory role and new epi-variates definition that combined will help us to create new biotechnological tools for forest trees productivity improvement.

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15.1 Introduction

Trees' long lifespan, growth in dynamic environments, and sessility make their development influenced by both biotic and abiotic factors. Contrary to annual plants, in most latitudes trees must survive to winter colds and to summer droughts in a loop that can last for thousands of years (think in some pines, firs, or junipers). During this time trees encounter unfavorable environmental situations such as temperature increasing, altered precipitation regime, soil salinity, and high light intensity due to climate change with the impossibility to move to more favorable environments. In consequence, they have to be able to adapt rapidly to new conditions to ensure their survival. The integration of external stimuli, which should be reflected in a reprogramming of gene expression that leads to adaptation, can follow different pathways but in last term they are coordinated by a differential epigenetic modulation (Köhler and Springer 2017).

This modulation is also coordinated with the change of stress-responsive genes expression, becoming a crucial process for responding to environmental stimuli and regulating cell growth and differentiation (Chinnusamy et al. 2014). DNA methylation and histone post-translational modifications (PTMs) have been revealed as key mechanisms for controlling chromatin structure and function (Kouzarides 2007) and regulating cell growth and differentiation (Valledor et al. 2007; De Carvalho et al. 2010; Feng et al. 2010; Bräutigam et al. 2013; Lafon-Placette et al. 2013). Furthermore, the role of small RNAs is getting more importance for modulating epigenetic responses. This regulation is composed of a highly interactive network of sRNA-directed DNA methylation, histone, and chromatin modifications, all of which control transcription (Simon and Meyers 2011). These mechanisms are dynamic and can be reverted or adapted to particular environmental situations for maintaining gene and genome activities (Kim et al. 2015a), constituting a link between genotype and phenotype (Schmitz and Ecker 2012). The study of global changes in DNA methylation or specific histone PTMs has allowed the characterization and monitoring of several processes such as flower development (Zluvova et al. 2001; Meijon et al. 2010), or stress response (Chinnusamy and Zhu 2009; Correia et al. 2013). Furthermore it was recently discussed how epigenetic variation complements natural genetic variation as a source of phenotypic and functional variation in plants (Kawakatsu et al. 2016; Slotkin 2016).

In the current context of climate change, the study and understanding of the stress response and acclimation mechanisms in plants became mandatory for their stress response improvement. Principal physiological mechanisms in response to stress and later adaptation have been described in many forestry species. Good examples of well-described physiological stress response and acclimation are heat and UV stress response in *Pinus radiata* (Escandón et al. 2017, 2018; Pascual et al. 2017) and drought and heat stress response in *Eucalyptus globulus* (Jesus et al. 2015; Correia et al. 2018). In contrast, the regulation of these processes at chromatin level and those mechanisms that are implied in long-term stress responses and acclimation are still poorly described in most cases.

The recent characterization of some environmental signals that influence on epigenetic marks to control, i.e., flowering, and on the resultant changes in phenotype as a consequence of gene expression has raised a significant interest in stress-responsive epigenetic mechanisms. In addition, environmental stresses can occur repeatedly, so it has been suggested that plants have a stress memory supporting the stress adaptation. Epigenetic memory is based on the defense priming by playing a more rapid and stronger response to abiotic stress (Kinoshita and Seki 2014; Lämke and Bäurle 2017). Stress memory is still supported by few researches in forestry species (Le Gac et al. 2018), and it has been proposed as a way to improve environmental stress adaptation of cultivars (Springer and Schmitz 2017). Knowing these epigenetic marks will provide important information about how natural populations will survive in the current climate change context. Nevertheless, forests are composed by genetic and environmentally heterogeneous populations that make the epigenetic underlying mechanisms poorly understood (Bräutigam et al. 2013).

Environmentally generated epigenetic variation has gained increasing attention over the last years as one of the main sources of quick phenotypic variation and evolutionary change. This variation is closely related to the epigenetic memory, the transgenerational inheritance of epigenetic marks, discussed long-time by scientific community but a widely accepted fact nowadays. Although the underlying mechanisms in natural populations are still poorly understood even in model species because of the difficulty of characterizing epigenetics in genetically and environmentally heterogeneous populations, some advances have been recently achieved in forest species. This chapter provides some of the last advances in forest epigenetic mechanisms and memory, and its implications as potential new tools for plant breeding and conservation as a way to select or induce new epi-varieties adapted to changing ecosystems.

15.2 Epigenetic Regulation in Plant Development and Environmental Responses

15.2.1 Epigenetics Implications in Tree Leaf Development

Plants are subjected to a series of transitions in their development cycle. Three clearly defined stages are embryonic, postembryonic, and growth. The latter is characterized by different patterns of growth in terms of differentiation of cells, organs (heteroblasty), and the gain of physiological competence. The heteroblasty has been described for woody species of the genus *Pinus* (Climent et al. 2006), *Acacia* (Forster and Bonser 2009), and *Eucalyptus* (James and Bell 2001). The most described type of heteroblastic variation is the change of the vegetative phase (Poethig 1990), which implies changes in the characteristics of leaves, stems, and buds (Rasmussen 1986).

In recent years, the mechanisms implied in the regulation of the leaf juvenile-to-adult transition have been discovered in model herbaceous species *Arabidopsis* (Wilson-Sánchez et al. 2014) and some of their players were uncovered also in forest trees, a first step for understanding the control of this essential developmental event in the life cycle of plants.

