# Epigenetic Mechanisms and its Role in Plant Growth and Development

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## ABSTRACT

The plant epigenetics has been the contemporary topic of research and possible sources for obtaining the required traits for improvement of plant, genetic advancement and plant breeding. Generally, three different mechanisms are practiced to initiate and sustain epigenetic gene regulation. Different epigenetic concepts, mechanisms and their component, along with their relationship led to revolution in plants, resulting epigenetically controlled phenotype, and enabling their higher survivability and benefit to mankind. Thus, this review briefly depicts different components of epigenetics and their relationship, their mechanisms in accordance to crop improvement, which ultimately tries to relay its significance for human value.

**Keywords:** Plant growth; DNA; Acetylation

## INTRODUCTION

Necessity is there to know the term of epigenetics in order to understand its mechanisms and its pivotal role in crop development. Basically, study of evolution and development are the history of epigenetics, and was primarily termed to present development of a mature, complex individual from a fertilized zygote [1]. Conrad Hal Waddington, Scotch embryologist and geneticist, dated back to 1939, defined the casual interactions between genes and gene-product and bringing the phenotype into being [2], and definition involves the heritability of a phenotype passed on through either mitosis or meiosis [3]. Over the following years, its meaning, too, has undergone an evolution, gradually narrowing its meaning, thus the mechanism for stout and study maintenance of changes in gene expression involving physically marked DNA or its related proteins, allowing genetically identical cells to be phenotypically different, for instance phenotypically distinct neuron cell and heart cells in an individual human [4]. However, Sweatt and his team noted, as a common theme in definition, it is a mechanism for storing and perpetuating memory at a cellular level and must be perpetuated through various cell divisions, where mechanism for cellular memory does not depend on DNA sequence alterations. They stressed epigenetic mechanisms are responsible for distinct phenotypes of each cell and can be detected only through the expression of mRNA and protein in specific cell. Thus [5] defined Epigenetics as a gene regulation activities run through biochemical processes that do not entail changes in the genomic DNA code, but instead modify the physical accessibility to the genome. ATP-dependent chromatin-remodeling multi-subunit complexes alter the chromatin structure by changing the conformational state or the mobilization of the nucleosomes.

### Epigenetic Regulation in Plants

As the explanation given by [6], phenotype of an individual passed either through mitosis or meiosis, [7] stated different changes, variations and altered attributes of the cell should be memorized after each cell division. The epigenetic modification in plants, mostly, are comprehensively studied by DNA methylation [8,9] and the process includes addition of methyl group to the fifth carbon position of a cytosine ring. Histone modifications may be influenced by transposable elements that are often methylated and small RNAs capable of directing DNA methylation and chromatin remodeling at their target sites [10]. Availability of DNA is then altered by chromatin structure to transcription factors, and affects if genes could be expressed [11].

Different studies suggested requirement of different enzymes in each context, such as Met1 DNA methyltransferase,

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methyltransferase Chromomethylase3 – CMT3 and Domains Rearranged Methyltransferase – DRM1/DRM2 or CMT2 methyltransferase for CG, CHG and CHH methylation respectively [12]; Also, RNA directed DNA Methylation (RdDM) are guided by siRNAs which are derived by RNA Polymerase IV from long dsRNAs transcription in the nucleus and processed by dicer-like 3 (DCL3). Thereafter these RNAs are exported to the cytoplasm to be incorporated into RISC complex containing ARGONAUTE 4 (AGO4). Then [13] studied alignment of siRNA-AGO4 with their target, a nascent scaffold transcript from RNA Polymerase V, after entering in nucleus and then recruit DNA methyltransferase to silence its target. Therefore, [14] also stressed DNA methylation can create transposons silencing resulting in a protection of genome integrity.

Epimutations befit heritable epialleles, epigenetically equivalent to genetic alleles, which might rose from errors in methylation maintenance [15], called as de novo methylation [16] or from other structure of chromatin transformation [11] or by a certain environment stimulus [17]. Nevertheless epigenetic variation can occur without genetic variation but it can influence epigenetic variation and epimutations in several ways. For instance, whether a gene is subject to epigenetic silencing can be influenced by variation in the presence of methylated cytosine [18], transposable elements [19], sRNA production [20] and genes controlling histone modification and chromatin structure [11].

## Mechanism of Epigenetics for Plant Improvement

DNA methylation and demethylation: The transfer of methyl moiety from S-adenosyl methionine to 5th position of the cytosine residue of DNA from catalyzed enzymes, and is heritable epigenetic enzymatic modification is called DNA methylation. Mostly found in the prokaryotes act as defense mechanism in host, also found in eukaryotes for controlling the transposable element in genome. The level of methylation varied extremely between organisms that display this modification. [21] Suggested available of methylated cytosines (5 mC) in plants ranges from 4 to 37 % depending upon the species [22] which promotes the transcriptional repression by preventing the activators from binding to their target sites. 5 mC content get varied due to plants and fungal genomes having methylated symmetric cytosines in CpNpG (where N can be any nucleotide) or random asymmetric C nucleotides often associated with DNA outside nuclear genes, which in definite plant species encompasses most of the cellular DNA. DNA methylation occurs in CG sites, CHG sites (H indicates A, C or T), and CHH sites (asymmetrical site), catalyzed through enzymes DNA methyl-transferase (MTase). CGsites can be copied after the DNA replication, on other side, de novo gets established after complete cycle of DNA replication in asymmetrical methylation process [23] However, distinct these two processes finally hide variety of unique situation and response to endogenous and exogenous cues. In plants, tissue and development specific stage and stress induced variation were reported in DNA methylation [24].

