

## A REVIEW OF THE DYNAMICS ON DNA METHYLATION AND ITS ROLE IN PLANT DEVELOPMENT

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### Abstract

In plants, the most prevalent and significant phenomenon is DNA methylation. *De novo* methylation in plant genome is generally lower than in animals. Natural environments controlled by biotic stresses also play a critical evolutionary role in controlling the pathways that regulate responses to stress, gene activity, and plant growth. The present review discusses various features of developmental changes resulting from genetic methylation in plants and particular genes involved in this process. Methylated DNA sequences primarily control changes from the vegetative to the reproductive cycle. In plants, DNA methylation regulates different developmental functions such as the morphology of leaves, flowering time, identity of different floral organs, ability to fertilize, restriction of transposable elements, and activation or blockage of transgenes multiple genes, their receptors, and protein products. For the development of endosperm and planning of the flowering period, also monitor time by this mechanism. DNA methylation genes for plant immunity have defensive functions that address environmental stimuli such as a parasite, pathogen or cold, stress, heat, and drought. These genes describe how complete DNA methylation manages a plant's whole development and progression. This overview provides insight into progress in DNA methylation in regulating developmental pathways of *Arabidopsis thaliana* and the evolution of other plant species. Our findings suggest that research in proteomic and genomic analysis can empower scientists to understand the methylation patterns and the role of methylation variation in biological processes during evolution. In addition, it provides collective information on molecular mechanisms that underlie various plant functions.

**Key words:** DNA Methylation; *Arabidopsis thaliana*; Epigenetics; Gene Expression; Omics.

### Introduction

Plants are biological systems that are important for us and our environment. In prokaryotes and eukaryotes, DNA methylation is the conserved epigenetic modification (Beaulaurier *et al.*, 2019). In plants, *de novo* methylation of DNA occurs when the methyl is added to the cytosine C5 to form 5-methylcytosine (5mC). This form of epigenetic modification is closely related to the silence of transposable elements (TE), the stability of the genome, and the inactivation of the X chromosome. DNA methylation regulates gene expression in the promoter region and is essential for plant development and species improvement (Jost *et al.*, 2013). DNA methylation is very well conserved in plants (Bird *et al.*, 2002). All other organisms, including animals and fungi, exhibit a distinct variation of cytosine methylation (Law *et al.*, 2010; Chan *et al.*, 2005). It does not modify the original DNA sequences or the DNA arrangement but affects gene expression in an inherited form (Nagyimihaly *et al.*, 2017).

The typical development of all living things requires well-tailored, sequenced, and precise gene expression patterns. *Cis* and *trans*-DNA sequences control spatial and appropriate coordination in gene expression. Studies have shown that special epigenetics conventions are part of developmental and progression mechanisms. These changes during DNA methylation are mitotically and meiotically hereditary and reversible in gene expression associated with plant development (Steimer *et al.*, 2004).

Of the significant alteration of epigenetics, cytosine methylation is associated with and linked to many cellular apparatus (Chan *et al.*, 2005; Gehring *et al.*, 2007). In plants, cytosine methylation regulates development and growth, gene expression, genome integrity, and responses to various

environmental stimuli (Grant *et al.*, 2005). The methylation develops from a methyl transfer group to the fifth carbon of cytosine. Within plants, three arrangements are present for cytosine methylation, which affect both the systems and the methylation capacity. The most significant pre-arrangement of the modulation is inside a CpG. Under stress and during development, gene expression regulates by cytosine methylation (Bird, 2002; Zhang *et al.*, 2016; Zilberman *et al.*, 2007). There is sufficient evidence that cytosine methylation controls gene regulation, which controls various biological pathways through plant development (Gehring *et al.*, 2009). There are different levels, patterns, and designs that cytosine methylation contains to start several processes, such as an increase in the size of the plant, its flowering season and time, its reproductive cycle, and its vegetative cycles (Johannes *et al.*, 2009).