The role of DNA methylation has been reported in different tree systems and developmental stages. As a general rule DNA methylation levels increase with age in meristematic regions, while juvenile meristems are less methylated than adults, as it was described in *Prunus persica* (Bitonti et al. 2002) or *Pinus radiata* (Fraga et al. 2002). Vegetative to reproductive phase change seemed to be the triggering point for increasing DNA methylation in these species. Developing *Pinus radiata* needles also showed a lower DNA methylation level compared to mature needles (Valledor et al. 2010). Epigenetic marks of specific developmental stages have been reported for promoters of key genes implied in needle development (Valledor et al. 2015). In *Arabidopsis*, the changes in its epigenome during leaf development were clearly identified, and also the effects of knocking out methyltransferases (*met1* and *drm1 drm2 cmt3*) in 5 weeks (Zhang and Jacobsen 2006) and wild plants of 25 days

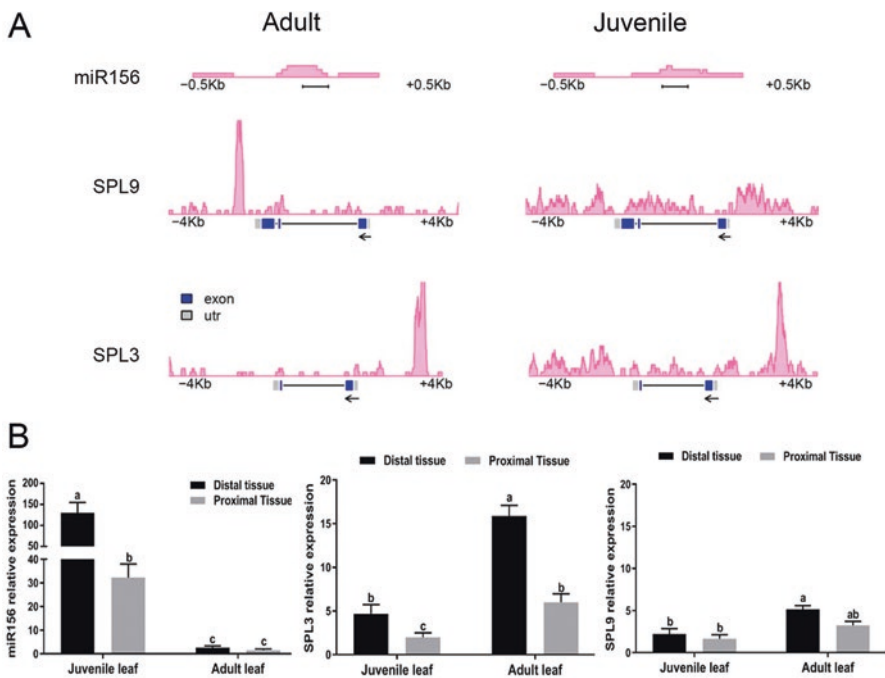


Fig. 15.1 (a) Methylation pattern at vegetative phase control genes. Normalized signal plots (pink, MEDIP) for adult (left) and juvenile (right) leaves. Arrows indicate direction of transcription. (b) Expression levels of miR156, SPL3, and SPL9 in juvenile and adult leaves of *E. globulus*, distal and proximal zones. Expression was normalized with miR171a (miR156) or EF1a and actin (SPL3 and SPL9). Averages of three replicates and three trees are shown for each tissue, two-way ANOVA

(Zilberman et al. 2007) identifying the differential methylation of SQUAMOSA PROMOTER PROTEIN-LIKE SPL10. These results suggest that the genes of the pathway miR156-SPL could be regulated by DNA methylation (Xu et al. 2018).

In *Eucalyptus globulus*, the levels of transcribed miR156 (associated with juvenility) were reduced during phase transition, with an increase of the transcriptional levels of SPL3 and SPL9, involved in adult traits. The comparison of the transcription expression profiles with DNA methylation profiles (Hasbún et al. 2016), both in adult and juvenile leaves, revealed a correlation between methylation of putative promoter regions and transcript abundance. Methylation levels on juvenile tissues correlated with lower transcription levels of the SPL3 and SPL9 genes (Iturra 2018) (Fig. 15.1).

Conversely the epigenetic mechanism that has been addressed in more reports is the modifications of histones. In Arabidopsis, an increase in the trimethylation of the histone H3 lysine 27 (H3K27me3), mediated by the chromatin remodeler CHD3 PICKLE (PKL) (Zhang et al. 2008), could contribute to the addition of H3K27me3 to genes miR156. This associated with a temporary decrease in the acetylation of H3K27 would contribute to the decrease of miR156 (Xu et al. 2015). Considering the developing *Pinus radiata* needle, primordia show a greater abundance of marks related to the expression of euchromatin genes, such as AcH3 or H4K4me3. These marks are progressively lost during needle development and replaced for repressive marks such as H4K9me3 (Valledor et al. 2010). Furthermore, these marks can be associated with gene specific methylation status and gene expression (Fig. 15.2).

In Arabidopsis, a decrease in the transcription of miR156 is related to an increase in the histone mark H3K27me3 and a decrease in the mark H3K27ac in the region after the beginning of the transcription (TSS) (Xu et al. 2016b, c). It is also reported that a decrease in the monoubiquitination of histone 2a (H2Aub) and H3K27me3 in the TSS region of miR156 prolongs the juvenile phase (Picó et al. 2015). Arabidopsis H3K4 methyltransferase TRITHORAX7 (ATXR7) joins a region adjacent to the TSS of miR156 and deposits the H3K4me3, which activates the transcription miR156 (Xu et al. 2017). The ambient factors (light and photoperiod) play an important role in the regulation of the genes SPLs and in the induction of miR156, by means of the acetylation of acetyltransferases of histones (HAT) of the type Spt-Ada-Gcn5-acetyltransferase (SAGA), they could be an important aspect in the transition of vegetative phase. This mechanism of control is prior to the post-transcriptional regulation mediated by miR156 in the phase change (Kim et al. 2015b).