The conserved enzymes DNA methyltransferase, that catalyzed

theDNA methylation, shows the ancestral origin of DNA modification, are categorized as,

i.Maintenance methylation: that possesses the responsibility for maintaining the stable cytosine methylation pattern through successive cell generation.

ii.De nono methylases: works as carrier to transfer methyl groups to cytosines of unmethylated DNA including Methyltransferases 1 (MET1), Chromomethylase 3 (CMT3) and DRM [25]. MET1, a homologue of the mouse Dnmt1 (DNA methyltransferases, likely to function as maintenance but also pays role in de novo methylation. CMT3 (methylates CpNpG sequence particularly in centromeric repeats and transposon [26] which transmits the symmetrical methylation (CpG and CpNpG) imprints on the parental DNA [27].

iii.Domain-rearranged methylases (DRMs): rapt by short RNA and specifically methylated homologous genes in process termed as RNA directed DNA methylation (RdDM). It consists of three types of methyltarnsferase, a homologue of Dnmt1 plants, DRM1, DRM2, and DRM3 catalyzed de novo methylationof cytosine at asymmetrical CpNpNp sites [28,29]. DRM2, and DRM3 both are responsible to controls RNA directed methylation via pathways that regulates plant specific RNA polymerase V in Arabidopsis [30].

Mechanism of DNA demethylation occurs in active or either in passive process. In the passive process, methylated cytosine is replaced with non-modified cytosine during DNA replication [31]. Incorporation of unmodified cytosines, which may be due to the loss of activity of maintenance DNA methylases i.e.MET1 and CMT3, during DNA replication [32]. As result of enzymes inactivity, there is subsequently loss of DNA methylation in the subsequent generation [33]. In active mechanism, there is the participation of specific protein that demethylate DNA sequence, which was reported in mammals and birds [34]. During this procee, DNA methylation involves a base excision repair pathway undertaken by various DNA methylase which possess either only glycosylase activity (monofuntional DNA glycosylase) or both glycosylase and lysase activity (bifunctional DNA glycosylase). The 5 mC is removed as a free base and a single base gap left behind is filled by non-methylated cytosine by DNA polymerase and ligase. Four Arabidopsis mutations, one interfere the maternal expression of an imprinted gene and other induced transcriptional gene silencing (TGS) of transgenes and endogenous homologous loci, were charted to the genes DEMETER (DME1) and ROS1 respectively, which code DNA glycosylases and remove 5 mc from DNA. These DNA demethylases also prevent the formation of stable hyper-methylated epi-alleles in plant genomes and thus helps to maintain equilibrium between methylated and unmethylated DNA.

The most conspicuous roles of methylation as proposed are (i) to provide a heritable epigenetics mark that could direct the development program of organism, (ii) to provide a means of genomes defense against the activity of parasitic mobile elements,

(iii) to reduce background transcriptional noise in organism that have large number of genes and (iv) to "memorize" pattern of gene activity by stabilizing gene silencing brought about by other mechanism. These hypothesis is found to be true, depends on organism considered and may be not mutually exclusive. Methylation if, in fact, is evolutionary tools, it would be expected to serve a variety of functions and to play different roles both within and between organisms.

## Implication of DNA Methylation in Plants Improvements

DNA methylation for Abiotic stress response: According to the location of methylation, DNA methylation shows variety of behaviors which is positively correlated to repression of gene and leads to the silencing that are existing in transposable elements and promoter regions of genes and shown positive to regulates the gene expression with in gene body (Liu et al. 2015). When the plant expose to stress condition, increase in DNA methylation may down regulate expression of the transcriptome which slow down the metabolism of the plant and make enable to conserve energy, and enhance for overcoming the temporary challenges and dormancy. However, resistance related genes with declination of methylation favors for chromatin activation and the expression of novel gens that increases the resistance in plant for long period.

CAM plants switch over for C3 photosynthesis cycle to CAM pathways, so that to reduce the water and increasing the resistance when it faces the water deficit problem. This process is coupled with promotion of genomic methylation and hyper-methylation of satellite DNA. This hyper-methylation response for synthesis of chromatin structure that control the expression of a number of genes simultaneously, to adapt for water stress. Similarly, hyper-methylation detected under the root tip of pea when exposed to water deficit.

When maize is exposed to chilling, 1.8 kb designated ZmMI2, part of putative protein coding gene for indicating active role in cold tolerance, was demthylated (under normal condition shows 38% methylation). Drought exposed rice express the level of OsCMT2 that codes mRNA, and induce total of 12.1% methylation difference in tissue, genotypes and development stages. Another heat stress expose to rice, reduce the seed size which is controlled by OsFIE1. The expression of OsFIE1 is controlled by two factors DNA methylation and H3K9me2 methylation, but found in declined number. The level of methylation increased in heat stress condition than in drought stress condition in Populus tricocarpa. DNA methylation that found in Tobacco, aid to control cell growth and expansion under heats stress and Exposure to moderate heat (35°C) inhibited 70% growth accompanied with decrease in transcript level of NtCycA13, NtCyc29 and CDKB1-1 transcripts and increase in NtCycD3-1 level. McrBC assay based DNA methylation analysis revealed hypomethylation in NtCycD3-1 genes whereas hypermethylation in NtEXPA-5. NtcycA13 and CDKB1-1 showed no change in methylation pattern under heat stress and normal condition (Centomani et al. 2015).

