Epigenetics and the Production of Cultivars

Subjects: Biology

Contributor: Haley Turcotte, Jean-Sébastien Parent, Julia Hooker, Bahram Samanfar

As the global population continues to grow, food demand will be reaching levels which current agricultural practices cannot meet. This projected demand combined with the negative impacts of climate change on crop production calls for more careful breeding efforts to develop better adapted plants more tolerant to climate fluctuations. Fortunately, the development of molecular biology techniques like genome, transcriptome and epigenome sequencing now offer new approaches to help classical breeding meet these challenges.

Keywords: epigenetics ; DNA methylation ; epi-markers ; plant breeding

1. Introduction

Plant breeding has allowed for tremendous advancements in crop quality and yield improvement for more than a century. However, rapidly growing global population and intensifying climate change magnify the need for high quality, high-yield crops with low requirements for inputs such as fertilizers and pesticides [1]. As the global population will approach 10 billion people over the next 30 years, it is increasingly evident that global food demands are reaching levels that current agricultural practices cannot meet [1]. Areas for improvement will include, but will not be limited to pest and disease management, reduced inputs (ex.: nitrogen and phosphorus) and adaptation to new growing areas and changing environments. In order to do this, new approaches need to be integrated into classical breeding programs. These include molecular approaches such as genomics, transcriptomics, and epigenetics.

2. Epigenetics and Its Potential Applications in Plant Breeding

2.1. Introduction to Epigenetics

Most cells in a given organism share the same genes yet they can be drastically different from one another. This is because not all genes are activated in all cells at any given time. The epigenetic context of a gene, or the accessibility of a gene to transcription, can reveal if the gene is likely to be active or not [2]. Indeed, the nuclear genome is found as a dense mixture of long strings of DNA wrapped around specialized proteins called histones to form superstructures known as nucleosomes [3]. These nucleosomes can be further packed together to make even denser 3D structures at times visible under the microscope which inspired the name “chromatin”. The modifications and proteins associated with the DNA are called epigenetic modifications, or marks, and they can influence gene expression within the cell. Epigenetic modifications can occur spontaneously in the genome and can be lost just as randomly presenting the concept of stability. It follows that the influences of epigenetic modifications on gene expression can only be passed from mother cell to daughter cell and from one generation to the next if the underlying modifications are actively maintained. As a strategy for adaptation, both genome sequence-dependent (genetic) and independent (epigenetic) variability are used in combination to create variability at the phenotypic level [4]. This, in turn, maximizes the chances of survival of at least some progeny under different conditions and provides a wider pool of phenotypic variation to be used for plant breeding [4][5].

2.2. Molecular Mechanisms

Because DNA is densely packed in the nucleus, for any gene that needs to be actively transcribed into mRNA, local DNA has to be unpacked and made accessible to the transcriptional machinery [2][6]. Epigenetic modifications therefore regulate accessibility to the chromatin and gene activity. This can take the form of direct modification to the DNA, the addition of histone variants, histone post-translational modification, chromatin super-structure, etc. [7]. Because of their role, these modifications can provide useful information about gene activity (or lack thereof) at the transcriptional level. Contrary to the actual DNA sequence, epigenetic marks can be removed over time but some of them are maintained through cell divisions and even passed down to the next generation. These epigenetic marks serve in a variety of processes including DNA replication and repair, stem cell maintenance, tissue and organ development and differentiation, as well as initiating responses to environmental stimuli [8]. Indeed, the flexibility of epigenetic modifications makes them an

ideal mechanism to rapidly adapt the transcriptional program of a cell to changes in environmental conditions. As such, epigenetic context might be particularly useful when trying to map environment related traits. Finally, repressive epigenetic modifications are used to silence repeated sequences like transposable elements that can jeopardize genomic integrity [9].