The mechanism associated with chromatin remodeling has been addressed in recent years. It has been described that the transcription of miR156 is promoted by the remodeler nucleosomal BRAHMA (Xu et al. 2016c) and the complex SWR1, which exchanges the variant of histone H2A.Z by H2A (Choi et al. 2016). This indicates that H2A.Z promotes juvenile vegetative identity, and would also promote the expression of MIR156 by facilitating the deposition of H3K4me3 (Xu et al. 2017). The remodeling protein of nucleosomes PICKLE (PKL) is associated with the remodeling and deacetylation complexes of nucleosome (Ho et al. 2013), which binds to the adjacent TSS region of MIR156 by repressing its transcription (Xu et al. 2016b).

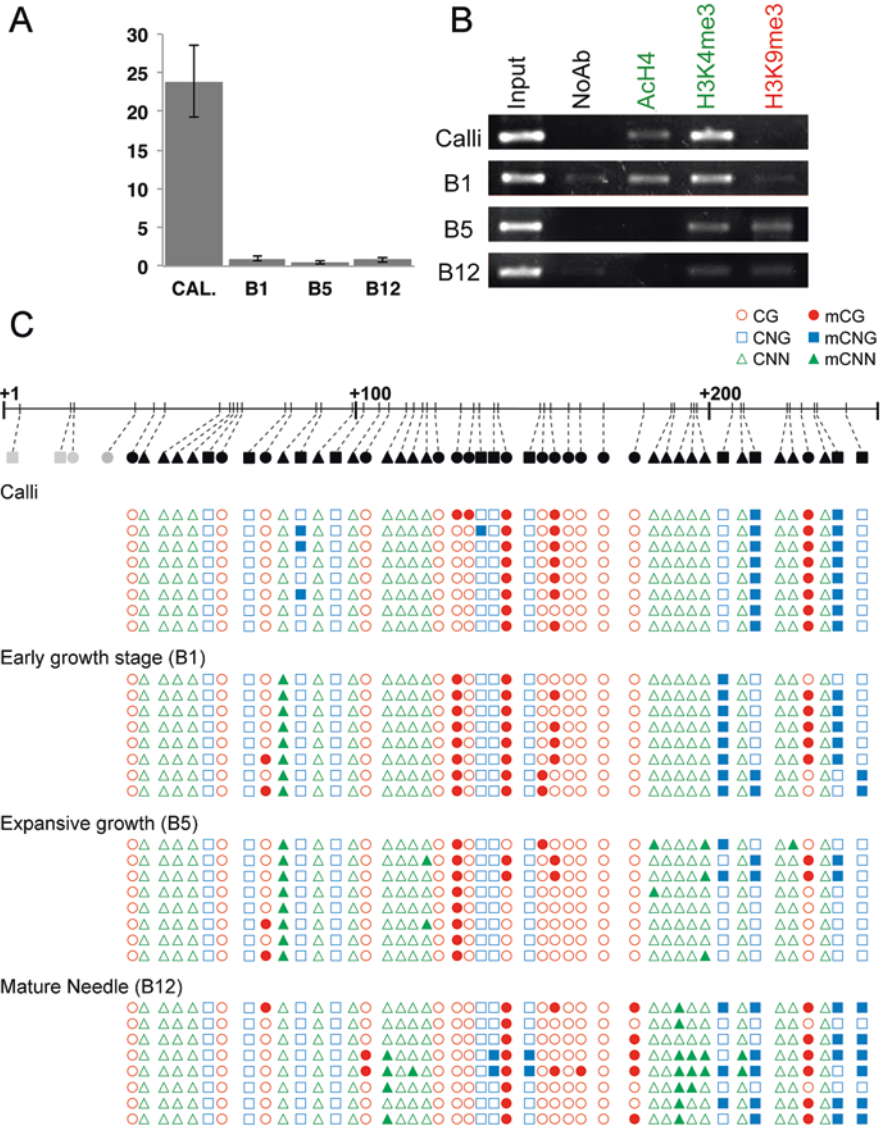


Fig. 15.2 (a) Expression level of *Pinus radiata* *CSDP2* gene in four developmental stages (cal., calli; B1 leaf primordia; B5 needles in expansive growth; B12 mature needles). (b) Chromatin immunoprecipitation of promoter region of *CSDP2* in the same tissues. Two marks associated with gene expression (AcH4 and H3K4me3) and one to repression (H3K9me3) have been tested. Amplification band represents the presence of this mark in the analyzed region. (c) Site specific DNA methylation of promoter region of analyzed gene. All cytosines were analyzed, CG were represented as circles, CNG as squares, and CNN as triangles. Filled symbols represent a mC. This figure was adapted from Valledor et al. (2015)

15.2.2 *Epigenetic Regulation of Floral Transition*

Transition from vegetative to floral buds is a critical physiological change during plant development that determines the survival of the flowering species. Floral transition is achieved through a complex genetic network and regulated by multiple environmental and endogenous cues. A striking example of how epigenome reacts to environment involves the induction of flowering by exposure to low winter temperatures in *Arabidopsis* and many other flowering plants. These epigenetic modifications include DNA methylation, histone modifications, and the production of small RNA (sRNA) that mediate epigenetic modifications (Hepworth and Dean 2015).