## DNA Methylation for Plant Breeding/Selection

DNA methylation and gene action: DNA methylation plays crucial role in gene expression in eukaryotes plants, where induction of several endogenous genes in certain tissue has been linked to loss of cytosine methylation, and correlating hypo-methylation of these gene with transcription in the respective tissues that required optimal amount of coded protein. Quantitative change in gene activity has positive correlation increasing hybrid vigor and performance of F1 hybrid maize either by individual protein level, or the enzymatic activity level or RNA level. DNA methylation as generator of epimutations have importance role in phenotypic consequences, which give rise to more permanent mutant alleles at a locus, while mutations only rarely lead to new epialleles. Similarly on other hand, regulation of gene activity related to transposable elements due to DNA methylation may be benefit to host plant and in sense for the element's benefits, as well.

## DNA Methylation, Genetic and Epigenetic Variation

Heritable epigenetics as source of phenotypic variation plays crucial role in plant development. Polymorphism in DNA methylation occurs in the genotypes of species, organ or tissue of species, interfere with gene expression and consequently become associated with phenotypic variation. This is also called somatic variation due to mimicking the genetic variation even next generation with meiosis division, which helps for the evaluation and selection of superior genotypes through expression of phenotypes. It also helps in increasing genetic variation by generating the new mutation. Assessing the importance of methylated epialleles in plant breeding as reviewed by requires determination of (i) the extent of variation in methylation patterns among individuals within the selection population (ii) the degree to which methylation patterns affect phenotypes (iii) the extent to which methylation variants linked to superior phenotypes are stably inherited. The potential association between individuals of methylation pattern thus helps to estimate the levels of methylation associated epialleles diversity and it related phenotypic diversity. Significance study and understanding should be done for finding the role of DNA methylation as source of polymorphism in plant evolution, domestication and breeding.

Somaclonal variation attributed generally to tissue culture induced heritable genetic changes. First evidence of methylation found in crown gall tumor lines where changes in T-DNA methylation was associated with phenotypic variation as well as demethylation in reactivation of Ac elements following tissue culture. Most queries issues was its divergence, and epigenetics state stable in mitotically and meiotically regenerated from somatic cells and even meristematic cells of plant become gametes. The plant growth regulators, antibiotics and the number of subculture generation influence soma clonal variation in Torenia spp. and this variation in Torenia have resulted from epigenetic changes.

## DNA Methylation, Heterosis and Hybrid Breeding

Variability of gene expression assessed through polymorphism

of RNA amount (RAP) and individual protein amount (PAP), that responsible for bio-chemical and physiological process and essential for manifesting phenotypic diversity and phenotypic expression of genetic difference like heterosis and hybrid vigor. The better hybrid had significant number of gene expressed over the better parent at different stages. Monitoring the methylation involvement for manifestation stated (i) inbred line are probably higher in methylation than hybrids one (ii) heterotic hybrids are less methylated than related non-heterotic hybrids (iii) old, low yielding inbreds are highly methylated (iv) more modern inbreds, especially those selected for high yield and consistent and stable under dense spacing in the isolation environment, have lower percentage of methylation in comparison with old progenitor lines. Similarly, F1 hybrid is higher in yield and stable in different environment and stress condition. These mays be due to methylation involvement in the expression of genes and varies with genotypes, the development stages, and condition of growth of methylation status of genomic DNA. Stressful growth condition results in more resistance to such density induced methylation and suppression of genome activity in their genomic DNA, and consequently avoidance of suppression results to higher yield and more importantly stable yield. In shift of finding the higher yielding and stable F1 yield, there was concomitant shift in parental lines giving less heterosis, and inbreeding depression and with less methylation.

### Manipulation of Parental Imprinting

Epigenetic mechanism that control parental imprinting help in manipulation of development of endosperm that is the desirable trait of seed crops. The seed development through apomixes reveals the exploration of epigenetic mechanism. In case of this mechanism, hybrids can be regenerated indefinitely, thus overcome on the current limitation of plant breeding to maintain hybrid vigor for more than one single generation.

