Chapter 6

Epigenetics in horticultural crops: consequences and applications in abiotic stress tolerance

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6.1 Introduction

The term "epigenetics" refers to heritable changes in gene expression (which may include changes in the magnitude, the temporal and/or spatial expression pattern of a gene) that are not caused by alterations in the DNA sequence. Epigenetic marks associated with gene expression are DNA methylation, histone modifications, and histone variants, all of which affect chromatin structure and accessibility. Genetic and genomic studies mainly in *Arabidopsis thaliana* have generated valuable information on the functions of these epigenetic marks, and many of the factors involved in the regulation of epigenetic marks have been identified (Pikaard and Mittelsten Scheid, 2014; Law and Jacobsen, 2010).

DNA methylation occurs on carbon number five of a cytosine base, resulting in 5-methylcytosine (5mC), whereas mammalian 5mC was observed predominantly at CG sites, in plants, cytosine methylation is found in any sequence context, functionally divided into CG, CHG, and CHH methylation (where H is A, T, or G) (Pikaard and Mittelsten Scheid, 2014; Law and Jacobsen, 2010). DNA methylation is deposited by DNA methyltransferases (MTases) that are divided into three types: MET (DNA METhyltransferase), DRM (Domains-Rearranged Methyltransferase), and the plant-specific CMT (ChromoMeThylase) (Pikaard and Mittelsten Scheid, 2014). Genome-wide identification of DNA methyltransferases was conducted in rice (Teerawanichpan et al., 2009), maize (Qian et al., 2014a,b), wheat (Theiss et al., 1987), tomato, pepper, and potato (Kumar et al., 2016). DNA methylation patterns can be maintained during DNA replication by MET1 in the case of CG methylation or by CMT2, CMT3, and DRM2 in the case of non-CG methylation (i.e., CHG and CHH). De novo synthesis of DNA methylation is mediated by small RNA and DRM2 methyltransferase through the RNA-dependent DNA methylation (RdDM) pathway (Maeji and Nishimura, 2018). The RdDM pathway has been well characterized and includes several key enzymes: RNA-Dependent RNA polymerase2 (RDR2), Dicer like 3 (DCL3), and Argonaute (AGO) proteins as well as the plant-specific RNA polymerases Pol IV and Pol V. siRNAs are loaded into AGO proteins, guiding the methylation machinery to the target DNA to be methylated. The removal of DNA methylation marks, that is, DNA demethylation, can be achieved by either passive or active mechanisms. Passive mechanisms involve repression of DNA methylation activities, causing genome-wide reductions in DNA methylation levels during DNA replication. Active demethylation occurs through the DNA glycosylation activity of Demeter (DME), Repressor Of Silencing 1 (ROS1), Demeter like 2 (DML2), and DML3 enzymes (Maeji and Nishimura, 2018).

Histone modifications include methylation, acetylation, phosphorylation, and ubiquitination of specific amino acid residues in the N-terminal tails of the histone core proteins H2A, H2B, H3, and H4. They are important for gene regulation and were shown to play a role in plant response to abiotic stresses (Begcy and Dresselhaus, 2018; Zhao and Zhou, 2012). Genome-wide distribution of histone modification patterns and concomitant transcriptional alteration in response to environmental stresses have been extensively studied in cereal crops (Zong et al., 2013; Zhao et al., 2014; Wang et al., 2015a,b; Xue et al., 2018; Zheng et al., 2019a,b). In addition, several studies link histone modifications with

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abiotic stress response in dicot crops such as tomato, cotton, bean, and soybean (Yu et al., 2018; Wei et al., 2017; Yolcu et al., 2016; Huang et al., 2016).

Histone methylation marks appear on lysine and arginine residues in histone tails, including H3K4, H3K9, H3K27, and H3K36 (Liu et al., 2010; Qian et al., 2019a,b). SET domain proteins are known as histone lysine methyltransferases, which methylate specific lysine residues in N-terminal tails of histone H3 or H4 using methyl groups from a cofactor *S*-adenosylmethionine (Liu et al., 2010; Qian et al., 2019a,b; Yadav et al., 2016). In maize, 43 SET domain−containing proteins were identified. Most SET domain proteins were shown to be responsive to drought or salt stress, indicating they have potential roles in histone methylation under stress conditions (Qian et al., 2014a,b). Histone methylation marks can be removed by two types of histone demethylases: Jumonji C (JmjC) domain−containing proteins and lysine-specific demethylase1 (LSD1) (Liu et al., 2010). JmjC domain−containing proteins (JMJs) remove methyl group from histone lysine residue by hydroxylation, whereas LSD1 catalyzes amine oxidation to remove lysine methylation (Liu et al., 2010; Chowrasia et al., 2018; Qian et al., 2019a,b).

Histone acetylation is one of the most studied histone modifications, which is involved in chromatin decondensation and gene activation (Kim et al., 2015). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) have been shown to play a role in abiotic stress response in various plant species (Kim et al., 2015; Pandey et al., 2002). In *Arabidopsis*, 12 HATs were identified and grouped into four different families: CBP, GNAT, MYST, and $TAF_{II}250$ (Pandey et al., 2002). Similarly, eight HATs were characterized in rice (Liu et al., 2012; Fang et al., 2014). Eukaryotic HDACs can be categorized into three major families: RPD1/HDA1 superfamily, Silent Information Regulator 2 (SIR2) family, and the plant-specific HD2 family (Pandey et al., 2002; Kim et al., 2015). The *gcn5* histone acetyltransferase mutant (*AtHAG1*) in *Arabidopsis* exhibited growth inhibition and defects in cell wall integrity under salt stress. *TaGCN5*, a wheat ortholog of *GCN5*, was upregulated by salinity similarly to *GCN5* in *Arabidopsis*. Heterologous expression of *TaGCN5* in *gcn5* mutants restored the seedling growth and the cell wall integrity impacted by salinity, indicating GCN5 function under salt stress is conserved in dicotyledonous and monocotyledonous species (Zheng et al., 2019a,b). In addition, *TaGCN5* expression was responsive to heat stress as well as salinity. *TaCGN5* expression reestablished thermotolerance in *Arabidopsis gcn5* mutants susceptible to heat stress. Another example of the involvement of histone acetylation in response to abiotic stress is the *SlHDA5* deacetylase gene in tomato (*Solanum lycopersicum*), where reduced expression of *SlHDA5* by RNAi to around 50% of the normal level resulted in increased sensitivity to salt treatments (Yu et al., 2018).

Monoubiquitination of H2A and H2B histone tails plays a critical role in transcriptional regulation (Turner, 2002; Kouzarides, 2007). In rice, H2B monoubiquitination (H2Bub1) mediated by ubiquitin E3 ligases OsHUB1 (rice Histone monoubiquitination1) and OsHUB2 has been reported to be involved in anther development and yield potential (Cao et al., 2015; Du et al., 2016). In addition, H2Bub1 was found to cause abscisic acid (ABA) sensitivity and drought resistance during the seedling stage in rice (Ma et al., 2019).