Dynamic changes between chromatin states facilitating or inhibiting DNA transcription regulate the expression of floral induction pathways in response to environmental and developmental signals. The regulation of the *FLOWERING LOCUS C (FLC)* in *Arabidopsis* shows how chromatin-modifying systems have emerged as important components in the control of transition to flowering. Genetic and molecular studies have revealed three systems of *FLC* regulation: vernalization, the autonomous pathway, and *FRIGIDA (FRI)*. All these involve changes in the state of *FLC* chromatin by DNA methylation and/or histone modification (Farrona et al. 2008; Hepworth and Dean 2015). Histone methylation participates in repression of expression of inhibitor of flowering FLC during cold. This epigenetic change is mediated by a conserved repressive complex, POLYCOMB REPRESSIVE COMPLEX 2 (PRC2). An intronic non-coding RNA, called COLD ASSISTED INTRONIC NON-CODING RNA (COLDAIR), is required for the vernalization-mediated epigenetic repression of *FLC*. COLDAIR physically associates with PRC2 and targets PRC2 to FLC (Heo and Sung 2011). In annual species, such as *Arabidopsis*, this histone methylation is stably inherited through mitosis after returning from cold to warm temperatures allowing the plant to flower continuously during spring and summer until it senesces. However, in perennial species, histone modifications rapidly disappear when temperatures rise, allowing expression of the floral inhibitor to increase and limiting flowering to a short interval. In this case, epigenetic histone modifications control a key adaptive trait, and their pattern changes rapidly during evolution associated with life-history strategy (Turck and Coupland 2014). In perennial and woody species, such as *Azalea (Rhododendron sp)*, Meijón et al. 2010 showed that DNA methylation and histone H4 acetylation (Fig. 15.3a) have opposite and particular dynamics in the apical buds during the transition from vegetative to reproductive phase. The description of the global DNA methylation and histone H4 acetylation levels and immunodetection of 5-mdC and AcH4 in addition to a morphological study have delimited four basic phases in the development of the azalea bud identifying a stage of epigenetic reprogramming which showed a sharp decrease of whole DNA methylation (Fig. 15.3b). DNA methylation and histone modifications have been revealed as hallmarks that establish the functional status of chromatin domains and confer the flexibility of tran-

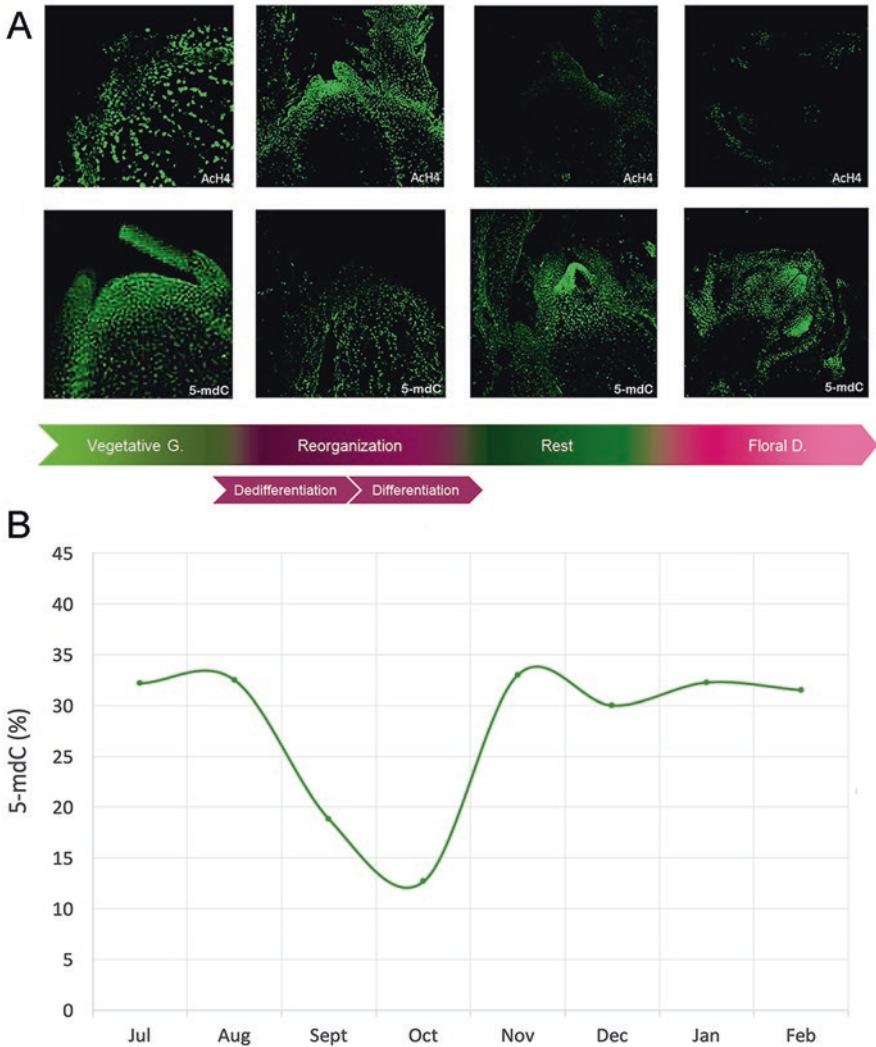


Fig. 15.3 (a) Immunodetection of 5-mdC and AcH4 along annual azalea bud development and (b) their global levels of DNA methylation (5-mdC %) from July to February. Adapted from Meijón et al. (2010)

scriptural regulation necessary for plant development and adaptive responses to the environment (Hepworth and Dean 2015).

In the last years, a crucial link between the epigenetic modifications associated with floral transition and those associated with stress tolerance was found (Yaish et al. 2011). Plants interact with their environment and accordingly modify their flowering programs. This research showed that plants use common and parallel epigenetic modification pathways in order to modify the expression of genes that are

involved in stress tolerance and flowering processes. Together these changes underlie intricate mechanisms that ensure plant survival and optimize reproductive success under a variety of stress conditions (Yaish et al. 2011).

On the other hand it has been recently demonstrated that these epigenetic modifications may also provide a mechanistic basis for a stress memory, enabling plants to respond more efficiently to recurring stress or even to prepare their offspring for potential future assaults (Lämke and Bäurle 2017).