2.3. DNA Methylation

Epigenetic marks come in many flavors but this entry will focus specifically on the most widely studied epigenetic mark, DNA methylation. DNA methylation is one of the most widely studied epigenetic modifications owing to its abundance and stability as well as the ease at which it can be detected and assessed using various methods [7][10]. In short, a methyl chemical group is covalently bound to the position 5 carbon atom of the aromatic ring of the cytosine base (that therefore becomes 5-methylcytosine). This reaction is catalyzed by specialized enzymes called DNA methyltransferases and can only be reversed by another set of specialized enzymes, making it a very stable modification once applied. However, methylation must be propagated when new strands of DNA are being synthesized during replication or methylation will be lost due to dilution (a form of demethylation) [11]. In plants, DNA methylation is found to occur in three different sequence contexts, CG, CHG, and CHH (H= A, T or C) all of which have their own underlying mechanisms and biological impact [8][12][13]. Consequently, the different contexts are useful to decode the epigenetic information encountered in different genomic locations. Indeed, while mCG is found both on expressed genes and repressed sequences, mCHG and mCHH are mostly found on the latter.

2.4. Quantifying DNA Methylation

Because it is covalently associated with DNA, DNA methylation is more readily quantified than other epigenetic marks and there are different convenient techniques to do this. In order to choose an appropriate technique to quantify DNA methylation, one must consider: (1) the type of biological sample being analyzed, (2) the desired outcome (identification of unknown epigenetic marks vs. assessment of methylated regions within genes of interest), and (3) the availability and cost of the technology [14][15]. Restriction enzyme-based approaches involve the use of methylation-sensitive restriction enzymes whose activity are influenced by the presence of the methyl group on DNA [14]. Restriction endonucleases used in this method can include MspI, HpaII, NotI, SmaI and McrBC. For example, HpaII recognizes the CCGG restriction sites in genomic DNA when it is unmethylated however, the addition of methyl group (CmCGG or mCCGG) to the restriction site prevents HpaII activity. Thus, the different treatment of methylated and non-methylated sequences can be used to assess the methylation status of a locus, as long as the restriction sequence is present. Cleavage of the DNA molecule prevents PCR amplification thus creating a signal that can easily be detected for the presence or absence of DNA methylation. Alternatively, genomic DNA can be treated with sodium bisulfite resulting in the conversion of unmethylated cytosines to uracils [14]. During PCR amplification of the region of interest, uracils are read by DNA polymerase as thymines which can be detected by melting curve analysis, Sanger sequencing or Amplicon sequencing.

When there are many sequences of interest or if they are unknown, a genome-wide approach needs to be considered. Affinity enrichments-based approaches use antibodies designed to bind to methylcytosines and to pull them down so that methylated DNA is enriched [14]. Once the methylated DNA has been isolated, bisulfite sequencing is typically used to sequence the methylated regions. This reduces the cost of sequencing by focusing on regions where high methylation is present. Whole-genome bisulfite sequencing (WGBS) is still the predominant approach used to precisely quantify DNA methylation genome-wide. This method is frequently used because it is easy, fast, and provides highly accurate results that can be used to identify all differentially methylated regions (DMRs) existing between two samples [16]. Although bisulfite sequencing has been used as the gold standard approach for methylome analysis, there are limitations to this technique. Sodium bisulfite is a reactive chemical that damages the DNA molecules which can lead to inaccurate interpretations or destruction of limited samples. Enzymatic methyl-seq is a new approach designed to minimize DNA damage by using enzymes (including TET2 and APOBEC2) rather than chemicals to convert the methylated bases [17][18]. Using this method, DNA integrity can be better maintained resulting in more accurate readings and mapping efficiencies. This newly-established approach is a promising alternative to bisulfite sequencing.