Histone modifications are closely linked to transcriptional gene regulation and chromatin structure. In general, it is widely accepted that H3K9 and H3K27 methylation marks are associated with gene silencing and heterochromatin formation, while histone methylation of H3K4 and H3K36 and histone acetylation are involved in gene activation (Begcy and Dresselhaus, 2018; Liu et al., 2010; Kim et al., 2015). Likewise, histone modification−modulated gene regulation has been proposed in cereal crops. Rice submergence−responsive genes *ADH1* and *PDC1* are induced in a biphasic manner during submergence. During the first induction period, H3K4me2 was decreased, but H3K4me3 was increased in the coding regions of both *ADH1* and *PDC1*. On the other hand, H3 acetylation was increased in the promoters and coding regions of both genes during the later induction period. These H3K4 trimethylation and H3 acetylation reverted to the original states by the escape from submergence, indicating submergence-induced epigenetic regulation of *ADH1* and *PDC1* occurs transiently (Tsuji et al., 2006).

Polycomb Repressive Complex2 (PRC2) is known to regulate gene silencing by promoting the deposition of the repressive histone mark H3K27me3 (Pikaard and Mittelsten Scheid, 2014). In cereals, homologous of the *Arabidopsis*, PCR2 members have been identified and some of them characterized. Barley, for instance, has four PRC2 genes *HvFIE*, *HvE(Z)*, *HvSu(z)12a*, and *HvSu(z)12b*. After fertilization, transcription levels of *HvFIE* and *HvSu(z)12b* are increased. However, *HvFIE* and *HvE(Z)* expression also increase in response to ABA, indicating their potential function in abiotic stress responses (Kapazoglou et al., 2010).

The impact of environmental stresses on plant development and productivity has long been recognized as a major agricultural challenge. Recent studies have demonstrated that DNA methylation and chromatin-related processes play an important role in plants resistance and tolerance to different abiotic stresses such as extreme temperatures, drought, salinity, or osmolality stress, pointing out the importance of epigenetic regulations for these traits (Kim et al., 2015; Chang et al., 2020). For example, a specialized histone H1 variant was shown to be required for the adaptive response

to combined light and water deficiency and a substantial part of DNA methylation associated with environmental-stress conditions in *Arabidopsis* (Rutowicz et al., 2015). Numerous studies reported genome-wide alterations in DNA methylation status occurring under abiotic stress conditions, or between tolerant and sensitive varieties. For instance, drought and salt stresses were used to compare methylation profiles between stress-tolerant and sensitive varieties in rice (Wang et al., 2011a,b, 2015a,b; Karan et al., 2012; Guo et al., 2019a,b; Rajkumar et al., 2020). Genome-wide changes in DNA methylation profiles were reported in response to heat stress in cotton (Ma et al., 2018; Zhang et al., 2020).

Epigenetics is studied predominantly in the contexts of development, response to the environment, and evolution. In terms of plant stress response and adaptation, several recent publications review key aspects in which epigenetics is involved, such as stress priming, stress memory, and inheritance (Chang et al., 2020; Varotto et al., 2020; Perrone and Martinelli, 2020). In this chapter, we discuss the epigenetic pathways and factors that are involved in the basal response to abiotic stresses (i.e., heat, cold, drought, water deficit, salinity, and osmotic) in various crop plants. In order to discuss possible applications of epigenetics knowledge and tools for breeding purposes (epibreeding), this chapter focuses on the rapidly growing data coming from different crop species. The accumulating data on the regulatory relationships between epigenetic mechanisms and agriculturally important traits such as abiotic stress tolerance will facilitate the development of new molecular breeding techniques and resistant crop varieties.

6.2 Epigenetic changes related to temperature stress

Changes in ambient temperatures greatly affect plants' physiology and development. With the current global climate change, growth temperatures are gradually changing and temperature extremes occur more frequently. As temperature is a central factor governing plant growth and development, either high or low temperatures will limit productivity and yield. Genetic mechanisms controlling plants' responses to different temperatures and the molecular events contributing to tolerance and acclimation have been widely studied (Ritonga and Chen, 2020; Ding et al., 2020; Driedonks et al., 2015). In the last two decades, molecular, biochemical, and cellular evidences have shown the link between plants' response to the surrounding temperature and epigenetic processes (Ueda and Seki, 2020; Rai et al., 2020; Begcy and Dresselhaus, 2018).

6.2.1 Transcriptional response of the epigenetic machinery to temperature stress

Transcriptional response to high and low temperatures has been observed in several plant species. The survey for DNA methyltransferase genes resulted in the identification of 9 genes in tomato (*S. lycopersicum*), 8 in the wild relative of tomato (*Solanum pennellii*), 7 in potato (*Solanum tuberosum*), and 13 in pepper (*Capsicum annuum*). The transcriptional level of the tomato methyltransferases was analyzed under different conditions, and it was found that *SlDRM7, SlDRM8, SlCMT4, SlMET1, SlDRM5, SlDRM6*, and *SlDNMT2* were upregulated by heat stress. Interestingly, *SlMET1* was also upregulated by cold stress (Kumar et al., 2016). Similarly, several MBD (methyl-binding domain)−containing genes were found to respond at the transcriptional level to cold and/or heat stress in tomato (Parida et al., 2018). In rice, induced transcription was found for *CMT2* under cold stress conditions (Sharma et al., 2009; Ahmad et al., 2019). Another example for upregulation of genes associated with a DNA methylation process is the induction of 17 *AGO* genes in response to heat stress in maize (Zhai et al., 2019).

Contrary to DNA methylation−related genes that are mostly upregulated in response to temperature stress, genes involved in histone modification processes show mixed trends. For instance, histone methylase SET domain−containing genes were identified in *Gossypium raimondii*, a diploid cotton species. Most of the 52 identified genes were found to respond transcriptionally to heat stress treatments of 12, 24, or 48 h at a temperature of 38°C. Unlike most genes identified in this study, which were downregulated in response to heat stress, *GrKMT1A1b, GrKMT1B3c*, and *GrKMT6B2* were upregulated, reaching a peak at 12 h after heat stress (Huang et al., 2016). In maize, 19 JmjC domain−containing histone demethylase genes were characterized in response to heat stress. Ten JmjC genes (*ZmJMJ1*, *ZmJMJ3*, *ZmJMJ5*, *ZmJMJ6*, *ZmJMJ8*, *ZmJMJ9*, *ZmJMJ10*, *ZmJMJ14*, *ZmJMJ17*, and *ZmJMJ19*) were significantly upregulated, whereas six JMJ genes including *ZmJMJ2*, *ZmJMJ4*, *ZmJMJ7*, *ZmJMJ11*, *ZmJMJ12*, and *ZmJMJ16* were repressed (Qian et al., 2019a,b). The transcriptional response of histone acetylation genes in rice was found to vary between temperature-stress conditions, whereas cold stress generally suppressed expressions of *HATs* except for *OsHAG704*; the expressions of *OsHAC701*, *OsHAG702*, and *OsHAM701* were increased in response to heat stress, while *OsHAC704* and *OsHAG704* were rather repressed (Liu et al., 2012). Similarly, differential expression patterns of *HDACs* in rice under different stress conditions (cold, mannitol, and NaCl) showed that *HDA704*, *HDA712*, and SIR2 family members (*SRT701* and *SRT702*) decreased under all three abiotic stresses, while *HDA702* transcription was activated.