15.2.3 *Bud Dormancy*

Bud dormancy is an adaptive mechanism allowing plants to endure periodic/seasonal chilling or drought in a quiescent status (de la Fuente et al. 2015). This response adjusts to seasonal variation through the perception of change into different environmental variables (Shim et al. 2014). Irradiance is the inducing factor for tropical species (Borchert et al. 2015), while for temperate ones it is the photoperiod and/or temperature (Lloret et al. 2018). This process is best characterized into temperate species where short days and/or temperature change induces bud set, growth arrest, and final endodormancy (Cooke et al. 2012). Moreover, low temperature exposition time and degree mediate these species endodormancy break and their variation affects phenological traits and productivity (Kumar et al. 2017).

Based on the same triggering stimuli, signaling system after bud dormancy holds onto different elements. Between these, phytohormones (Chao et al. 2017) and developmental factors such as *FLOWERING LOCUS T (FT)* in angiosperms (Porto et al. 2015; Maurya and Bhalerao 2017), and *FLOWERING LOCUS T/TERMINAL FLOWER1* like (*FTLI*) in gymnosperms (Gyllenstrand et al. 2007; Klintenas et al. 2012; Carneros et al. 2017) stand out. These genes are the most representative elements into temperature and day length dependent vegetative growth control, bud set and for angiosperms also floral induction control (Gyllenstrand et al. 2007; Hsu et al. 2011; Maurya and Bhalerao 2017; Chen et al. 2018). Bud endodormancy is *FT/FTLI* related (Hsu et al. 2011; Avia et al. 2014) but also with MADS-box *FLOWERING LOCUS C (FLC)* like genes known as *DORMANCY ASSOCIATED MADS-BOX (DAM)* genes. *FLC* controls flower repression (Gu et al. 2013) and vernalization (Bastow et al. 2004) in *Arabidopsis*. Likewise, some related *DAM* genes expression correlates with dormancy imposition and its vernalization like chilling dependent break in forest species (Lloret et al. 2018).

Environment induced fluctuations on the aforementioned genes along the bud developmental process imply the differential regulation of large gene sets (Porto et al. 2015; Chao et al. 2017) and a concomitant proteome (Xu et al. 2016a) and metabolome (Michailidis et al. 2018) reshape. In the previous section the epigenetics mechanisms that regulate bud phase change have been described; however, histone fold status might be also involved into the dormancy break required chilling sensing (Friedrich et al. 2018). Dormancy induction is associated with a global increase in repressive DNA methylation (Santamaría et al. 2009) and generally

repressive histone modifications with some histone deacetylases, methylases, and ubiquitinases upregulating on this stage (Maurya and Bhalerao 2017). Conversely, dormancy break is related to DNA methylation decrease and histone acetylation increase, correlating with DNA methyltransferases downregulation and histone acetylases upregulation (Santamaría et al. 2009; Kumar et al. 2016). Breaking this general scheme, histone acetylases as chestnut *GCN5L* upregulate under bud dormancy (Santamaría et al. 2011) and histone deacetylase genes upregulate during dormancy break pointing to the existence of specific epigenetic regulation mechanisms over regulatory genes. As an example of both this system specificity and epigenetic control over bud dormancy, some FLC-like DAM genes develop a promoter focused enrichment in H3K27me3 marks towards dormancy break allowing its silencing during the growth phase (de la Fuente et al. 2015). This resembles FLC repression upon chilling exposition during vernalization in *Arabidopsis* (Bastow et al. 2004). Interestingly, dormancy related DNA methylation has shown their chilling intensity sensitiveness. In apple, dormancy break is linked to a more intense demethylation after high chilling than after low chilling, and related to productivity and fruit quality (Kumar et al. 2016, 2017).

Although this developmental process shares multiple elements between species, there are also multiple evidences of its variability. Most of these changes can be addressed to genotypical variation, from profound changes as those separating gymnosperms and angiosperms bud development regulation to small ones like the latitudinal differences in FT-like *PaFT4* mediated growth arrest and bud set regulation in Norway spruce populations (Gyllenstrand et al. 2007). Besides this, epigenetic mechanisms are behind observed differences in some cases. *Picea abies* have shown the capacity to change its bud phenology upon environmental conditions during embryonic development through differential and stable epigenetic regulation of FT-like *PaFT2* between other targets (Carneros et al. 2017). miRNA mediated epigenetic regulation would be after this temperature dependent epigenetic tuning of *Picea* bud phenology (Yakovlev and Fossdal 2017). Moreover, methylation pattern and methyltransferases coding genes expression have been found differential between different *Pinus sylvestris* ecotypes and related to embryogenic environmental conditions (Alakärppä et al. 2018).

15.2.4 Epigenetic Regulation in a Changing Environment

Through this chapter we have shown the link between the different environmental inputs plants can sense and how they regulate gene expression by inducing epigenetic changes. These environmental inputs have slowly changed for thousands of years, and plants have evolved upon their changes. However, the negative effects of climate change in the form of altered temperatures and rains can be considered especially worrying for forest outcome, since these climatic variations are happening in a very short timeframe, impeding species to fully adapt. Thus, description of the mechanisms underlying bud set, dormancy, and dormancy release is mandatory

to identify key elements into this process allowing plants to tune their phenotype to a changing environment. The easiness, specificity, stability, or inheritance, and relation to bud dormancy make epigenetics mechanisms a promising place to start. Understanding and tuning bud phenology would enable us to prepare for and avoid climate associated problems selecting/engineering tolerant genotypes. The potential generation of epitypes or the directed selection of natural variants would also increase plant biomass production or release some crops from their phenological boundaries.