### Avoiding Transgene Silencing in GM Crops

Silencing can be done at both transcriptional and post transcriptional level in plants [35] that is correlated with methylation of the corresponding transgene. Methylation of the transgene promoter correlates with transcriptional gene silencing, coding sequence of methylation related with post transcriptional gene silencing. Silencing has been observed in plants, constituting a major commercials risk and hampering the general economic exploitation of transgenic plants (Tables 1 and 2) (Figure 1)
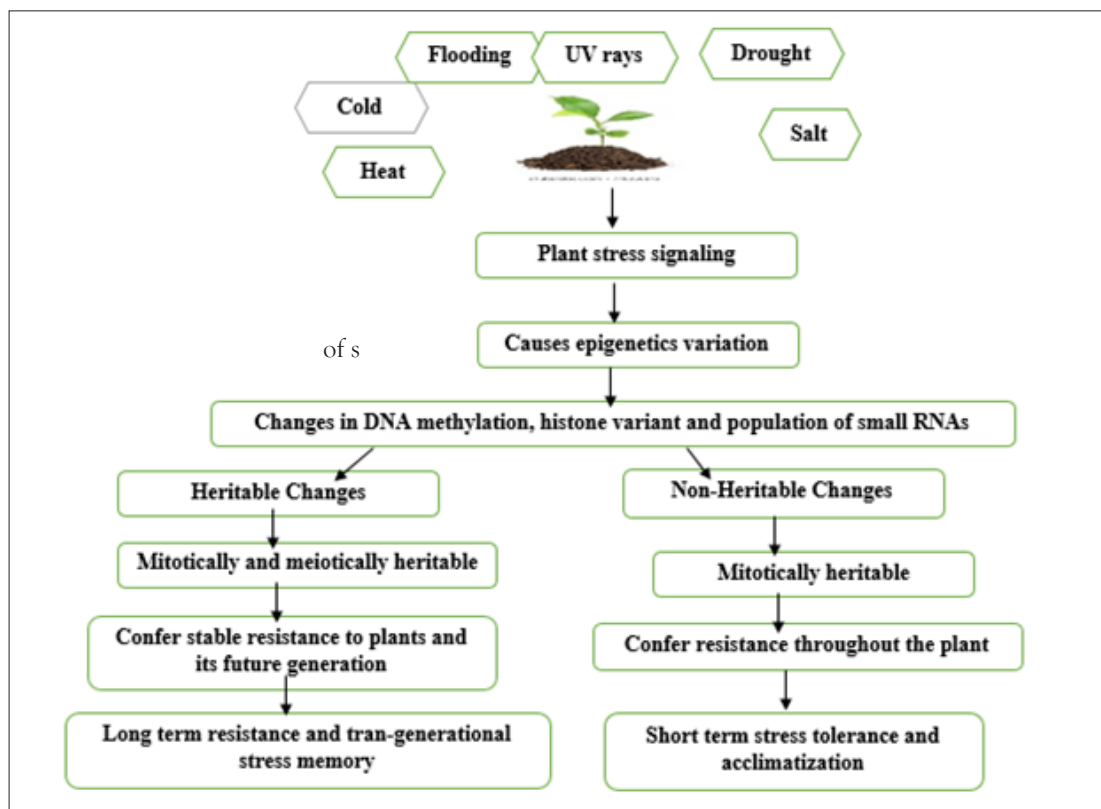


**Figure 1:** Flowchart of DNA methylation during abiotic stress in plants

**Table 1:** Different gene responsible for histone modification in various crops

| Enzyme | Gene | Function | Plant | Reference |
|---|---|---|---|---|
| HATs | AtABO1 | Drought and oxidative stress tolerance | Arabidopsis | (Chen et al. 2006) |
| | OsHAG702 | Heat stress tolerance | Rice | (Liu et al. 2012) |
| | OsHAG703 | Drought stress tolerance | Rice | (Liu et al. 2012; Fang et al. 2014) |
| | OsHAG704 | Heat stress tolerance | Rice | (Liu et al. 2012) |
| | OsHAM701 | Drought stress tolerance | Rice | (Liu et al. 2012; Fang et al. 2014) |
| | OsHAC701 | Heat stress tolerance | Rice | (Liu et al. 2012) |
| | OsHAC703 | Drought stress tolerance | Rice | (Liu et al. 2012; Fang et al. 2014) |
| | OsHAC704 | Heat stress tolerance | Rice | (Liu et al. 2012) |
| | OsHAF701 | Drought stress tolerance | Rice | (Liu et al. 2012; Fang et al. 2014) |
| | AtATX1 | Drought stress tolerance | Arabidopsis | (Ding et al. 2011) |
| HMTs | HvtX1 | Drought stress tolerance | Barley | (Papaefthimiou and Tsaftaris 2012) |
| | HvPKDM7 | Drought stress tolerance | Barley | (Papaefthimiou and Tsaftaris 2012) |
| | AtMSI1 | Drought stress tolerance | Arabidopsis | (Alexandre et al. 2009) |
| HDMs | AtCHR12 | Drought and Heat stress tolerance | Arabidopsis | (Alexandre et al. 2009) |
| | AtBRM | Drought stress tolerance | Arabidopsis | (Berr et al. 2012) |

**Table 2:** DNA methylation modification involved in biotic and abiotic stresses in different plant varieties

| Gene | Plant | Stress | Methylation status | Reference |
|---|---|---|---|---|
| Asr1 | Tomato | Drought stress | CG hyper-methylation and CHH hypo-methylation | (Gonzalez et al. 2011) |
| Asr2 | Tomato | Drought stress | CHH hypo-methylation in regulatory region | (Gonzalez et al. 2013) |
| NtGPDL | Tobacco | Cold | Hypo-methylation | (Choi and Sano 2007) |
| ZmMll | Maize | Cold | Root-specific hypo-methylation | (Steward et al. 2002) |
| Glyma11g02400 | Soybean | Salinity | De-methylation | (Song et al. 2012) |
| Glyma6g27950 | Soybean | Salinity | Hypo-methylation | (Song et al. 2012) |
| Glyma20g30840 | Soybean | Salinity | Hypo-methylation | (Song et al. 2012) |
| Genome wide | Maize | Cold stress | Global methylation shift | (Tan 2010) |
| Genome wide | Rice | Drought stress | Genotype-dependent differential methylation | (Wang et al. 2011) |