Additionally, cold stress induced the expression of *HDA706*, *HDA714*, and *HDT701*, whereas these genes were repressed by salt stress, indicating that *HDA706*, *HDA714*, and *HDT701* function in different pathways under different stress conditions (Fu et al., 2007).

6.2.2 Changes in DNA methylation related to cold or heat stress

Comparing DNA methylation and transcriptional changes between cold tolerant (P427) and susceptible (Nipponbare and 93-11) rice lines under cold stress and control conditions showed that the number of cold-induced methylation-altered genes is higher in the tolerant line. For 29 genes in the tolerant P427 line, the change in DNA methylation reversely correlated with transcription level, hinting at a possible cold-induced regulation by DNA methylation. Most of these methylation changes occurred at promoter regions (Guo et al., 2019a,b). Interestingly, a homolog of open stomatal1 (OST1) was upregulated under cold stress accompanied by decreased methylation level in its promoter region. OST1 is a well-known Ser/Thr protein kinase in ABA signaling and acts upstream of C-repeat-binding factors to positively regulate freezing tolerance (Ding et al., 2015). Thus demethylation and activation of OST1 under cold stress might play a role in cold tolerance in rice (Guo et al., 2019a,b).

Heat-stress treatment in maize seedlings resulted in 325 differentially methylated genes. Those genes are involved in processes, including spliceosome function, homologous recombination, RNA transport, ubiquitin-mediated proteolysis, and carbon metabolism pathways (Qian et al., 2019a,b).

Several agriculturally important crops such as wheat, cotton, canola, and peanut are polyploids. The effect of temperature shift and polyploidy level on DNA methylation patterns was tested in the polyploid alpine perennial herb *Ranunculus kuepferi* (Syngelaki et al., 2020). The researchers tested the changes in DNA methylation in diploid and autotetraploid individuals of *R. kuepferi* exposed to cold (7°C/2°C day/night; frost treatment: −1°C cold shocks for 3 nights per week) and warm (15°C/10°C day/night) conditions. Their findings show that methylation patterns differed between cytotypes, and that both cold and warm shifts had affected the magnitude of DNA methylation variation (Syngelaki et al., 2020).

6.2.3 Changes in chromatin and histone modification patterns under temperature stress

It has been reported that abiotic stress−induced DNA methylation alteration causes chromatin structural changes. Using fluorescence in situ hybridization in rice plants under heat and salinity stresses, it was shown that both treatments induced extensive decondensation of 45S rDNA chromatin as well as an increase in the distance between two homologous 5S rDNA loci (Santos et al., 2011).

The involvement of HDACs in abiotic stress responses in cereals was investigated using a HDAC inhibitor treatment. Trichostatin A (TSA), a HDAC inhibitor, has been shown to cause global histone hyperacetylation. TSA treatment of maize plants under cold stress conditions inhibited the induction of cold-responsive *ZmDREB1* and *ZmCOR413* genes. On the other hand, the expression of *ZmICE1* was less affected by TSA. Among these genes, only the promoter region of *ZmDREB1* appears to be hyperacetylated by TSA. The authors, therefore, concluded that HDACs selectively regulate the expression of *ZmDREB1*, which, in turn, modulates *ZmCOR413* expression in response to cold stress (Hu et al., 2011).

Studies investigating the well-known transcription factors dehydration-responsive element-binding (DREBs) proteins in response to different abiotic stresses (Liu et al., 1998; Dubouzet et al., 2003) showed that overexpression of *OsDREB1b* conferred cold tolerance in rice (Ito et al., 2006). Upon cold stress, acetylation levels of H3K9, H3K14, and H3K27 in the upstream region of *OsDREB1b* increased, and the chromatin structure was subsequently changed, suggesting histone acetylation accounts for cold-induced *OsDREB1b* expression (Roy et al., 2014).

In relation to heat stress, the activity of histone modifications was explored in maize seedlings. Heat stress caused an increase in the global acetylation levels of H3K9, H4K5, and H3, whereas H3K9 dimethylation decreased. In response to cold stress, H3K27ac and H3K36ac tend to increase in rice seedlings (Xue et al., 2018). TSA treatment of normal seedling resulted in increased O_2^- levels, which resembles cellular heat stress response, indicating that histone acetylation is involved in heat stress−induced programmed cell death (Wang et al., 2015a,b).

Heat stress was proposed to inhibit lateral root primordium formation in maize by histone acetylation−mediated transcriptional regulation. Although heat stress induces a global change of H3K9ac and H4K5ac, acetylation levels of the promoter regions of lateral root development−related genes *ZmHO-1* and *ZmGSL-1* were rather decreased under heat stress, resulting in downregulation of both genes (Zhang et al., 2018).

Heat stress applied to flowering rice plants postfertilization led to a reduction in seed size and aberrant endosperm cellularization, a similar phenotype observed when rice FIE homolog, *OsFIE1*, was constitutively expressed in rice. *OsFIE1* expression was increased under heat-stress conditions, consistent with a sharp decrease in DNA methylation and histone modification H3K9me2 in its promoter. Moreover, *OsFIE1* represses the expressions of syncytial stage-specific type I MADS-box genes (*AGL36*, *OsMADS82*, and *OsMADS87*) by elevating H3K27me3 levels in their promoter regions, which suggest that *OsFIE1* plays a key role in the persistence of the syncytial stage of rice endosperm development (Folsom et al., 2014).