Besides representing a key mechanism during growth and developmental processes, adjustment and control of gene expression is especially important when plants are subjected to unavoidable environmental stressors (Yaish 2017). The phenotypic plasticity offered by reversible epigenetic marks constitutes an essential factor especially considering the adaptive capacity of long-lived organisms such as woody plants and the framework of rapid climate change (Plomion et al. 2016). Since 2014, when the main studies dealing with epigenetic alterations occurring in important tree species were reviewed by our group (Pascual et al. 2014), many others have been conducted. The advances in next-generation sequencing technologies have considerably boosted the research focusing on the involvement of epigenetic alterations to stress conditions. Most of the available studies are, therefore, conducted on species/genus with a sequenced genome. For example, *Populus trichocarpa* genome was the first sequenced tree genome, and most of the studies considering the subject of epigenetic alterations due to environmental stress were conducted with *Populus spp.*

The first single-base resolution methylome of *Populus trichocarpa* under control and drought stress conditions (Liang et al. 2014) revealed that the methylation levels of cytosines, upstream 2kp, downstream 2kb, and in repetitive sequences significantly increased after drought treatment. They suggested that DNA methylation may not be associated with cis-splicing but rather with trans-splicing and found a relation in transcription factors with transposable elements showing reduced methylation and expression levels or increased methylation and expression levels after drought treatment.

A different group examined the involvement of epigenetic mechanisms in phenotypic plasticity towards soil water availability in *Populus × euramericana* employing microarray chips (Lafon-Placette et al. 2018). Several regions were identified for each water regime and associated with differential gene expression. Interestingly, highest variations of both gene expression and DNA methylation were associated with rewatering. These authors observed that changes in methylation were particularly in the body of expressed genes and to a lesser extent in transposable elements, revealing that phenotypic plasticity was accompanied by coordinated variations in DNA methylation, gene expression, and specific genes involved in hormone pathways (Lafon-Placette et al. 2018).

An acute drought stress (7 and 11 days after water withholding) and relief (2 h and 3 days after rewatering) were monitored in leaves of *Eucalyptus globulus* by quantifying several biochemical markers of oxidative stress and DNA methylation patterns (Correia et al. 2016). Water withholding imposed a mild oxidative stress, an

increased global 5-methylcytosine distribution, and a high number of specific demethylation events, while rehydration showed a decreased global DNA methylation and lipid peroxidation shortly after 2 h (Correia et al. 2016). These results showed a parallel induction of redox and complex DNA methylation changes occurring during drought stress imposition and relief in eucalypts (Correia et al. 2016).

In order to decipher the molecular mechanisms that *Pinus halepensis* (Aleppo pine), one of the most drought-tolerant pine species, uses to withstand drought, Fox et al. (2018) performed large-scale physiological and transcriptome analyses at six physiological stages: pre-stomatal response, partial stomatal closure, minimum transpiration, post-irrigation, partial recovery, and full recovery. These authors found a strong transcription of retrotransposons during recovery from drought and argue that the activation of transposable elements might be partially related to the differential expression of several methylation-related transcripts, which implies an epigenetic regulation of gene expression during drought stress in *P. halepensis* (Fox et al. 2018).

The effect of temperature stress in *Populus simonii* was analyzed by using methylation-sensitive amplification polymorphisms and quantitative reverse transcriptase PCR (RT-qPCR) to uncover changes of methylation and expression of miRNA genes (Ci et al. 2015). The authors found that 25.38% of methylation sites changed in response to abiotic stress and identified 1066 sites that were differentially methylated in response to heat and cold stress, from which seven were miRNA genes (Ci et al. 2015). Their results suggest that DNA methylation may regulate the expression of miRNA genes, likely through the gene-silencing function, as a strategy to maintain cell survival under abiotic stress conditions (Ci et al. 2015).

Also using *Populus* (specifically, roots of *Populus* × *canadensis* I-214), Ariani et al. (2016) compared changes in epigenetic modifications under excess Zn using chromatin immunoprecipitation sequencing (ChIP-Seq) for two histone modifications associated with highly expressed genes (H3K4me3) and repressed genes (H3K27me3) together with RNA-Seq transcript abundance to examine how epigenetic modifications would affect gene expression. On one hand, their analyses revealed that genes with an H3K4me3 modification are generally highly expressed, and that H3K4me3 modifications were enriched in genes involved in carbon (C) catabolism, nitrogen (N) metabolism, and in regulation of sub-cellular vesicular trafficking in roots under excess Zn condition. On the other hand, genes with an H3K27me3 modification on the 50-UTR are mainly low expressed and H3K27me3 modifications were enriched primarily in genes involved in photosynthetic processes (Ariani et al. 2016). As the authors stressed, the understanding of epigenetic modifications in response to excess Zn in *Populus* roots constituted a starting point for improving phytoremediation potential of this species (Ariani et al. 2016).

In 2018, Volkova et al. analyzed genetic and epigenetic changes in Scots pine (*Pinus sylvestris* L.) populations from areas that were chronically irradiated for more than 30 years. By using amplified fragment length polymorphisms and ultra-performance liquid chromatography coupled with mass spectrometry, their results

showed that the genetic diversity was significantly higher at the radioactively contaminated areas in comparison to the reference site, and that the genome of pine trees at 4 of the 7 affected sites was also significantly hypermethylated (Volkova et al. 2018).

The importance of dynamic epigenetic mechanisms, such as DNA methylation and histone modifications, in plant adaptation to different biotic stresses has also been explored (Espinosa et al. 2016). Although most studies addressing this matter focus on model species like *Arabidopsis* or tomato, a few studies on woody species are already available. Gene expression patterns of miRNAs in *Populus trichocarpa* plantlets inoculated with the poplar stem canker pathogen, *Botryosphaeria dothidea*, were analyzed by miRNA Array, real-time quantitative PCR for miRNAs and their targets, and miRNA promoter analysis (Zhao et al. 2012). The authors found 12 upregulated miRNAs and any downregulated in the stem bark of *P. trichocarpa* and provided a potential co-regulatory network and a putative miRNAs-transcription factors feedback regulatory network, which were developed to describe post-transcriptional regulation in the pathological development of poplar canker disease (Zhao et al. 2012).