## Histone Modification

Histones are basic, as well as most abundant proteins in eukaryotes, especially rich in lysine and arginine. Chromatin structure and genome accessibility can be altered by formational state or nucleosomes mobilization, or by modification of covalent and post-translations of the N-terminal histones tails which consists acetylation, methylation, ubiquitination, sumoylation, phosphorylation, ADP-ribosylation, glycation, and carbonylation. 'Histone code' is expressed through various combinatorial nature of histone modifications, thus considered to integrate intra- and extra-cellular signals to regulate the process of accessible of DNA information from genetic code. Also, these histone codes are affected by alternative composition of amino acid in histone variants and its relation with post-translational modifications.

The different types of histone modifications includes acetylationn, methylation, ubiquitination, sumoylation, phosphorylation, ADP-ribosylation, glycation, and carbonylation, and indicated by an abbreviation of the histone involved and followed by amino acid residue and modifications made on each amino acid, such as H3K4Me3 indicates trimethylation of lysine 4 on H3 histone, monoubiquitination of H2B as H2BUb, acetylation as Ac and phosphorylataion.

### Ubiquitination and Sumoylation

Ubiquitin (Ub), a highly conserved 76 amino acid polypeptide, are targeted to proteasome-mediated proteolysis and involved in several cell functions such as DNA repair, stress responses, signalling networks, intracellular trafficking, protein-protein interactions, and transcription. Ub attached covalently to the lysine residues through isopeptide bonds of the target proteins with its C-terminal glysine, and consists three important steps catalyzed by unlike enzymes, E1, the ubiquitin-activating enzyme (Uba), E2, the ubiquitin-conjugating enzyme (Ubc), and E3, the ubiquitin ligase. E1 activates C terminal glysine of Ub forming a thiol ester with its carboxyl group, which is carried by E2. The covalent ligation of the ubiquitin is mediated by E3 ligase to the lysine residue of the substrate, thus determining the substrate specificity in association with its related Ubc.

Ubiquitination mostly target Histones and the most abundant ubiquitin conjugates found in higher eukaryotes are monoubiquitinated histones H2A and H2B, wheras H1 and H3 occur very occasionally H2A was the first protein found in plants observed to be post-translationally modified by covalent ligation of ubiquitin. Histones ubiquitination is a transient modification which holds only a small proportion of histones in an ubiquinated stage at a given time depicting the importance of histone de-ubiquitination in the process, for instance reported 5–15% and 1-2% respectively are the proportion of ubiquitinated forms for H2A and H2B. De-ubiquitination occurs by hydrolysis of peptide linkage between the substrate lysine and C-terminal glysine of ubiquitin, noted two types, the ubiquitin C-terminal hydrolases (UCHs) and the ubiquitin-specific processing proteases (UBPs).

The process sumoylation competes with ubiquitin modifications on histones which is mediated by similar enzymes as those for ubiquitination. In yeast, E1 activates small- ubiquitin-like-modifier (SUMO) protein, composed of a heterodimer between Aos1 and Uba2 proteins whereas two homologs of Aos1 and one homolog of Uba2 have been identified in plants forming a functional E1 protein complex. Several E3 ligase enzymes specific for sumoylation process have been discovered.

Nathan reported antagonist activity of histone sumoylation to activating lysine modifications of histones, like acetylation and ubiquitination. These histone sumoylation involved in transcriptional repression and act to maintain low basal levels of transcription of a broad range of genes via dynamic interplay with histone acetylation and ubiquitination. All core histones H2A, H2B, H3 and H4 shows sumoylation with site specificity. Alike to ubiquitination, it is also short-lived and labile under innate conditions and shown to associate with transcriptional regulation during stress indicating pathways in plants.

### Methylation

Through the enzymatic addition of a methyl group from donor S-adenosylmethionine (SAM), histones can be methylated on their arginine and lysine residues, catalyzed by histone methyltransferases (HMTs) which can be divided in three classes such as, the lysine-specific SET domain comprising HMTs with a specificity for K4, K9, K27, and K36 of histone H3 and K20 of histone H4, the non-SET domain-containing lysine HMTs that are involved in methylating K79 of histone H3, and the arginine HMTs which methylate R2, R17, and R26 of histone H3 as well as R3 of histone H4.

A remarkable characteristic of this histone modification is that more than one methylation mark can be supported by one amino acid residue, for instance lysines could be mono-, di- or trimethylated, while arginines can be mono- or dimethylated. According to Strahl and Kouzarides, transcriptional repression and silent heterochromatin is responsible for methylation, however methylation of some residues such as H3K4Me3 denotes an triggering mark for transcription, and this antagonistic role is depicted by the members of Poly-comb Group (PcG) and Trithorax Group (TrxG), belonging to the class of the SET domain-containing lysine HMTs and playing crucial role during development by maintaining balanced expression of homeotic genes, especially in Drosophila and mammals.