6.3 Epigenetic changes related to drought and water deficit stress

Similar to other abiotic stresses, drought represents a considerable threat to plant growth, development, and productivity. Crop yields are negatively affected by suboptimal water supply due to physical, physiological, and biochemical disruptions. Several drought-responsive genes, including transcription factors, aquaporins, late embryogenesis abundant proteins, cytoskeleton, heat shock proteins, and dehydrins, have been identified. These genetic factors enable plants to respond and withstand unfavorable conditions of drought or water deficit (Joshi et al., 2016; Kaur and Asthir, 2017; Ghatak et al., 2017; Fahad et al., 2017). However, information on the epigenetic mechanisms operating in crop plants in response to these stresses is still very limited. Drought response was associated with the RdDM pathway in foxtail millet (*Setaria italica*) (Liu et al., 2016). EMS-driven *SiAGO1b* mutant (*siago1b*) exhibited drought-stress susceptibility during seedling stage and four leaves stage as well as morphological changes, including stunted growth and reduced yield. In addition, C-terminal motif lacking mutant protein ΔSiAGO1b encoded by *siago1b* allele failed to interact with SiHYL1, whereas yeast two-hybrid and BiFC showed the protein interaction between normal SiAGO1b and SiHYL1. This indicates that the C-terminal motif of SiAGO1b might be essential for AGO1 function, which influences plant development and drought tolerance in foxtail millet (Liu et al., 2016). Similarly, *ago18b* mutant in maize showed severe leaf yellowing under drought stress, indicating *ZmAGO18b* positively affects drought tolerance in maize. In addition, the *ZmAGO18b* gene showed considerable increase in transcriptional activity during early response to drought stress (Zhai et al., 2019).

6.3.1 Transcriptional response of the epigenetic machinery to drought or water-deficit conditions

Several studies in crop plants have described transcriptional changes of DNA methyltransferases and demethylases in response to desiccation or drought stress (Kumar et al., 2016; Moglia et al., 2019; Kapazoglou et al., 2013). In tomato, it was found that *SlDRM8, SlCMT4, SlCMT3, SlDRM6*, and *SlDNMT2* were upregulated by a desiccation treatment. Interestingly, the induction of *SlCMT3* was specific to desiccation and did not occur under other stress treatments (Kumar et al., 2016). In eggplant (*Solanum melongena*), all five demethylases were found to be upregulated by drought stress, whereas for the methyltransferase genes, *SmelMET1* and *smelCMT2* were upregulated and *SmelCMT3b* was downregulated following the drought treatment (Moglia et al., 2019). Similarly in barley, a cereal crop, transcriptional levels of *HvDME* demethylase showed drought stress−related induction. Moreover, induction was higher in the drought-tolerant variety Demetra compared with the drought-sensitive variety Caresse, indicating a putative function for HvDMEs in response to water deficit that could contribute to drought tolerance (Kapazoglou et al., 2013).

The expression of histone methylation SET domain−containing genes was shown to respond to desiccation stress in maize and foxtail millet, indicating they might be involved in histone methylation in response to water-deficit conditions (Yadav et al., 2016; Qian et al., 2014a,b). Among the 53 putative SET domain−containing genes characterized in foxtail millet, 19 SiSET genes showed increased expressions in response to dehydration, and other abiotic stress treatments (Yadav et al., 2016). In maize, most of the 43 SET domain−containing genes were shown to be responsive to desiccation; however, their expression was either increased or decreased (Qian et al., 2014a,b).

One example of the involvement of histone acetylation in drought response is the induction of drought sensitivity by altering the HDAC gene *SlHDA5* in tomato. Reduced expression of this gene by RNAi to around 50% of its normal expression resulted in increased sensitivity to drought treatment (Yu et al., 2018).

Histone ubiquitination may as well play a role in response to drought, as suggested by a study in rice. Overexpression of histone monoubiquitination2 *(OsHUB2)*, an E3 ligase for H2Bub1, caused ABA sensitivity and drought resistance during seedling stage. Knockdown of *OsHUB2* exhibits an opposite phenotype (Ma et al., 2019). Under ABA treatment and drought-stress condition, OsHUB2 physically interacted with OsbZIP46, a positive regulator of drought tolerance (Tang et al., 2012; Ma et al., 2019).

ATP-dependent chromatin remodeling complexes affect transcriptional activation by altering the position of nucleosomes and controlling the composition of histone octamers by replacing one histone variant with another (Han et al., 2015; Hu et al., 2013; Pikaard and Mittelsten Scheid, 2014). Various chromatin remodeling factors were proposed to be linked to abiotic stresses in cereal crops. For instance, 40 members of the SNF2 protein family, one of the major ATP-dependent chromatin remodeling factors, were identified in rice. Among these genes, four (*CHR712*, *CHR720*, *CHR728*, and *CHR742*) were upregulated by drought and salinity, whereas *CHR735* expression was repressed by both stresses (Hu et al., 2013), indicating these genes participate in chromatin rearrangement during drought and salt stresses in rice.

6.3.2 Changes in DNA methylation related to drought or water-deficit stress

Sesame (*Sesamum indicum*) is considered a drought-tolerant crop. Changes in DNA methylation after drought stress strongly induced de novo methylation, while most of the loci were demethylated during the recovery phase. Interestingly, the transcript levels of the differentially methylated regions highly correlated with the methylation changes, that is, upregulation in the hypomethylated loci, and downregulation in the hypermethylated regions (Komivi et al., 2018). In other studies, drought stress was applied to rice plants to compare methylation profiles between stress-tolerant and sensitive varieties (Wang et al., 2011a,b; Garg et al., 2015; Rajkumar et al., 2020). Changes in methylation due to the stress accounted for about 10% of the total site-specific DNA methylation in tolerant transgression lines compared with parental varieties. About 29% of the drought-induced methylation alterations remained after rewatering, suggesting that drought conditions may induce stress memory that could facilitate stress priming (Wang et al., 2011a,b, 2015a,b). In addition, drought conditions lead to gene-specific methylation alterations, as differential DNA methylation was identified in 14 zinc-finger protein (ZFP) genes in rice plants submitted to salt and drought stresses (Ahmad et al., 2019). Similarly in pea, water deficit of 82% resulted in the identification of three methylation polymorphism sites (Labra et al., 2002).

Investigation of the effect of polyploidization on DNA methylation patterns revealed that the percentage of DNA methylation increased following drought treatment in diploid *Brassica oleracea* and *Brassica rapa* as well as in the newly generated polyploid *Brassica napus*. After drought stress, the percentage of low-methylation fragments in *B. napus* F1 was lower than both parents, whereas the low-methylation level in F2, F3, and F4 was in between the diploid parents (Jiang et al., 2019).