Sollars and Buggs (2018) performed a genome-wide DNA methylation analysis in several ash trees (*Fraxinus excelsior*) and Manchurian ash (*F. mandshurica*) genotypes, with different susceptibility to ash dieback (caused by the fungus *Hymenoscyphus fraxineus*). The authors found that the overall level of cytosine methylation in the leaf methylome of *Fraxinus excelsior* is similar to leaves of *Populus trichocarpa*. They also found higher methylation in transposable elements as opposed to non-mobile elements and identified 1683 significant differentially methylated regions between the high and low susceptibility genotypes of *F. excelsior* trees.

15.3 Epigenetics and Natural Variation: New Insight to Unveil Adaptive Mechanisms

The capability of different provenances of the same species to adapt to very different niches demonstrates the enormous plasticity of genotypes. To date, hundreds of polymorphisms or genes related to natural variation have been identified, mostly in model species (*Arabidopsis* and some crops). In *Arabidopsis*, recent studies involving the re-sequencing of thousands of ecotypes and the availability of genome-wide association tools allowed to elucidate the molecular bases of phenotypic differences related to plant adaptation to distinct natural environments and to determine the ecological and evolutionary processes that maintain this variation (Alonso-Blanco et al. 2016). Natural variation can thus be defined as the intra-specific phenotypic variation caused by spontaneously arising mutations that have been maintained in nature by an evolutionary process such as artificial and natural selection

(Alonso-Blanco et al. 2009). In the same way as gene variants, epigenetic regulation can also explain the observed differences between populations (Dubin et al. 2015; Kawakatsu et al. 2016; He et al. 2018). With the discovery that epigenetic regulation of gene expression can be inherited across cell lineages or even across organismal generations, enormous interest has been generated in the potential evolutionary consequences of epigenetic inheritance (Skinner 2015).

Various environmental signals and stresses can induce persistent changes in epigenetic modifications, thereby creating a flexible memory system for short or prolonged periods of time (Whittle et al. 2009; Yakovlev et al. 2010). In this context of environmental challenges, such epigenetic modifications may be thought of as relatively plastic yet heritable marks that allow for rapid responses and adaptations and, at the same time, might avoid excessive genetic diversification (Boyko and Kovalchuk 2008; Lira-Medeiros et al. 2010).

15.3.1 Epigenetic Control of Natural Variation

The determination of the sources and the role of natural variation has always been recognized as a priority for plant evolutionary biology studies (Richards et al. 2017; Henderson and Salt 2017), embracing the enormous diversity present within wild plants (Alonso-Blanco et al. 2009). Analyzing and understanding the natural variation in wild species was the starting point to elucidate the molecular bases of phenotypic differences related to plant adaptation to distinct natural environments. This genetic variation also exists in more domesticated species, which allow the exploitation of desirable traits in agriculture (Henderson and Salt 2017). Several functional polymorphisms and genes involved in natural variation have been identified in crops development and physiology and associated with important plant traits including genes related to plant morphology, architecture, fruit and seed structure, yield, and quality traits improved by successive breeding (Alonso-Blanco et al. 2009). Phenotype diversity has also been reported in forest trees and the observation of high levels of within-stand phenotypic and molecular diversity has been a concern in forest population genetic studies (Scotti et al. 2016). As static but long-lived organisms growing under temporal and spatial contrasting environment conditions, trees are particularly exposed to many challenging situations during their life span (Avramidou et al. 2015). The maintenance of genetic and phenotypic variability may be a question of adaptation and survival for forest trees (Scotti et al. 2016).

The question in debate is to what extent genetic information contributes or influences a specific phenotypic trait. What do we currently know on the question of natural variation?

It has recently become clear that heritable phenotypic variation results from the mutual yet differential contribution of genetic and epigenetic variation. The notion that variation does not need to be based just on DNA sequence polymorphism is

already commonly accepted (Balao et al. 2018). Epigenetics increasingly occupies a pivotal position in our knowledge of inheritance, natural selection, and consequently, evolution with implications across many fields of biology (Burggren 2016; Richards et al. 2017). The theory claims that epigenetic marks can control adaptive phenotypes but it is not presently understood the relative potential of epigenetic variation in comparison to genetic variation for the contribution to certain traits, especially across generations (Burggren 2016). Epigenetic regulatory mechanisms can facilitate changes in gene activity and fine-tune gene expression patterns, thus enabling plants to survive and reproduce successfully in unpredictable environments. Current knowledge is based upon studies in the model species *Arabidopsis* (Alonso-Blanco et al. 2016; Richards et al. 2017; Aller et al. 2018) taking advantage of its short life cycle and benefiting from genome sequence, powerful genomic resources, and access to high-throughput phenotyping platforms (Richards et al. 2017). These works push up a new research field in which the theoretical ability of epigenetic variation to influence the heritable variation of complex traits is gaining power in the study of plant adaptation (Rodríguez-Leal et al. 2015; Aller et al. 2018). The use of model species allows the discover of the underlying mechanisms of epigenetic dynamics but these studies still have a limited ecological realism (Richards et al. 2017) and are carried out under controlled conditions. Available works with non-model species (that lack extensive genomic resources) in natural environments are still insufficiently explored and thus welcome. Extensive variation of DNA methylation patterns within a species has been uncovered from studies of natural variation (Zoldoš et al. 2018) and suggests that epigenetic variation might be important for ecological studies. Unfortunately, most species used for epigenomic studies are annual herbaceous plants, and epigenome dynamics has been poorly investigated in perennial woody plants (Fortes and Gallusci 2017). The dynamics between epigenetic variation in addition to genetic variation and environment as a mechanism of adaptive plasticity in natural plant populations still needs further research (Richards et al. 2017; Aller et al. 2018; Lele et al. 2018; Zoldoš et al. 2018). Environmentally shaped phenotypic plasticity is thought to play an important role in the adaption of plants to contrasting habitats particularly after postembryonic development (Pikaard and Scheid 2014); however, the transient or heritable nature of the variation (thus potential adaptive) should be clarified. It is also premature to establish whether or not the reported epigenetic profiles are under genetic control and the degree of such control (Richards et al. 2017). One thing is clear, plants are unable to escape their surroundings and are forced to cope with changeable and often unfavorable growth conditions; therefore, epigenetic changes and transgenerational epigenetic inheritance might play an important role in plant response to stress and ecological adaptation (Meyer 2015) that deserves attention. This may be especially important for long-lived organisms with complex life cycles such as forest trees (Bräutigam et al. 2013).