Switching patterns of histones from methylated to demethylated and vice-versa with varied degree of epigenetic mark (mono-, di- and tri-) signifies a fine-tuning system to control gene transcription and chromatin status. Therefore, Fisher considered histone methylation as an original biological language spoken in different forms by different eukaryotes. Also, Bernstein and Allis focused rising of complexity of histone modification due to mechanistic link between histone methylation, DNA methylation and RNS

interference machineries in heterochromatin formation.

## Acetylation

In gene activation and repression, acetylation and deacetylation of lysine of core histones plays a functional role. Generally histones are acetylated at lysine residue of N-terminal tail, a reversible modification, which is the product of balanced actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetyl-Coenzyme A beget acetyl group in histone acetylation which can again divert back to Coenzyme A or to ADP-ribose in histone deacetylation by NAD-dependent deacetylases Denu. Positive charge of histone tails is neutralized by the addition of an acetyl group on lysine residues and reduces its affinity for DNA, therefore increasing the availability of transcriptional regulatory proteins to chromatin template and indicates increased transcriptional activity leads to histone acetylation. Struhl reported confirmation of histone acetylation and transcriptional activity relationships by identifying HAT domains in proteins or transcriptional activators. Also, Fukuda specified association of histone acetylation with transcriptional activation and histone deacetylation in gene repression.

Individual HATs and HDACs show distinct characteristics. Pandey reported presence of four families of HATs and three families of HDACs in Arabidopsis, consisting 12 and 16 genes respectively through sequence and domain analysis of its genome. Similarly, Lusser conveyed plant specific HD2 family of HDACs indicates functional diversification of histone-modifying proteins in plants while other indication includes different gene copies numbers in some classes of gene and low specific functional domains.

## Phosphorylation

Several cell activities such as mitosis, meiosis, cell death, DNA repair, recombination, replication and transcription are correlated with histone phosphorylation. In DNA, all of the four core histones are known to be phosphorylated on specific serine and threonine residues, and these phosphorylated histones act in cell cycle progression, and mitotic and meiotic cell divisions whereas, only serine 10 of histone H3 relates with transcriptional activation. As this serine plays an opposite roles during cell cycle interphase (transcriptional activation) and mitosis (chromosome condensation), its effect is thought to be context dependent and could be influenced by other histone modifications.

## Ribosylation, Glycation and Carbonylation

Kraus and Lis and Hassa reviewed unequivocal recognition of histone ribosylation in DNA repair, however the function of this alteration in transcriptional regulation is still not entirely explicated. A family of proteins represented by Poly ADP-ribose polymerase (PARP) catalyzes the attachment of ADP-ribose units from NAD+ molecules to diversified target proteins, resulting in linear or branched polymers of poly (ADP-ribose). Similar to other histone modifications, ADP-ribosylation is reversible, and plays an important role in both transcriptional activation and repression depending on chromatin environment, for instance PARP-1 is involved in transcriptional activation and chromatin decondensation in some highly inducible genes while found in transcription repression as well by condensing some repetitive elements condensed, thus adding complexity due to dual nature of action.

Glycation and carbonylation are non-enzymatic histone modifications process and occurs spontaneously in living cells. Glycation alters proteins related with high concentration of sugar and generally linked with several animal diseases whereas carbonylation involves basic amino acid residues, such as lysine and arginine, and is a marker for protein-oxidative alterations that intensifies with age. All histones except H4 are carbonylated and methods includes direct oxidation of formerly glycated residues or by chemical mechanisms n or reaction of amino acid residues with reducing sugars or sugar-oxidizing products; and the availability of ADP-ribose is a key factor in entire process where these sugars are interacted with histone, particularly H1 as in glycation process.

## Histone modification in plant development

Different chromatin research showed almost every process in plant development is influenced by histone modifications mechanism. Tai and Long reported necessity of histone code during embryogenesis, earliest phase of plant development, to establish the correct body plan; whereas its modification influence patterning, phase change, flowering time and maternal imprinting. According to Zhou, histone modifications are also correlated with activities of plant hormones such as, ethylene, jasmonic acid, salicyclic acid and abscissic acid. In addition, several alterations in environment resulting biotic and abiotic stress, light penetration and vernalization are controlled by epigenetic regulation. Also, both the histone variants and covalent modifications of core histone tails affect all facets of plant development.