Once information on methylated sites is obtained, downstream analysis can proceed to identify DMRs. This involves comparing methylated sites ratios between samples using a specific distance criteria and statistical tests [19]. Once DMRs have been identified, they can be linked to quantitative traits segregating in a population much like QTLs. Identification of DMRs from sequencing data requires consideration of various factors to optimize accurate interpretation and reduce bias. This includes (1) considering spatial correlation between methylation levels of neighboring methylated sites to more accurately estimate corresponding hypomethylated sites, (2) considering sequencing depth which takes into account sampling variability during sequencing, and (3) considering biological variation among samples which will minimize the number of false positives in the results [19].

2.5. Variation in DNA Methylation Patterns

With all these techniques, it was found that DNA methylation patterns are dynamic and exhibit considerable variation in the levels of methylation and the nature of the sequences being methylated. Indeed, much like the DNA sequence in the genome, the epigenome can also accumulate heritable changes in the lifetime of an organism. However, these epigenetic changes occur at a much faster rate than mutations in the DNA sequence [20]. This has led to the hypothesis that heritable epi-mutations can be selected naturally or artificially like their DNA mutation counterparts. Interestingly, methylation patterns are also shaped by the environment and thus can change in response to abiotic and biotic stress exposure [21][22][23][24]. This brings the possibility to store information about the conditions an organism encountered in its development. Consequently, pattern differences can be divided into two main groups; (1) developmental, whereby individuals exhibit specific patterns associated with different stages of growth and development and (2) acquired, whereby individuals exhibit specific or random patterns related to specific conditions encountered in their environment.

2.5.1. Developmental Epigenetic Modifications

DNA methylation is a feature of constitutive heterochromatin in many eukaryotes where it plays an important structural role. In some organisms, like in flowering plants, it is also involved in developmental processes including reproduction, seed development, germination, tissue differentiation and growth. [25][26][27]. Understanding methylation patterns at different developmental stages is important in linking traits to particular methylation markers. For traits relating to a particular tissue, like seed size, it may be worth looking for specific epigenetic changes in that tissue and even selecting a precise time window when it is most abundant for higher resolution. Indeed, it was recently published that DNA methylation levels differ greatly at different stages of seed development in a variety of species [26][28][29][30][31]. Specifically, DNA methylation levels seem to increase throughout the stages of seed development. Fruit ripening is another developmental trait that comes with important epigenetic modifications and has been studied in various plant species including tomato, apple, and orange [32][33][34].

DNA methylation also exhibits extensive variation among different tissues and cell types particularly in vegetative organs such as leaves, shoots, and roots. Within Arabidopsis, an analysis of the methylation status within the root apical meristem revealed differences in methylation status among various cell types [35]. For example, there is widespread hypermethylation within the columella cells when compared to the epidermis, cortex, endodermic, and stele cells [35]. In soybean, differences in methylation patterns between single-celled root hairs and multicellular stripped roots were assessed [36]. Analyses revealed significant differences in DNA methylation patterns, particularly in the CHH methylation context, between the two types of cells [36]. DNA methylation patterns also greatly differed between roots and shoot tissues in Arabidopsis and also other Brassicaceae [37][38]. In inbred lines of maize, patterns of DNA methylation greatly differed among tassel, bracteal leaf, and ear leaf tissues [39]. Moreover, during fruit development in tomato, the fruit and the leaf tissues displayed different methylation levels [32]. In addition to epigenetic variation among vegetative organs, there also seems to be epigenetic variation among different tissues within the seed. Particularly in seeds of Arabidopsis and rice, the endosperm displayed lower levels of DNA methylation relative to the embryo [40][41]. These examples demonstrate that DNA methylation can play an important role in tissue/cell differentiation. By comparing levels of DNA methylation among various developmental stages and tissue/cell types, researchers can appreciate the magnitude of epigenetic variation in a developmental and tissue-specific manner. This information is particularly useful when trying to produce high resolution epigenetic studies especially when targeting a specific trait.