15.3.2 *Linking Epigenetic and Phenotypic Variation in Forest Species*

Trees are long-lived organisms that have to deal with heterogeneous habitat conditions. This imposes limits on natural selection under rapidly changing climate conditions. Phenotypic plasticity is the ability of a genotype to generate and display different phenotypes in response to variation in the environment (Forsman 2015). For the adaptation, phenotypic plasticity plays an important role in the environmental adaptation of trees, so epigenetic variation as a mechanism of adaptive plasticity in natural plant populations needs further research as epigenetic marks and their relation to phenotypic traits are still unexplored to date (Verhoeven et al. 2016; Lele et al. 2018). There are several studies in *Arabidopsis* about the relationship between epigenetic and phenotypic variation in response to environmental stimuli including also traits of transgenerational inheritance (Cortijo et al. 2014; Kooke et al. 2015; Liu et al. 2015; He et al. 2018), but just a few in trees (Bräutigam et al. 2013).

In tree forest species, phenotypic plasticity due to epigenetic variation plays a key role in long-term abiotic stress adaptation. Analysis of the involvement of epigenetic mechanisms in the winter-dormant shoot apical meristem of poplar (*Populus × euramericana*) clones in memory of the growing conditions faced during the vegetative period showed that variations in global DNA methylation between conditions were genotype dependent and correlated with biomass production capacity (Le Gac et al. 2018). Also in poplar (*Populus simonii*), it has been described that variation in genomic methylation in natural populations is associated with leaf shape and photosynthetic traits (Ci et al. 2016). This study provides an association analysis to study the effects of DNA methylation on plant development indicating that epigenetics, environmental, and genetic factors are linked and affect both poplar growth and development.

Conifers from the temperate and boreal regions, such as Norway spruce and Scots pine, have developed systems to modify their performance (phenotype) to tolerate seasonal changes in climatic conditions. They are able to acclimate from active growth to frost-tolerant winter dormancy and *deacclimate* back to active growth in a cyclic manner, synchronized with seasonal changes in temperature and day length. There are studies indicating that adaptive phenomena cannot be explained only by traditional Mendelian genetics, but are likely influenced by non-genetic inheritance (NGH) or epigenetic mechanisms (Kvaalen and Johnsen 2008; Rohde and Junttila 2008; Bräutigam et al. 2013; Salinas et al. 2013; Vivas et al. 2013). Alakärppä et al. (2018) have reported variations in global DNA methylation and gene expression between three Scots pine suggesting a contribution to the local adaptation and the enhancement of fitness of trees under rapidly changing climatic conditions. Studies at transcriptional level in Norway spruce embryos from the same genotype exposed to different epitype-inducing temperatures showed several epigenetic regulators with principal role in epigenetic memory, supporting that both DNA and histones methylation and sRNAs are crucial for the epigenetic memory establishment (Yakovlev et al. 2016).

The long generation time of trees is a drawback and reduces the number of available studies considering epigenetic inheritance. So far, we have learnt that breeders must take care about the different lots of seeds generated for progeny selection from the same parent genotype, since the temperature and day length conditions should be similar for each lot in order to obtain similar progenies. This phenomenon is not only of important for breeding but has evolutionary significance for conservation of forest genetic resources. Contrary to crop and herb model species, there are only a few published examples linking tree epigenetics and environment being necessary more experiments. Nevertheless, some of them are ongoing for understanding both stress adaptive mechanisms underlying epigenetics and phenotypic variation in forest species however much more work is needed to gain enough knowledge to understand these processes.

15.4 Concluding Remarks and Further Perspectives

Since our first review (Pascual et al. 2014), the evidences proving the pivotal role of epigenetic regulation linking environment, gene expression, and phenotype in forest species have increased. Epigenetics mechanisms can respond to internal (i.e., leaf development through hexoses accumulation) and external (long-term cold exposure) sensing mechanisms. Furthermore, recent advances in epigenetics demonstrate how some traits can be modulated during embryogenesis, seedling stages, or even before fecundation. This last effect is particularly striking since, although priming effects are well known, the possibility of a transgenerational inheritance of these marks and the definition of new epi-varieties could quickly help to improve tree stress tolerance. Despite the difficulty of working with tree species, many fundamental questions have been answered in the recent years as it was described above. However, deepening in the epigenetic regulation of complex adaptive traits such as long-term metabolic adaptation to stressful environments or in transgenerational epigenetics effects will possibly provide an unprecedented breakthrough and will be supposedly reached in the near future. All this knowledge will be applicable to develop new biotechnological tools for breeders and forest managers towards the improvement of the efficiency and productivity of our forests.

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