A calculated number of 5-methylcytosine is present in the plant genome. Over 20-30% of flowering plant genomes in angiosperms methylated cytosines were found (Niederhuth *et al.*, 2016). Only a small quantity of methyl cytosines, such as CpG (5' C phosphate G 3') and CpNpG (a trinucleotide), is present at other sites (Meyer *et al.*, 1994). The degree of methylated cytosine in low-level plants. That represents more than 30% and is not present in the CpG and CpNpG positions. However, it also occurs in DNA sequences in asymmetrical sequences (Meyer *et al.*, 1994). For example, the Guanosine cytosine (GC) content in tobacco is about 40.3%, and in tomatoes is about 40.71% (Messegueur *et al.*, 1991). It is comparatively higher in animals compared to plant genomes. Its levels are higher at the original codon but lower than the coding region.

Proteomics is a universal field of research that contributes to understanding the natural and environmental stresses at subcellular levels. Various studies reported that

plants respond differently to activation signals to deal with environmental stress. Researchers contributed their work on *Arabidopsis thaliana* as a model organism in this area. They revealed different aspects of signaling networking, post-translational modifications (PTMs), and an abundance of proteins involved in stress tolerance (Wienkoop *et al.*, 2010; Singh & Jwa, 2013). Harsh environmental conditions and abiotic and biotic stresses impact plant growth and crop productivity. Therefore, this study investigates the mechanisms plants adopt against harsh environmental conditions that trigger the regulatory pathways for plant survival, specifically in *Arabidopsis thaliana*. Moreover, the data explore the genetic regulatory factors such as DNA methylation, epialleles stability, and post-translation modification and their transcriptional factors to help the plant cope with biotic and abiotic stresses.

#### **Some significant components of plant development:**

Novel elements of plant development (Walbot *et al.*, 1996) provide a framework for understanding the effects of genome methylation control on plants. Through the controlled activity of meristem, most of the structures in plants carry out a variety of functions during embryo development. They are sets of undifferentiated cells ready at the top of roots and growing shoots (Hewezi *et al.*, 2017). During the developmental time, apical meristem changes create structures (Poethig *et al.*, 1990). The moderately late dissimilarity of vegetative (substantial) and flower (conceptive) ancestries permits innate changes that happen through substantial development and transmits to ensuing ages. The formative versatility and natural connection requirement suggest plants' widespread use of epigenetic administrative methods. Epigenetic systems may likewise explain in plants due to the chance to choose and transmit to the people to come; metastable epigenetic states settle through development in the biological tissues. On the other hand, the general resilience of aneuploidy and genic imbalance in plants (Guo *et al.*, 1994) predicts that adjusting DNA alteration will profoundly impact plant development. The work so far provided underpins both perspectives.

#### **Levels of DNA methylation changes during development:**

During DNA replication, the static form of DNA methylation changes passively to DNA demethylation through DNA transferases or actively through the base excision repair mechanism. Methylation levels vary from vegetative meristem to other development processes (Agorio *et al.*, 2007). During embryogenesis, demethylation occurs in re-methylation in animals at the implantation stages. For the improvement and development of immature plants, re-methylation takes place instead of demethylation (Finnegan *et al.*, 1996). In *Arabidopsis thaliana*, mutated and antisense methyltransferase causes low DNA methylation levels, which is not reversible in the species during the crossing.

When acquired, transposable elements (TEs) in maize plants, Spm and Ac, differ from male and female gametes, indicating that methylation levels were dynamic during gamete development. These TEs present in primary leaves are less methylated than those of the highest plant parts; this indicates that DNA methylation of

TEs increases during development. *Arabidopsis* and tomato had dynamic levels of DNA methylation. Studies indicate that DNA methylation in young and developed seedlings differs from 20%, lower in young seedlings (Messegueur *et al.*, 1991). Developed and young seedlings of *Arabidopsis* plants face an enormous pattern of less methylation in two. It is unclear whether this dynamic pattern also increases during the development of plants or not (Cokus *et al.*, 2008).