6.3.3 Changes in chromatin and histone modification patterns under drought and water deficit stress

The correlation between gene transcription and the activation mark H3K4me3 under drought stress was analyzed in rice seedlings. Combining RNA-seq and ChIP-seq analyses, a gene cluster was found, including four dehydrin genes, that were regulated by the accumulation of H3K4me3 mark in their promoters (Zong et al., 2013). Following these results, the role of ZIP23 transcription factor in drought stress response was demonstrated. In an *OsbZIP23* knockdown line, H3K4me3 levels were reduced in the promoters of the four dehydrin genes, indicating that OsbZIP23 positively affects H3K4me3 accumulation, therefore, regulating the expression of dehydrin genes under drought stress (Zong et al., 2019). Similarly, the MODD (mediator of OsbZIP46 deactivation and degradation) protein interacts with OsTRP3-HDA702 corepressor complex to reduce histone acetylation of the OsbZIP46 transcription factor, which leads to transcriptional repression of OsbZIP46. By that, MODD negatively regulates ABA signaling and drought resistance (Tang et al., 2012, 2016). OsbZIP46 transcription factor regulates target genes by interacting with HUB2 histone ubiquitinase. OsHUB2−OsbZIP46 complex induces H2Bub1 accumulation in OsbZIP46 target genes, thereby increasing their transcription. Intriguingly, MODD, a repressor of *OsbZIP46*, recruits an OsOTLD1 deubiquitinase to decrease H2Bub1 levels in OsbZIP46 target genes, suggesting that a reversible histone H2Bub1 mark fine-tunes OsbZIP46-mediated drought response in rice (Ma et al., 2019). Another example for a chromatin-related regulation of drought response in rice comes from the *oschr4* mutant that exhibits morphological phenotypes, including narrow and rolled leaves, stunted plant growth, and reduced tiller number as well as drought-stress tolerance due to enhanced cuticle wax accumulation. Noteworthy, *oschr4* mutants, defected in *OsCHR4*a, a CHD3-family chromatin remodeling factor, showed increased expressions of seven wax biosynthetic genes (*WSL4*, *CER7*, *LACS2*, *LACS7*, *ROC4*, *BDG*, and *GL1-4*) and four auxin biosynthetic genes (*YUC2*, *YUC3*, *YUC5*, and *YUC6*). Consistently, active H3K4me3 marks were induced and repressive H3K27me3 marks were decreased in the promoter regions of upregulated

genes (Guo et al., 2019a,b), suggesting that epigenetic regulation by OsCHR4 is required for multiple developmental processes including plant growth, leaf morphology, and cuticle wax synthesis, which are important for drought tolerance.

6.4 Epigenetic changes related to salinity and osmotic stress

High salinity conditions cause ion toxicity and hyperosmotic stress, which inhibit plant growth development and productivity. The physiological, biochemical, and molecular aspects of Na^+ and Cl^- uptake and transport were studied in regard to salinity stress, and prospects for engineering stress-tolerant crops were recently discussed (Isayenkov and Maathuis, 2019; Wani et al., 2020). Genetic and biochemical studies have shed light on several signaling pathways in salt tolerance. The Salt Overly Sensitive signaling pathway plays a key role in maintaining ionic homeostasis, whereas the mitogen-activated protein kinase cascades mediate ionic, osmotic, and reactive oxygen species (ROS) homeostasis (Yang and Guo, 2018). Several publications in *Arabidopsis* demonstrate the involvement of DNA methylation and various histone modifications in regulating salt tolerance−related genes (Chang et al., 2020).

6.4.1 Transcriptional response of the epigenetic machinery to salinity or osmotic stress

Among the eight DNA methyltransferases in maize, *MET* (*ZmMET1a* and *ZmMET1b*) and *CMT* (*ZmMET2a* and *ZmMET2b*) methyltransferase genes show reduced transcription under osmotic and salinity stresses (Qian et al., 2014a, b). Similarly, the transcriptional activity of *MET* and *CMT* genes in rice tended to decrease in response to salinity stress, whereas *CMT2* transcript showed higher accumulation compared with normal conditions (Sharma et al., 2009). The transcriptional level of tomato methyltransferases was analyzed under different conditions, and it was found that *SlDRM7, SlDRM8, SlCMT4, SlMET1*, and *SlDRM6* were upregulated by a low-salinity treatment. *SlDRM7, SlDRM8*, and *SlCMT4* were upregulated by a high-salinity treatment as well (Kumar et al., 2016). Similarly, several MBD-containing genes in tomato were found to respond to salinity at the transcriptional level (Parida et al., 2018). In eggplant, three demethylases were found to be upregulated by salinity stress, whereas *SmelDRM2* and *smelDRM3* methyltransferase genes were upregulated and *SmelCMT3a* and *SmelCMT3b* downregulated following the salinity treatment (Moglia et al., 2019).

The transcriptional response of histone modification enzymes to salinity was studied in maize, rice, and foxtail millet (Qian et al., 2014a,b; Chowrasia et al., 2018; Fu et al., 2007; Yadav et al., 2016). Most of the SET domain histone methyltransferases identified in maize were responsive to salt stress, indicating a potential role for histone methylation under salinity conditions (Qian et al., 2014a,b). Additionally, among the 53 putative SET domain−containing genes characterized in foxtail millet, 21 *SiSET* genes showed altered expressions in response to salinity (Yadav et al., 2016). Expression of the *JMJ* histone demethylase genes under salinity was analyzed in the salt-tolerant rice variety FL478, showing that most of the *JMJ-C* genes were upregulated under salinity at the seedling stage and during reproductive development (Chowrasia et al., 2018).

Histone acetylation genes were also found to be responsive to salinity and osmotic stresses. Differential expression patterns of *HDACs* in rice under different stress conditions (cold, mannitol, and NaCl) showed that *HDA704*, *HDA712*, and SIR2 family members (*SRT701* and *SRT702*) decreased under all three abiotic stresses, while *HDA702* transcription was activated. Additionally, *HDA706*, *HDA714*, and *HDT701* were repressed by salinity, indicating that *HDA706*, *HDA714*, and *HDT701* function in different pathways under different stress conditions (Fu et al., 2007). HDT701 is a plant-specific HD2-type HDAC known to negatively regulate resistance to rice pathogens by modulating H4 deacetylation (Ding et al., 2012). Under ABA, NaCl, and PEG treatment, *HDT701* expression was repressed, although it is constitutively expressed throughout the entire life cycle. Interestingly, overexpression of *HDT701* in rice plants decreased tolerance to ABA, salinity, and osmotic stress during seed germination, but enhances salinity and osmotic stress tolerance during the seedling stage, suggesting that rice HDT701 functions differentially in abiotic stress depending on developmental stages (Zhao et al., 2015). Similarly, a member of the RPD3/HDA1-type HDACs *HDA705* also has a divergent role in stress responses according to plant growth stages. Overexpression of *HDA705* increased ABA sensitivity and decreased salinity resistance during seed germination by downregulating GA biosynthetic genes and upregulating ABA biosynthetic genes. During the seedling stage, on the contrary, overexpression of *HDA705* exhibits rather enhanced osmotic stress tolerance (Zhao et al., 2016). *OsHAC701*, *OsHAC703*, *OsHAC704*, *OsHAG703*, and *OsHAM701* were highly expressed under salinity accompanied by higher levels of H3K18ac (Liu et al., 2012).