2.5.2. Acquired Epigenetic Modifications

Epigenetic marks can also be acquired or lost in response to environmental cues, including abiotic and biotic stresses. These epigenetic modifications can be heritable and allow for plants to rapidly adapt their development to changing environments and play important roles in pathogen defense and abiotic tolerance (salinity, drought, high and low temperatures, etc.) [42]. Environmentally stimulated changes in DNA methylation have been reported in many crop varieties including rice [43], soybean [44], maize [45], and wheat [46]. In soybean, DMRs among root hair cells and stripped root cells were assessed following a heat treatment [36]. At room temperature, root hair cells showed hypermethylation at specific DMRs when compared to stripped root cells which showed hypomethylation. In response to a heat stress, hypomethylation occurred at DMRs in both cell types revealing that root hair cells may be more sensitive to fluctuations in temperature. Furthermore in wheat, individuals with a salt-tolerant genotype have higher methylation levels in the root tissues which has been associated with restricting Na+ entry from the soil into the root tissues [47]. Analyzing these changes can provide candidate markers for detecting plant stress responses within a population.

While these epigenetic changes can function in momentarily tuning gene expression to adapt to a new condition, they can also act as ”stress memory” [48]. This stress memory allows for individuals to respond more quickly to recurring stimuli by

priming gene expression patterns for more rapid adjustments [49]. Studies have shown that Arabidopsis exhibits heat-stress memory whereby heat-inducible genes remain activated through hypermethylation for at least two days following removal of the stress [50]. This primes the individual for a quicker response to recurring heat stress. Moreover, epigenetic cross-adaptation can also occur whereby exposure to one stress can lead to resistance to other stresses [51]. This was tested in cold-tolerant Brassica rapa, to see whether individuals also showed heat tolerance. DNA methylation patterns among four candidate genes were compared between control and cold-acclimated (CA) plants [51]. It was found that the promoter regions of these four candidate genes exhibited demethylation resulting in increased gene expression in CA plants [51]. This demethylation was linked to elevated levels of organic acids and enhanced photosynthesis which authors concluded were contributing factors to enhanced heat tolerance and higher growth rates in CA plants [51]. Such study suggests that DNA methylation plays a role in cross-adaptation within plants and can help generate plants resilient to extreme temperatures. They also show that certain epigenetic modifications are only revealed in certain specific conditions.