In *Arabidopsis*, the repetitive sequences of TEs face rapid demethylation during gametogenesis or in the development of seeds. There was a lower level of DNA methylation in developed leaves, while DNA methylation was higher in *Arabidopsis* and tomato seeds. Nuclear concentration may be higher in seeds than in leaves. As DNA methylation is higher in the seeds, it reflects increased methylation levels of duplicate sequences. However, hypermethylation and demethylation in the germination stages occur during gametogenesis, indicating reinitialized methylation patterns. TEs in Maize and *Arabidopsis* increase during developmental processes (Karban *et al.*, 2007), resulting in smaller meiosis recombination and decreased gametes (White *et al.*, 1994).

#### **Regulation of biological processes by DNA methylation:**

Gene expression regulates various developmental and physiological processes through the methylation of DNA (Kawakatsu *et al.*, 2017). Plant physiology depends on DNA methylation patterns in a vegetative cycle and development pathways.

#### **Imprinting and development of seed:**

In plants, genomic imprinting is a phenomenal epigenetic mechanism expressing differently from parental origin genes. Genetic analysis of imprinting genes suggests their role in seed development. Loss of function of *MEA*, *FIE*, and *FIS2* genes, which encode subunits of *PRC2* (Polycomb Repressive Complex 2) and are *MEGs* (maternally expressed genes) in *Arabidopsis*, has substantial effects, *i.e.*, suspensions in endosperm and seed development, increased cell proliferation in developmentally delayed endosperm and embryo lead to central cell division before ovule fertilization. The gene expression of the imprinted genes occurs in the endosperm, and it controls its development and can also regulate the nutrients in the seeds during seed development. If these genes have lost their function or mutated, this affects the seed's development, leading to the abortion of the seed as an embryonic result, and endosperm remains immature (Kiyosue *et al.*, 1999; Ohad *et al.*, 1996). The double fertilization strategy used by *Arabidopsis thaliana* relies on the multicellular nature of male and female gametes. The female gametophyte develops the egg and focal cells from the sperm cells in the pollen, which later develop the embryo and endosperm. Hypomethylation of DNA within angiosperms and embryos demonstrates by Rice and *Arabidopsis thaliana* (Hsieh *et al.*, 2009). Before fertilization, the dynamic demethylation of the plant *Arabidopsis* into the focal cells occurs, which took all the approbation (Park *et al.*, 2016). Sub-nuclear proteomic profile data can help understand the regulatory networks to manipulate the seed quality traits and weight.

DNA methylation regulates through endosperm DNA glycosylase DEMETER (DME) gene mutants (Ibarra *et al.*, 2012). During gametogenesis, DNA methyltransferase 1 (met1) transcriptional repression occurs. It is unclear whether it contributes to demethylation, as hypomethylation occurs in the wild-type endosperm. The typical plant methylation sites are CG, CHG, and CHH (where H is A, C, or T). During the development of the seed, an increase in the CHH gene's methylation occurs. During the germination process, methylation levels decline, and demethylation occurs, which indicates a specific action of DNA methylation in plant development (Kawakatsu *et al.*, 2017). In male somatic cells, the methylation of the *CHH* gene is lower than in sex cells. Numerous RNA-directed D.N.A. methylation (RdDM) factors trigger hypermethylation significant for meiosis 1 (Walker *et al.*, 2018). In the light of these findings, DNA methylation has a dynamic role in regulating gene expression in response to environmental stimuli.

#### **Vegetative growth and formation of leaf patterns:**

Meristems of plants have immature and undeveloped cells that form the tissues and organs of the plant body. The level of RNA-directed D.N.A. methylation (RdDM) (Chinnusamy *et al.*, 2009) factor is comparatively higher in tissues of meristem than that of other cells of plant body like in the single leaves or the hypocotyl region (Baubec *et al.*, 2014). All cells in plants correlate with one another, which provides evidence that the DNA methylation level is higher in columella cells and has more access to RdDM factors. These cells have less pericentromeric chromatin (Kawakatsu *et al.*, 2016). However, in *Arabidopsis* plant with RdDM mutants shows no defect in the meristem. Still, in rice, maize, and other plants, RdDM mutants show a greater variety of developmental abnormalities, indicating how important they are for the development and advancement of meristems, which affects the whole plant (Alleman *et al.*, 2006).