6.4.2 Changes in DNA methylation related to salinity and osmotic stress

One of the earliest studies on plant DNA methylation under abiotic stress conditions was performed on tobacco TBY-2 cell suspension culture that was subjected to salt and osmotic stresses (Kovařik et al., 1997). Two heterochromatic loci (HRS60 and GRS, characterized by repetitive DNA sequences) were inspected by methylation-sensitive restriction enzymes in combination with Southern blot hybridization. The findings suggested that an internal cytosine within the CCGG motifs remained fully methylated, while a reversible hypermethylation of the external cytosine occurred in cells grown under mild osmotic stress equal to a NaCl concentration of 10 g L^{-1} (Kovařik et al., 1997). The effect of salinity levels on DNA methylation patterns in three pepper (*Solanum capsicum*) cultivars was evaluated, showing that under saline conditions [control (0.7), 3.5 and 7 dS m^{-1}], DNA methylation was increased by up to 11.11% (Shams et al., 2020). In canola (*B. napus*), both de novo methylation and demethylation events in CCGG sites were detected in plantlets exposed to 10−200 mmol L^{-1} salt (Guangyuan et al., 2007). Using methylated DNA immunoprecipitation (MeDIP), differential DNA methylation was identified in 14 ZFP genes in rice plants submitted to salt and drought stress (Ahmad et al., 2019).

In rice, most methylation/demethylation changes that occurred under salt stress remained stable after recovery, implying a possible establishment of stress memory (Wang et al., 2011a,b, 2015a,b). In maize seedlings as well, osmotic and salt stress induced methylation alterations. Induced methylation was observed in two stress-specific fragments from leaves, LP166 and LPS911, shown to be homologous to retrotransposon Gag−Pol protein genes. These are evidence of the possible role of osmotic stress−induced methylation of retrotransposons. Under salinity, the first intron of maize protein phosphatase 2C (*ZmPP2C*) showed induced methylation in salt-treated roots, whereas methylation in maize glutathione *S*-transferases (*ZmGST*) was decreased in salt-treated leaves. The transcriptional analysis showed that salt stress downregulated *ZmPP2C* in roots and upregulates *ZmGST* in leaves, indicating that methylation in these loci controls their expression in different tissues (Tan, 2010).

In wheat, methylation changes under salinity stress were compared between a salt-tolerant variety SR3 and its progenitor parent JN177. Methylation levels of 13 loci in JN177 were induced only under salt stress, whereas the same loci were methylated in both stressed and nonstressed SR3. When a more detailed analysis was conducted, cytosine methylation of the promoter regions of *TaFLS1* (a flavonol synthase) and *TaWRSI5* (a Bowman−Birk-type protease inhibitor) showed a decrease in DNA methylation, concomitantly with their elevated expressions under salt stress in SR3. Furthermore, heterologous expression of both *TaFLS1* and *TaWRSI5* in *Arabidopsis* enhanced salt tolerance during seed germination and seedling stage (Wang et al., 2014). Compared to other cereal crops, foxtail millet is known to have a better tolerance to environmental stresses. A comparison of both salt-tolerant and -sensitive foxtail millet varieties in response to salinity showed a strong decrease in DNA methylation levels in the salt-tolerant line. Promoter regions and coding sequences of several genes, including ABC transporter, WRKY transcription factor, serine−threonine protein phosphatase, disease resistance, oxidoreductases, cell wall−related enzymes and retrotransposon, and transposase-like proteins, were unmethylated (Pandey et al., 2017).

Cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC) catalyzes a key reaction during glycolysis. GAPC was suggested to positively regulate stress responses in plants. In two different varieties of wheat, Changwu134 and Zhengyin1, *TaGAPC1* transcriptional levels were induced under osmotic and salinity stresses. These transcriptional alterations were accompanied by decreased methylation of CG and CHG cytosines in the promoter region of *TaGAPC1* in the Changwu134 variety (Fei et al., 2017).

Proline is a well-studied osmoprotectant and its cellular accumulation is a typical physiological response for osmotic stress, contributing to osmotic stress tolerance. Studies have shown that proline production is epigenetically regulated (Zhang et al., 2013). In higher plants, proline is synthesized by three key enzymes (P5CS, Δ^1-pyrroline-5-carboxylate synthetase; P5CR, Δ^1-pyrroline-5-carboxylate reductase; and *δ-OAT*, ornithine-δ-aminotransferase) (Verbruggen and Hermans, 2008). After osmotic stress, selfed progenies of rice plants exposed to osmotic stress exhibited higher accumulation of proline compared with selfed progenies of nonstressed plants. Higher proline levels in the progenies of osmotic-stressed plants were correlated with higher transcriptional levels of *P5CS* and *δ-OAT* as well as decreased DNA methylation in *P5CS* and *δ-OAT* when compared with control progenies, suggesting that DNA demethylation might play a key role in transgenerational proline accumulation in response to osmotic stress (Zhang et al., 2013). The rice *MYB91* gene demonstrates another example of epigenetic regulation of proline accumulation in response to salt stress. Exposure of rice plants to saline conditions during vegetative development led to a rapid removal of DNA methylation from the promoter region of *OsMYB91* promoting its expression. Overexpression of *OsMYB91* resulted in enhanced tolerance to salt stress due to elevated proline content and enhanced ROS scavenging capabilities (Zhu et al., 2015).

6.4.3 Changes in chromatin and histone modification patterns under salinity and osmotic stress

Most of the reports dealing with histone modification changes in response to salt stress in plants provide information regarding histone acetylation, as many salt-stress responsive genes were found to be regulated by histone acetylation and deacetylation. On a genome-wide scale, osmotic stress was reported to induce genome-wide accumulation of H3K9ac and H4K5ac in maize seedling (Zhao et al., 2015). In rice, salt-dependent differentially expressed genes (DEGs) were positively correlated with the active chromatin marks H3K9ac and H4K12ac in seedlings and a repressive mark H3K36me3 in roots. Interestingly, H3K27me3 levels had a highly positive correlation with the repression of DEGs in roots, whereas H3K4me3 mainly modulated downregulated genes in seedlings, which suggests a mutual functional exclusion between H3K4me3 and H3K27me3 in seedlings and roots (Zheng et al., 2019a,b).

OsBZ8, a rice bZIP transcription factor, is highly expressed in the salt-tolerant variety Nonabokra but only induced by salt treatment in salt-susceptible variety IR64. Nonabokra and IR64 showed no apparent difference in genomic sequence and nucleosome arrangement in the *OsBZ8* locus. However, the promoter region of *OsBZ8* exhibited high levels of H3K9ac, K3K27ac, and H3K4me3 and low accumulation of H3K27me3 regardless of NaCl treatment in Nonabokra, whereas histone acetylation in the upstream region of *OsBZ8* was induced by salt stress in IR64. Treatment with curcumin, a HAT inhibitor, led to hypoacetylation of the *OsBZ8* promoter and reduced transcription level of *OsBZ8* in both varieties, suggesting that histone modification contributes to the differential expression of *OsBZ8* between Nonabokra and IR64 (Paul et al., 2017).