References

1. Hickey, L.T.; Hafeez, A.N.; Robinson, H.; Jackson, S.A.; Leal-Bertioli, S.C.M.; Tester, M.; Gao, X.; Godwin, I.; Hayes, B.; Wulff, B.B.H. Breeding crops to feed 10 billion. Nat. Biotechnol. 2019, 37, 744–754.
2. Klemm, S.L.; Shipony, Z.; Greenleaf, W.J. Chromatin accessibility and the regulatory epigenome. Nat. Rev. Genet. 2019, 20, 207–220.
3. Samo, N.; Ebert, A.; Kopka, J.; Mozgová, I. Plant chromatin, metabolism and development—An intricate crosstalk. Curr. Opin. Plant Biol. 2021, 61, 102002.
4. Tirnaz, S.; Batley, J. Epigenetics: Potentials and Challenges in Crop Breeding. Mol. Plant 2019, 12, 1309–1311.
5. Gallusci, P.; Dai, Z.; Génard, M.; Gauffretau, A.; Leblanc-Fournier, N.; Richard-Molard, C.; Vile, D.; Brunel-Muguet, S. Epigenetics for Plant Improvement: Current Knowledge and Modeling Avenues. Trends Plant Sci. 2017, 22, 610–623.
6. Kono, H.; Ishida, H. Nucleosome unwrapping and unstacking. Curr. Opin. Struct. Biol. 2020, 64, 119–125.
7. Agarwal, G.; Kudapa, H.; Ramalingam, A.; Choudhary, D.; Sinha, P.; Garg, V.; Singh, V.K.; Patil, G.B.; Pandey, M.K.; Nguyen, H.T.; et al. Epigenetics and epigenomics: Underlying mechanisms, relevance, and implications in crop improvement. Funct. Integr. Genom. 2020, 20, 739–761.
8. Bartels, A.; Han, Q.; Nair, P.; Stacey, L.; Gaynier, H.; Mosley, M.; Huang, Q.Q.; Pearson, J.K.; Hsieh, T.F.; Charles An, Y.Q.; et al. Dynamic DNA methylation in plant growth and development. Int. J. Mol. Sci. 2018, 19, 2144.
9. Okamoto, H.; Hirochika, H. Silencing of transposable elements in plants. Trends Plant Sci. 2001, 6, 527–534.
10. Ito, T.; Nishio, H.; Tarutani, Y.; Emura, N.; Honjo, M.N.; Toyoda, A.; Fujiyama, A.; Kakutani, T.; Kudoh, H. Seasonal stability and dynamics of dna methylation in plants in a natural environment. Genes 2019, 10, 544.
11. Bochtler, M.; Kolano, A.; Xu, G.-L. DNA demethylation pathways: Additional players and regulators. Bioessays 2017, 39, 1–13.
12. Anderson, S.N.; Zynda, G.J.; Song, J.; Han, Z.; Vaughn, M.W.; Li, Q.; Springer, N.M. Subtle perturbations of the maize methylome reveal genes and transposons silenced by chromomethylase or RNA-directed DNA methylation pathways. G3 Genes Genomes Genet. 2018, 8, 1921–1932.
13. Cheng, C.; Tarutani, Y.; Miyao, A.; Ito, T.; Yamazaki, M.; Sakai, H.; Fukai, E.; Hirochika, H. Loss of function mutations in the rice chromomethylase OsCMT3a cause a burst of transposition. Plant J. 2015, 83, 1069–1081.
14. Pajares, M.J.; Palanca-Ballester, C.; Urtasun, R.; Alemany-Cosme, E.; Lahoz, A.; Sandoval, J. Methods for analysis of specific DNA methylation status. Methods 2021, 187, 3–12.
15. Kurdyukov, S.; Bullock, M. DNA methylation analysis: Choosing the right method. Biology 2016, 5, 3.
16. Gravina, S.; Ganapathi, S.; Vijg, J. Single-cell, locus-specific bisulfite sequencing (SLBS) for direct detection of epimutations in DNA methylation patterns. Nucleic Acids Res. 2015, 43, e93.
17. Vaisvila, R.; Ponnaluri, V.K.C.; Sun, Z.; Langhorst, B.W.; Saleh, L.; Guan, S.; Dai, N.; Campbell, M.A.; Sexton, B.S.; Marks, K.; et al. Enzymatic methyl sequencing detects DNA methylation at single-base resolution from picograms of DNA. Genome Res. 2021, 31, 1280–1289.
18. Suhua, F.; Zhenhui, Z.; Ming, W.; Steven, E.J. Efficient and accurate determination of genome-wide DNA methylation patterns in Arabidopsis with enzymatic methyl sequencing. Epigenetics Chromatin 2021, 13, 1–17.

19. Shafi, A.; Mitrea, C.; Nguyen, T.; Draghici, S. A survey of the approaches for identifying differential methylation using bisulfite sequencing data. Brief. Bioinform. 2017, 19, 737–753.

20. Noshay, J.M.; Springer, N.M. Stories that can't be told by SNPs; DNA methylation variation in plant populations. Curr. Opin. Plant Biol. 2021, 61, 101989.

21. Rajkumar, M.S.; Shankar, R.; Garg, R.; Jain, M. Bisulphite sequencing reveals dynamic DNA methylation under desiccation and salinity stresses in rice cultivars. Genomics 2020, 112, 3537–3548.

22. Ashapkin, V.V.; Kutueva, L.I.; Aleksandrushkina, N.I.; Vanyushin, B.F. Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. Int. J. Mol. Sci. 2020, 21, 7457.