In maize leaves, a dynamic pattern of DNA methylation is present in, on, or closer to the location of genes. Many genes are associated with processes such as remodeling chromatin, growth regulation, and cell cycle progression. It adversely affects physiology, which shows that it is essential for the proper development of leaves in Maize (Candaele *et al.*, 2014). In *Arabidopsis thaliana*, DNA methylation plays a vital role in forming patterns of epidermal cells of leaves. If the reactive oxygen species (ROS1) receptor, which encodes by gene *ROS1* gets mutated or lost, it causes the demethylation of the promoter region, which in turn activates the epidermal factor 2 (EPF2) factors which suppress the development of stomata on leaves, create over stomatal cells in *Arabidopsis thaliana* (Yamamuro *et al.*, 2014). These patterns may use to control leaf epidermal cell design. Suppose H3K9 (histone 3 lysine 9) got dysfunction. In that case, it will increase H3K9me2 and CHG methylation of DNA, suppressing *ERECTA* family genes which to the various deformities in stomata that occur in plants due to the dysfunction of *ROS1* mutant. It is could reverse by transforming RdDM factors in plants that are *ROS1* mutants which reverse these deformities (Wang *et al.*, 2016), or in HEK9 or CMT3 plants.

**Ripening of fruits:** During the development of tomato fruit, the pericarp 1% methylome of DNA adjusts inside it. It is a translational factor at which Active demethylation of DNA occurs (Zhong *et al.*, 2013). In the promoter region, both active and passive DNA demethylation occurs. In many fruit ripening genes, the promoter region contains binding sites for RIN (ripening inhibitor), which is required for the ripening of fruits. In the promoter region in many fruit ripening genes, the binding of RIN with a promoter and the expression of many genes adversely affects DNA methylation. During an experiment, if treated with the chemical inhibitor, it induced hypomethylation and resulted in colorless and non-ripening fruits. Later, it increased tomato fruits' aging (Zhong *et al.*, 2013). During fruit ripening in *Solanum Lycopersicum*, its DNA demethylases expression increases, resulting in the maturing of natural products (Liu *et al.*, 2015). DML2 in *S. Lycopersicum* targets the genes for maturing and the genes specific for ageing (Martinez *et al.*, 2014). That will create the demethylation and initiate the gene transcription by the promoter.

For the development of fruit in tomatoes, regulation of gene expression controls by DNA methylation. If *dm12* becomes dysfunctional, tomato fruits not be ripened (Lang *et al.*, 2017). Besides that, DNA methylation is also vital for maturing fruits and development. In the apple fruit, genome studies revealed anthocyanin collection is adversely affected by the methylation of DNA in *MYB10* genes in the promoter region and CHH hypermethylation in leaves and fruits. It indicates that lower DNA methylation levels are also responsible for the smaller sizes of fruits (Daccord *et al.*, 2017).

#### **Methylation mediates vernalization, the promotion of flowering by cold temperatures:**

As vernalization decreases DNA methylation, as evidenced by the work of many researchers. They have given the hypothesis that cold temperature results in the disruption and sometimes complete removal of DNA methylation because the long-term exposure of plant parts or seeds to cold or winter resulted in the loss of methyltransferases that carried out the DNA methylation. For the appropriate flowering, plants developing at high altitude regions require long-term presentation to low temperatures or cold weather. Vernalization also frames out the meristem of inflorescence. It can be achieved mitotically but does not transmit to progeny. In the maize plant, two types of TEs are present: Spm and Ac, which regulate the cycles of dormancy and activeness (Schwartz *et al.*, 1986). Vernalization is inversely proportional to DNA methylation, which affects methylation and termination. The molecular basis of vernalization indicates that DNA methylation regulates different processes to achieve plant development and advanced flowering (Dennis *et al.*, 1996). Vernalization results in the demethylation of DNA methylation, which increases the chances of more flowering. Those plants that are sensitive to a cold atmosphere or low temperature and are insensitive toward vernalization do not flower well (Brock *et al.*, 1994). DNA demethylation results in the inhibition of methyltransferases, resulting in advanced flowerings that diminished DNA methylation and good flowering (Dennis