In maize seedlings, salt stress causes root swelling due to cell enlargement in the elongation zone. Expansins (*EXPA1*, *EXPA3*, *EXPA5*, *EXPB1*, and *EXPB2*), in charge of cell wall loosening and a potential cell wall extension protein *XET1*, were induced in response to salinity, which leads to cell enlargement. Consistently, the promoter regions of *EXPB2* and *XET1* showed elevated H3K9 acetylation accompanied by global accumulation of H3K9ac and H4K5ac under salt stress as well as enhanced expressions of HATs such as *HATB* and *GCN5* (Li et al., 2014). The expression of the *ZmDREB2A* transcription factor under osmotic stress is also regulated by histone acetylation. Similarly, mannitol treatment significantly elevated the acetylation levels on H3K9 and H4K5 marks and chromatin accessibility of *ZmDREB2A* promoter region, thereby leading to a strong induction of *ZmDREB2A* under osmotic stress (Zhao et al., 2014).

Elevated levels of acetylation in H3K9 and H3K27 sites were identified in the coding region of a peroxidase (POX)-encoding gene, which was transcriptionally activated by salt treatments in beet plants. These marks were found to be linked with high *POX* transcript abundance in both sugar beet (*Beta vulgaris*) and wild beet (*Beta maritima*) plants, but the degree and the site of acetylation were different between the species (Yolcu et al., 2016).

On the chromatin level, it was reported that abiotic stress−induced DNA methylation alterations cause chromatin structural changes. In rice plants under salinity and heat stress, it was shown that both stresses induced extensive decondensation of 45S rDNA chromatin as well as an increase in the physical distance between two homologous 5S rDNA loci (Santos et al., 2011).

ZmCHB101, a maize SWI3-type chromatin remodeler, was proposed to affect alternative splicing in response to osmotic stress. This was based on the observation that the efficiencies of the second exon inclusion of *GRMZM2G103647* (*AtbZIP10* homolog) and the third exon inclusion of *GRMZM2G152757* (*AtSAL1* homolog) were enhanced in the wild type under osmotic stress, but not in *ZmCHB101*-RNAi plants. Consistently, osmotic stress−induced H3K36me3 levels, an epigenetic mark associated with exon inclusion, and repressed transcriptional rates in these exon regions only in the wild type, but not in *ZmCHB101*-RNAi lines. Furthermore, overexpression of intact *GRMZM2G103647* (the second exon inclusive) enhanced osmotic stress tolerance in *Arabidopsis*, but the truncated *GRMZM2G103647* (the second exon exclusive) failed to confer tolerance (Yu et al., 2019).

Using a transgenic hairy root system, Wei et al (2017) demonstrated that overexpression of GmPHD6, an H3 code reader, improves salt-stress tolerance in soybean (*Glycine max*) plants. GmPHD6 forms a transcriptional activation complex with LHP1 and affects the expression of dozens of stress-related genes. GmPHD6 is recruited to H3K4 methylation marks and recognizes the G-rich elements in target gene promoters, whereas LHP1 activates the expression of these targets. Among these, the ABA stress ripening induced CYP75B1 and CYP82C4; overexpression of each gene confers stress tolerance in soybean (Wei et al., 2017).

6.5 Perspectives for epigenetics in breeding for abiotic stress−resilient crops

Hundreds of years of genetic breeding have taught us that, in order for a biological system to be employed successfully in breeding schemes, it needs to:

1. be functionally associated with an important trait of agronomic and commercial value;

2. contribute substantially to phenotypic variation of the trait;
3. present a high fraction of high-penetrance variation, that is, transgenerational stability; and
4. have efficient tools for molecular identification, that is, molecular markers.

As described in the previous sections of this chapter, there is growing evidence that epigenetic mechanisms are involved in response to abiotic stresses in crop plants (Figs 6.1 and 6.2). Thus epigenetic factors may be exploited for

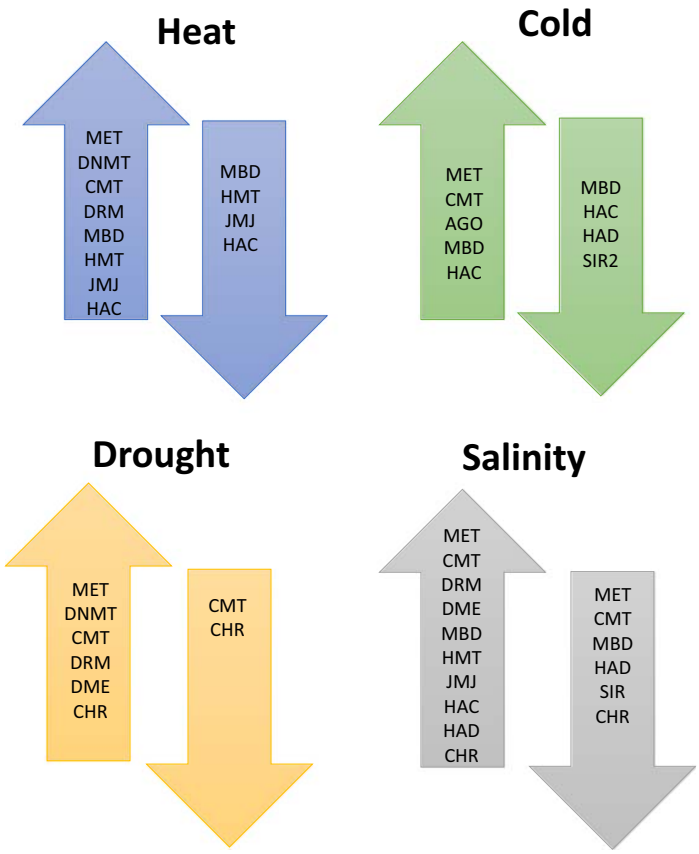

FIGURE 6.1 Transcriptional response of the epigenetic machinery to heat, cold, drought, and salinity stresses in crop plants. The observations reviewed in this chapter are graphically summarized by gene families.