23. Kong, L.; Liu, Y.; Wang, X.; Chang, C. Insight into the role of epigenetic processes in abiotic and biotic stress response in wheat and barley. Int. J. Mol. Sci. 2020, 21, 1480.

24. Guo, H.; Wu, T.; Li, S.; He, Q.; Yang, Z.; Zhang, W.; Gan, Y.; Sun, P.; Xiang, G.; Zhang, H.; et al. The methylation patterns and transcriptional responses to chilling stress at the seedling stage in rice. Int. J. Mol. Sci. 2019, 20, 5089.

25. Grover, J.W.; Kendall, T.; Baten, A.; Burgess, D.; Freeling, M.; King, G.J.; Mosher, R.A. Maternal components of RNA-directed DNA methylation are required for seed development in Brassica rapa. Plant J. 2018, 94, 575–582.

26. Kawakatsu, T.; Nery, J.R.; Castanon, R.; Ecker, J.R. Dynamic DNA methylation reconfiguration during seed development and germination. Genome Biol. 2017, 18, 171.

27. Ikeuchi, M.; Iwase, A.; Sugimoto, K. Control of plant cell differentiation by histone modification and DNA methylation. Curr. Opin. Plant Biol. 2015, 28, 60–67.

28. Lin, J.Y.; Le, B.H.; Chen, M.; Henry, K.F.; Hur, J.; Hsieh, T.F.; Chen, P.Y.; Pelletier, J.M.; Pellegrini, M.; Fischer, R.L.; et al. Similarity between soybean and Arabidopsis seed methylomes and loss of non-CG methylation does not affect seed development. Proc. Natl. Acad. Sci. USA 2017, 114, E9730–E9739.

29. Xing, M.Q.; Zhang, Y.J.; Zhou, S.R.; Hu, W.Y.; Wu, X.T.; Ye, Y.J.; Wu, X.X.; Xiao, Y.P.; Li, X.; Xue, H.W. Global analysis reveals the crucial roles of DNA methylation during rice seed development. Plant Physiol. 2015, 168, 1417–1432.

30. Bouyer, D.; Kramdi, A.; Kassam, M.; Heese, M.; Schnittger, A.; Roudier, F.; Colot, V. DNA methylation dynamics during early plant life. Genome Biol. 2017, 18, 179.

31. Rajkumar, M.S.; Gupta, K.; Khemka, N.K.; Garg, R.; Jain, M. DNA methylation reprogramming during seed development and its functional relevance in seed size/weight determination in chickpea. Commun. Biol. 2020, 3, 340.

32. Zhong, S.; Fei, Z.; Chen, Y.R.; Zheng, Y.; Huang, M.; Vrebalov, J.; McQuinn, R.; Gapper, N.; Liu, B.; Xiang, J.; et al. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat. Biotechnol. 2013, 31, 154–159.

33. Daccord, N.; Celton, J.M.; Linsmith, G.; Becker, C.; Choisne, N.; Schijlen, E.; van de Geest, H.; Bianco, L.; Micheletti, D.; Velasco, R.; et al. High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. Nat. Genet. 2017, 49, 1099–1106.

34. Huang, H.; Liu, R.; Niu, Q.; Tang, K.; Zhang, B.; Zhang, H.; Chen, K.; Zhu, J.K.; Lang, Z. Global increase in DNA methylation during orange fruit development and ripening. Proc. Natl. Acad. Sci. USA 2019, 116, 1430–1436.

35. Kawakatsu, T.; Stuart, T.; Valdes, M.; Breakfield, N.; Schmitz, R.J.; Nery, J.R.; Urich, M.A.; Han, X.; Lister, R.; Benfey, P.N.; et al. Unique cell-type-specific patterns of DNA methylation in the root meristem. Nat. Plants 2016, 2, 16058.