*et al.*, 1996). DNA methylation has an important role in regulating gene expression, growth and development, protection from environmental stresses as well as in genomic stability (Zilberman *et al.*, 2006; Mendizabal *et al.*, 2016; Kumar *et al.*, 2018)

**Responses of DNA methylation to environmental stimuli:** DNA methylation patterns are keys to controlling stress responses against environmental stimuli such as effects of parasites or pathogens and non-livings such as heat, cold, and drought factors. Investigations reports on differences in relationships between methylation of DNA and various local adjustments and epigenetics attributions towards loci (Schmitz *et al.*, 2013). Fig. 1a demonstrates that if a plant faces biotic or abiotic stress, it responds in two ways, either in a manner of gene expression in response to or changes in methylated carbon, and many more different epigenetic mechanisms result in reprogrammed epigenetic landscape and as a result inherited changes occur (stressed memory) (Downen *et al.*, 2012). Geographic stresses are associated with DNA methylation of different gene expressions brought by epialleles (Dubin *et al.*, 2015). However, further evidence shows that adjustment of DNA methylation occurs at single loci or over the whole genome (Fig. 1b).

**Environmental stress:** Abiotic stresses negatively affect economically important plant species (Shinwari *et al.*, 2020; Khurshid *et al.*, 2018; Jan *et al.*, 2017; Jan *et al.*, 2016). Different researches have explored that DNA methylation has a very effective action in plant responses to different environmental stimuli such as cold, heat, drought, high salinity level, different types of stresses like hyperosmotic or UV stress, deficiency in soil nutrients, change of climate and application of pesticides. A summary of some essential crops response is in Table 1. Under abiotic stress, i.e. salt concentration, wheat plant Lower the methylation level of the intolerant cultivar (Wang *et al.*, 2014). After removing the abiotic stress, i.e. heat, Grapevine shows transitional methylation inheritance (Baranek *et al.*, 2014). In response to cold, maize shows an increase in H3K9ac and H4K5ac in the promoter of cell cycle genes (Zhao *et al.*, 2014), and tomato may show a response in which loss of genes occurs, which are responsible for the biosynthesis of volatile flavors (Le *et al.*, 2014).

Like biotic stress, abiotic stress was examined over periods to verify the effect of DNA methylation and demethylation models. (Yong-Villalobos *et al.*, 2015). A report specified, building stress and memory are significant for DNA methylation. Demethylation in DNA makes ants susceptible so, abnormal growth occurs (Secco *et al.*, 2015).

**Disruption of DNA methylation and its effects on plant development:** DNA methylation plays a significant role in the typical development of the plant. Diminished levels of DNA methylation in *Arabidopsis thaliana* show many variations from the typical *Arabidopsis* plant, such as loss of apical predominance, diminished height, changed shape and size of the leaf, decreased length of root, change in organ shape, and loss of the ability to ripen (Kakutani *et al.*, 1995). These changes observed in other plants where DNA methyltransferases were mutated or lost (Finnegan *et al.*, 1996). Loss of DNA methylation has a positive effect on flowering because, in flowering, MET1 and *ddm1* mutants got lost due to the verbalization process. As a result, proper flowering occurs. Another type of change also occurs due to the disruption in DNA methylation, such as elevated rosettes, cauline leaves, and the inflorescence of bolt stem. Plants that have lost their antisense or mutants for DNA methylation change the standard plant's morphology, indicating various abnormalities due to the loss of DNA methylation (Kakutani *et al.*, 1996).