Jasencakova and Wako described histone acetylation, especially histone H4, represents an important mark during the regulation of cell cycle. Chua described correlation of active genes and highly acetylated histones and inactive genes and hypo acetylated histones in plants. Histone lysine acetylation, among all histone modifications, is found to be dynamic reversible switch for interconversion between active and repressed transcriptional states of chromatin domains, where strong acetylation activates transcription by relaxing chromatin structure while weak one to chromatin compaction and gene repression. For instance, Bertrand reported expansion of expression domains of regulatory genes WUSCHEL (WUS) and AG (AtGCN5), HATs, is necessary

to regulate flower meristem activity through WUS/AG pathway. Similarly, HD2 type of HDACs first discovered in maize, when silenced resulted seed abortion and coincided with a specific expression in flowers and young siliquas indicating its essential role in plant reproductive development. Also, HDAC proteins related to RPD3 leads early senescence, leaf serration, abnormal cotyledon, and leaf and siliqua formation (Figure 2).
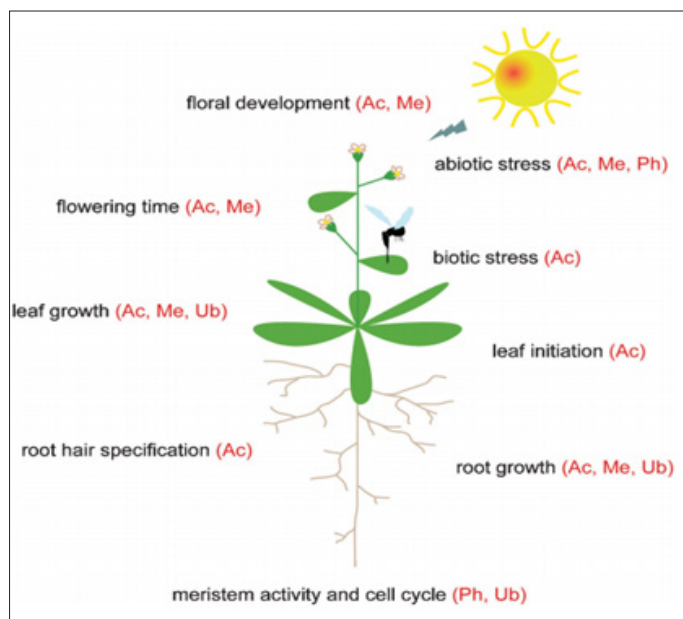


**Figure 2:** Plant processes affected by histone modifications

Nelisson reviewed impact of core histone modifications on plant growth and informed, in dicotyledonous plants, such as model plants Arabidopisis, a phenomenon called heteroblasty occurs during its life cycle. Different sorts of modifications of histones in different parts of plant for its growth and development, and its different phases of life are shown schematically in above Figure 2.

For a normal regulation of cell division and growth, HUB1 and HUB2, homologs of Bre1 ubiquitin E3 ligase plays an important role in plants, however hub1-1 mutants causes severe impairment of leaf and root growth where reduced cell production rates causes reduction of 2C content and increment of endoreduplication levels leading blockage of G2-to-M transition of cell cycle. Furthermore, the mutant hub1-2 showed an altered expression of dormancy related genes which reduce seed dormancy and seed longevity as well. Similarly, Alatzas and found increased detected signal in meristematic zone when H2AUb was detected immunohistochemically in maize roots, and these results elaborated the involvement of ubiquitinated histones in cell division regulator in plants in equivalence with other organisms, like Physarum, a slime mold, yeast.

Shen reported identification of NtSET1, a novel subgroup of plant SET-domain-containing protein, in tobacco which was capable to methylate both H3K9 and H3K27 in in vitro condition, and the activity was principally reliant on on C-terminal SET domain of protein. Also Yu added, if NtSET1 showed ectopic expression,

the amount of H3K9Me2 is increased, thus responsible for transcriptional repression; inhibiting growth of tobacco.

In Arabidopsis, the characteristics catalytic domain of AURORA (AUR) kinases is preserved in three Arabidopsis AUR-like-gens i.e. AUR1, AUR2 and AUR3, which can phosphorylate histone H3 in in vitro condition and can co-localize with phosphorylate histone H3 during mitosis, thus playing an important role in plant cell cycle.

Flower Locus C (FLC) gene, the key actor for flowering, a MADS box transcription box factor, whose expression is regulated by histone modification at the FLC locus and at other transcriptional regulators of FLC, plays crucial role for regulation of flowering time. He and Amasino described FLC repressed flowering quantitatively, therefore delayed flowering when highly expressed, and for floral transition, low FLC expression is required. However, the homologs components of human HDAC complex, Flowering Locus D (FLD) and Flowering Locus Ve (FVE) inhibit the FLC expression at this locus.

### RNA Interference

Activating RNA interference (RNAi), a post transcriptional gene silencing (PTGS), leads to RNA silencing, a novel gene regulatory mechanism that limits the transcript level. Here, a short double standard RNA (dsRNA) checks the expression of specific genes by degrading sequence of specific target mRNA in the cytoplasm. Napoli stated its mechanism to be first discovered in transgenic plant Petunia hybrida L. by enhancement of anthocyanin in Petunia by introducing chalcone synthase gene (CHS A) which encodes key enzymes in antocyanin biosynthesis pathway.