36. Hossain, M.S.; Kawakatsu, T.; Kim, K.D.; Zhang, N.; Nguyen, C.T.; Khan, S.M.; Batek, J.M.; Joshi, T.; Schmutz, J.; Grimwood, J.; et al. Divergent cytosine DNA methylation patterns in single-cell, soybean root hairs. New Phytol. 2017, 214, 808–819.

37. Widman, N.; Feng, S.; Jacobsen, S.E.; Pellegrini, M. Epigenetic differences between shoots and roots in Arabidopsis reveals tissue-specific regulation. Epigenetics 2014, 9, 236–242.

38. Seymour, D.K.; Koenig, D.; Hagmann, J.; Becker, C.; Weigel, D. Evolution of DNA Methylation Patterns in the Brassicaceae is Driven by Differences in Genome Organization. PLoS Genetics 2014, 10, e1004785.

39. Lu, Y.; Rong, T.; Cao, M. Analysis of DNA methylation in different maize tissues. J. Genet. Genom. 2008, 35, 41–48.

40. Zemach, A.; Kim, M.Y.; Silva, P.; Rodrigues, J.A.; Dotson, B.; Brooks, M.D.; Zilberman, D. Local DNA hypomethylation activates genes in rice endosperm. Proc. Natl. Acad. Sci. USA 2010, 107, 18729–18734.

41. Hsieh, T.F.; Ibarra, C.A.; Silva, P.; Zemach, A.; Eshed-Williams, L.; Fischer, R.L.; Zilberman, D. Genome-wide demethylation of Arabidopsis endosperm. Science 2009, 324, 1451–1454.

42. Gahlaut, V.; Zinta, G.; Jaiswal, V.; Kumar, S. Quantitative Epigenetics: A New Avenue for Crop Improvement. Epigenomes 2020, 4, 25.

43. Pan, Y.; Wane, W.; Zhao, X.; Zhu, L.; Fu, B.; Li, Z. DNA methylation alterations of rice in response to cold stress. Omics J. 2011, 4, 364–369.
44. Zhang, W.; Wang, N.; Yang, J.; Guo, H.; Liu, Z.; Zheng, X.; Li, S.; Xiang, F. The salt-induced transcription factor GmMYB84 confers salinity tolerance in soybean. Plant Sci. 2020, 291, 110326.
45. Qian, Y.; Hu, W.; Liao, J.; Zhang, J.; Ren, Q. The Dynamics of DNA methylation in the maize (Zea mays L.) inbred line B73 response to heat stress at the seedling stage. Biochem. Biophys. Res. Commun. 2019, 512, 742–749.
46. Gardiner, L.J.; Joynson, R.; Omony, J.; Rusholme-Pilcher, R.; Olohan, L.; Lang, D.; Bai, C.; Hawkesford, M.; Salt, D.; Spannagl, M.; et al. Hidden variation in polyploid wheat drives local adaptation. Genome Res. 2018, 28, 1319–1332.
47. Singh, A.; Bhushan, B.; Gaikwad, K.; Yadav, O.P.; Kumar, S.; Rai, R.D. Induced defence responses of contrasting bread wheat genotypes under differential salt stress imposition. Indian J. Biochem. Biophys. 2015, 52, 75–85.
48. Lämke, J.; Bäurle, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. 2017, 18, 124.
49. Kumar, G.; Rattan, U.K.; Singh, A.K. Chilling-mediated DNA methylation changes during dormancy and its release reveal the importance of epigenetic regulation during winter dormancy in Apple (Malus x domestica Borkh.). PLoS ONE 2016, 11, e0149934.
50. Bäurle, I. Plant Heat Adaptation: Priming in response to heat stress. F1000Research 2016, 5, 694.
51. Liu, T.; Li, Y.; Duan, W.; Huang, F.; Hou, X. Cold acclimation alters DNA methylation patterns and confers tolerance to heat and increases growth rate in Brassica rapa. J. Exp. Bot. 2017, 68, 1213–1224.