The minor methylation levels seriously influenced the plants, resulting in an unusual phenotype (Finnegan *et al.*, 1996). Research has shown a 70% decrease in the ripening of fruits in *Arabidopsis* plants after four-five ages. Different plants have different methylation levels ranging from young to mature leaves from young to mature seeds. There are also different mutations in different parts of the plant body.

**Proteomics and plant development:** The study of plant proteome is a powerful tool that can discover the attractive traits (phenotypic and genotypic) and biological behavior of different plant species. *De novo* methylation at cytosine (mC) is a genome modification that regulates the coding and non-coding expression and prevents the movement of jumping genes or TEs. Latest sequencing techniques in system biology have revolutionized the area of omics research. *Arabidopsis thaliana* served as the first model organism in studying quantitatively genome profiling, subcellular proteomics, signaling pathways, and biotic and abiotic stress responses. Wolny *et al.*, (2014) showed that epigenetic markers, i.e., H4K5ac, H3K4me2, and H3K4me1, are involved in gene expression swapping during embryo linked to physiological changes associated with seed desiccation, imbibition, and development.

Researchers have developed different bioinformatics tools to validate the quantitative work. In addition, proteomics analysis provides information on the proteins related to stress responses during developmental stages.

**Table 1. The response of important plant species to abiotic stresses.**

Abiotic stress	Crop	Responses	Reference
Salt	Wheat	Lower methylation level intolerant cultivar	Wang <i>et al.</i> , 2014
Heat	Grapevine	Transgenerational inheritance of methylation after removal of stress	Baranek <i>et al.</i> , 2014
Cold	Maize	Increase in H3K9ac and H4K5ac in the promoter of cell cycle genes	Zhao <i>et al.</i> , 2014
Cold	Tomato	Loss of genes responsible for the biosynthesis of volatile flavors	Le <i>et al.</i> , 2014