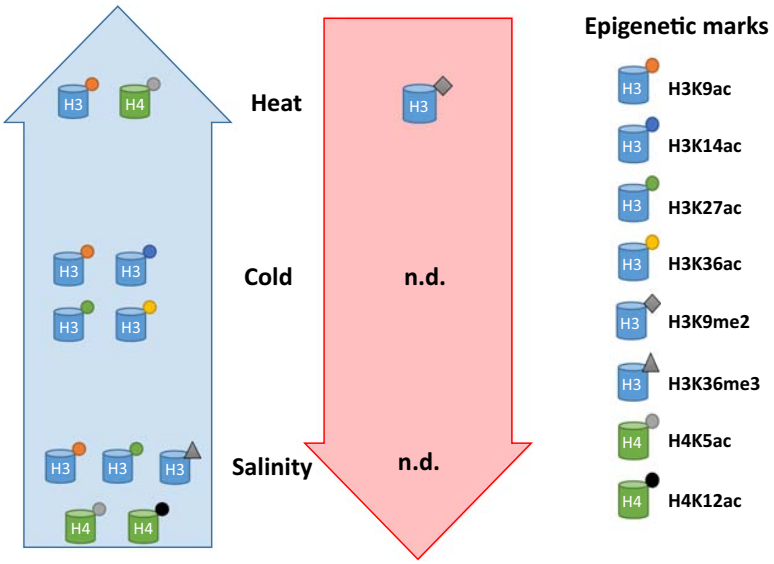

FIGURE 6.2 Changes in histone modifications in heat, cold, and salinity stresses reported in crop plants. The observations reviewed in this chapter are graphically summarized by types of histone modifications and direction of the response: increase (*blue arrow frame*) or decrease (*pink arrow frame*). No reports were found on histone modifications in response to drought stress in crops. *n.d.*, No data.

increasing crop tolerance to environmental challenges, which is timely needed in a changing climate scenario. Moreover, the dynamic nature of epigenetic marks could make them more readily responsive to changing environmental conditions. Considering agriculturally relevant environments, the response of the epigenome to combined stresses needs to be further explored.

The relevance of epigenetic regulation to crop breeding was demonstrated by its effect on growth vigor and yield in tomatoes (Yang et al., 2015). Silencing of the *MSH1* gene in tomato using RNAi resulted in enhanced plant growth and productivity, even in the absence of the transgene. Total fruit weight and number were increased under field conditions. In addition, under high-temperature field conditions, the MSH1-silenced line produced a higher proportion of red ripe fruits, similar to the FLA8044 heat-tolerant cultivar. These phenotypic changes were linked with DNA methylation as the methylation inhibitor 5-azacytidine (5-AzaC) repressed the observed phenotypes (Yang et al., 2015). In a later study, it was shown that MET1 and HDAC6 functions are necessary for the effect (Kundariya et al., 2020). In *Arabidopsis*, *msh1* mutants displayed enhanced tolerance to drought and salt stress and increased susceptibility to freezing temperatures (Raju et al., 2018). This example, albeit unique, highlights the link between epigenetics and multiple important traits, as well as the potential of epigenetics in crop breeding. It is to be expected that additional cases will be revealed in the coming years.

Several studies support the link between epigenetic marks, mainly DNA methylation, and phenotypic variation and heritability. The RNAi suppression of *MSH1* in tomato resulted in phenotypic variability for developmental and stress-response phenotypes that were stably inherited for several generations (Yang et al., 2015; Kundariya et al., 2020). This system was replicated in soybean, generating DNA methylation−altered lines with enhanced growth, increased yield, and higher stability across environments in field trials (Raju et al., 2018). In canola (*B. rapa*), the methylation inhibitor 5-AzaC was used to generate a stochastic chemically induced hypomethylated population that presented significantly higher phenotypic variation for several yield traits, including seed size, weight, oil, and protein content (King et al., 2010). The availability of methyltransferase mutants in *Arabidopsis* enabled the development of epigenetic recombinant inbred lines (epiRILs), which could be tested for phenotypic variation (Reinders et al., 2009; Johannes et al., 2009; Catoni and Cortijo, 2018). The *met1*-based epiRIL lines presented differences in phenotypic variation for flowering time, vegetative biomass, and response to salt stress and susceptibility to *Pseudomonas* infection (Reinders et al., 2009). In the *ddm1*-based epiRIL lines, an increased phenotypic variation, was observed for flowering time and plant height (Johannes et al., 2009). This population was used for the identification of bona fide epigenetic quantitative trait loci (epiQTL) for flowering time and primary root length. These epiQTL accounted for 60%−90% of the heritability for the two complex traits and was reproducible and successfully subjected to artificial selection (Cortijo et al., 2014). In addition, an extensive phenotyping of 6000 *Arabidopsis* plants harboring DNA methylation alterations revealed high heritable phenotypic diversity for seed-production traits (Roux et al., 2011). These studies pave the way for trait selection in crop breeding. In that aspect, it is to be noted that due to the dynamic nature of epigenetic marks, there is a possibility that the mark might revert in advanced generations; therefore a tight molecular monitoring and epigenotyping is crucial. Nonetheless, it is suggested that specific genetic features may determine the degree of transgenerational stability (Catoni et al., 2017). Identification and characterization of such features will be beneficial for trait selection in epibreeding.

To date, there are various tools for the detection and monitoring of epigenetic marks (Ay et al., 2014; Hossain et al., 2017; Perrone and Martinelli, 2020). On-going advances in whole-genome sequencing technologies allow fast and cost-effective data generation (Niederhuth et al., 2016). In terms of data accumulation, crop varieties are still lagging behind model plants, but not for long. Beyond generating epigenomic data, there is a need for the identification and characterization of potential targets for epibreeding. This target should be a specific, stable, epigenetic feature (e.g., DNA methylation pattern at a specific genomic site) that is associated with a desired trait. This is obviously a challenging task, mainly due to the dynamic nature of epigenetic features. Process-based models may be of assistance in the evaluation of the stability and heritability of epigenetic variations, as demonstrated for lycopene accumulation in tomatoes (Gallusci et al., 2017). Following the identification of targets for epibreeding, the remaining challenge would be a directed manipulation of epigenetic marks in crop plants. As successfully shown in mammalian systems and recently in *Arabidopsis*, the CRISPR-dCas9 system can be used to direct a methyltransferase or a demethylase to a specific locus in the genome, to enhance or reduce DNA methylation, respectively (Hilton et al., 2015; Gallego-Bartolomé et al., 2018; Papikian et al., 2019; Gallego-Bartolomé, 2020).

6.6 Summary

Over the past century, plant breeding relayed on artificial selection on natural and/or induced variation for the integration of desired traits into elite varieties. However, it is well established that modern breeding led to thinning plant

genetic diversity (Zamir, 2001). The narrow genetic base of crop species and rapid depletion of genetic variation in landraces and related species make further improvement of crop yield under changing environments increasingly difficult through traditional approaches. Therefore novel strategies to expand the sources of heritable phenotypic variation are necessary (Giovannoni, 2016). Although heritable phenotypic variation is primarily driven by genetic variation, accumulated data indicate that epigenetic factors such as DNA methylation and histone modifications affect gene expression in a heritable manner in model and crop plants (Cortijo et al., 2014; Reinders et al., 2009; Yang et al., 2015; Raju et al., 2018). These factors can provide an additional layer of variation on top of existing genetic variation and be used for future development of abiotic stress−resilient crop varieties.

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