The basic mechanism of RNA silencing acts on three different levels in plants, viz ds RNA based cytoplasmic silencing causing mRNA cleavage, micro-RNAs (miRNAs) based endogenous mRNAs silencing causing negative gene expression regulation by base-pairing to specific mRNAs resulting either RNA cleavage or post-transcriptional gene silencing (PTGS), and transcription suppression which is associated with sequence-specific DNA methylation. General procedure consists of cloning and insertion of interested gene into suitable plasmid forming a recombinant plasmid, which is then transferred to an appropriate vector, such as Agrobacterium mediated, micro projectile bombardment, and vacuum infiltration, syringing, and spraying infection suitable for plant transformation. Small interfering RNAs (siRNAs) with 21-24 nucleotide long staggered cut, formed through the cleavage activity of an enzyme called Dicer, initiates the general process. These are then introduced into RNA-inducing Silencing Complex (RISC) containing several proteins like AGO along with other siRNAs. The RISC activated by ATP unwinds the ds siRNA, where its sense strand is lost by the activity of RNA helicase enzyme, and anti-sense strand gets incorporated into nuclease which consists RISC complex. Thereafter, RISC with antisense siRNA sequence targets the homologous transcript by base pairing interaction and splits the mRNA blocking the translation causing protein synthesis inhibition (Figure 3).
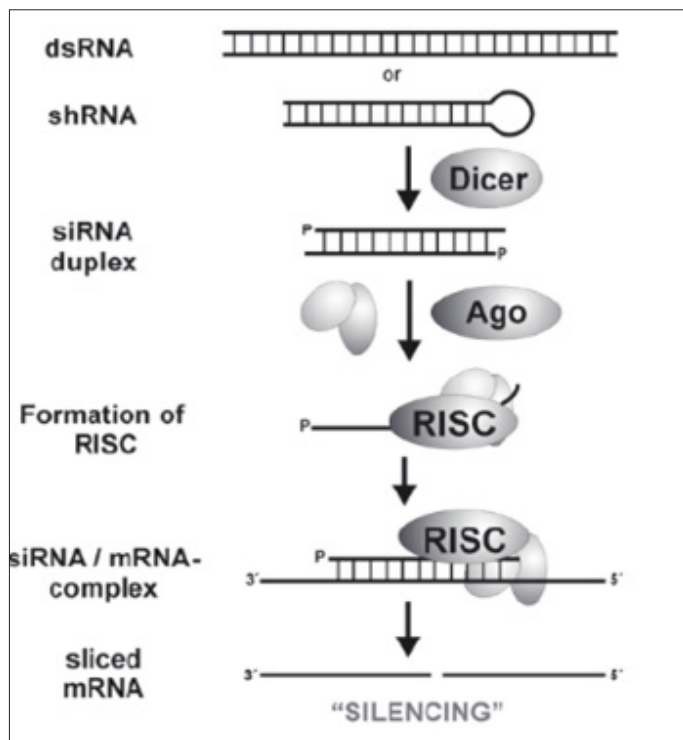
**Figure 3:** Mechanism of RNAi

### RNAi in Plant Improvement

Modifications of plant architecture through RNAi technology is being used nowadays, to improve crop productivity. For plant height, endogenous plant hormones along with gibberellins (GAs) and brassinosteroids (BRs) plays an important role, therefore, genetic manipulations belonging to GA biosynthesis are major hits for plant height alterations. Zhou demonstrated the task in rice where, RNAi checks the expression of OsGLU1 gene encoding a membrane-bound endo-1, 4b-D-glucanase gene, which affects the elongation of plant internode altering cell wall composition causing structural changes causing development of a dwarf phenotype. Also, OsGA20ox2 gene in rice encoding a regulatory enzyme GA-20-oxidase, syntheses of biologically active gibberellins, was suppressed by RNAi. Also, Hu studied that when HDAC genes is expressed, it displays certain expression patterns and divergent developmental functions when compared with closely related homologs in Arabidopsis, where most of them are responsive to drought and salt stresses. Similarly, in Arabidopsis seedlings, Sunkar and Zhu described different role of miRNAs in response to abiotic stresses such as, drought, cold, salinity and oxidative stresses, and reported that miR393 was strongly up-regulated by cold, dehydration, dehydration and ABA treatments. In addition to this, it was indicated that plants suffered from sulfer and phosphate starvation in Arabidopsis upregulate miR395 and miR399. Here, miR395 catalyzes the first step of inorganic sulfate assimilation as well as AST68 gene by targeting ATP sulfurylases genes and ubiquitin-conjugating enzyme (UBC24) was down regulated by miR399 by targeting

5' untranslated region (UTR), and Chiou stated this regulation as crucial for plant responses to Pi starvation by shifting the appearance of miR399 or UBC24 in Arabidopsis.

Similarly, being one of the major factor of biotic stress to crops, virus, possess RNA genomes and replicates through dsRNA intermediates, serving as potent inducers of RNAi during early replications and as silencing targets in later one. In addition, the RNAi has been used effectively for plant protection against phytopathogenic bacteria, especially A. tumefaciens, casual organism of crown gall disease, by targeting two genes, iaaM and ipt, responsible for inducing resistance to crown gall disease. This technology is also used as a genetic tool for engineering host resistance against parasitic weed, where a host plant is transformed and targeted against one or more vital parasitic genes by a plasmid encoding a ds hpRNA.

## CONCLUSION

Modern progress in molecular biology has generated steep prospects for the potential role of epigenetics for plant improvement where DNA methylation has become the mechanism of preference for scientists investigating gene function and manipulating plants to create novel characteristics, capable of enduring in different environmental conditions. Thus, the use of epigenetics can be enhanced, and transgene silencing could be mitigated with improved knowledge of the mechanisms, and most efficient strategy and thorough analysis of the transformants at molecular-level

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