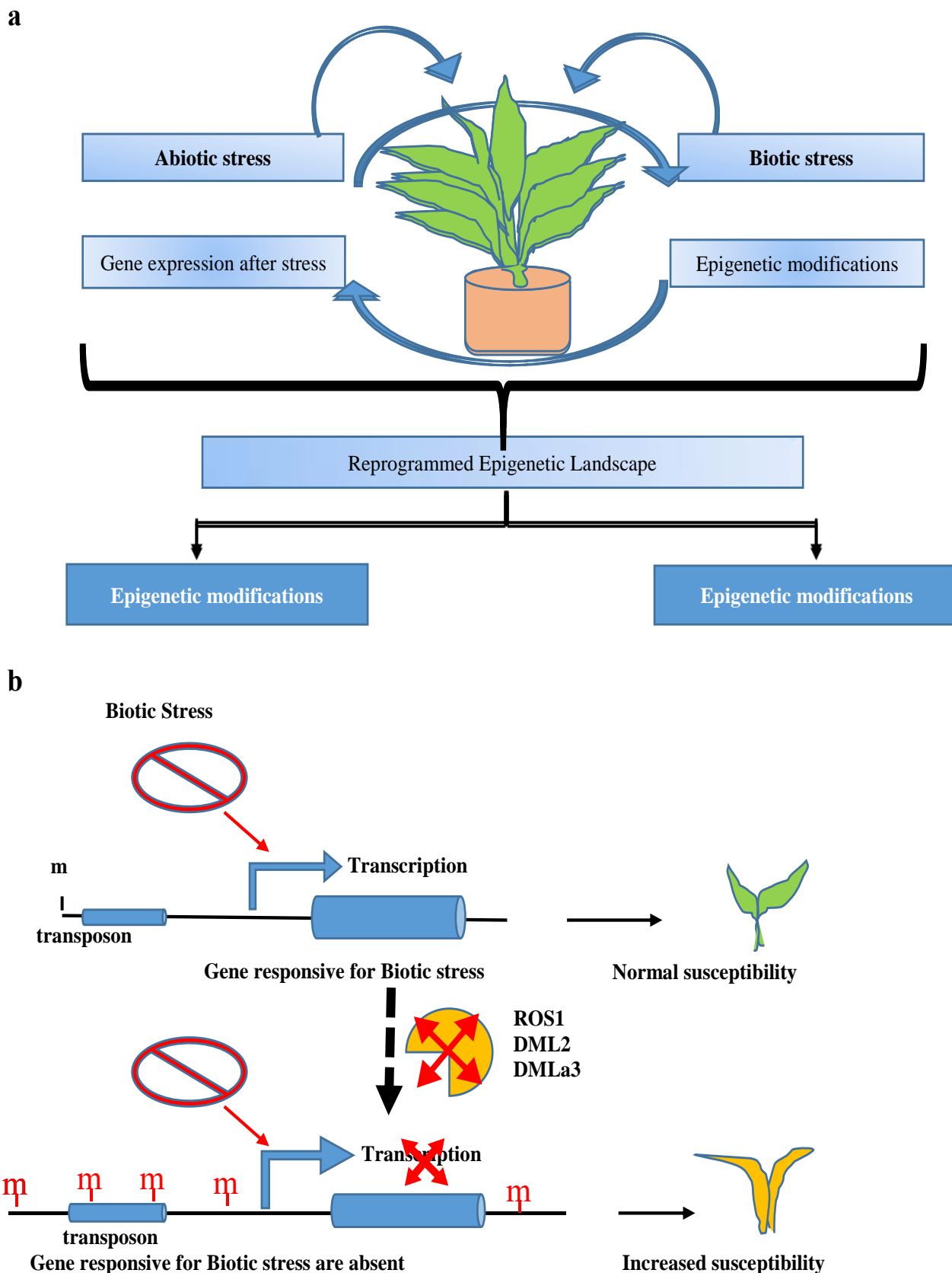


Fig. 1. Epigenetic modifications concerning responses to stresses. **a:** In plants, changes in 5mC DNA methylation are caused by abiotic or biotic stresses, corresponding to modified gene expression of stress response genes. These genes can cause changes in DNA methylation and epigenetic modifications. **b:** Genes named *ROS1* (receptor tyrosine kinase), *DML2* (Demeter like 2), and *DML3* (Demeter like 3) manage the biotic stresses by diminishing DNA methylation in that region. These are present in most plants, and if a plant has defected with any of three mutants, it demonstrates extended defenselessness to the parasitic pathogens. In wild-type leaves and plants, mutant *ROS1* and *RdDM* show extended proliferation (Schumann *et al.*, 2017).

## Conclusions

DNA methylation is essential for many plants to develop, such as *Arabidopsis thaliana*. DNA methylation is a highly conserved mechanism in plants. Its accurate patterns are crucial for developing plants and regulating biological processes such as imprinting and seed development, fruit ripening, vegetative growth, and pattern formation. However, processes do not regulate if DNA methylation is disturbed. It leads to many abnormalities such as developmental abnormalities, including failure of fruit maturation, poor plant growth, and loss of apical dominance. DNA methylation also involves plant responses to environmental stimuli, including biotic and abiotic stresses. *Arabidopsis thaliana* can serve as a model plant to investigate the basic mechanisms of methylation and demethylation of DNA. Further research on the exploration of novel proteins involved in stress tolerance would reveal different roles of DNA methylation, which will be very useful in understanding the methylation patterns in humans (Kim *et al.*, 2014). We have concluded that studies on omics profiling of plants concerning protein abundance and gene expression will provide the clarification of molecular mechanisms involved in DNA methylation in plant species.

The genomic techniques used to analyze and study DNA methylation have given us a masterwork of data and information on plant genomes. However, *de novo* methylation models need to investigate epigenomic changes. The discovery of DNA methylation models in plant species assist breeders in improving their feed quality and quantity in the selected breeds. Using DNA methylation approaches, food production in large quantities could be achievable. The modern approaches and applications of practical tools will reveal the role of epigenetics in the adaptation and evolution of species in stressed environments. Furthermore, diverse omics applications can disclose the other upcoming discoveries needed to understand plant functional and developmental mechanisms.

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