The Role of Epigenetics in Plant Breeding

Abstract

Epigenetics is upcoming new breeding strategy which will show a new direction in plant breeding. It creates phenotypic variation without involving any changes in DNA sequences and these variations or epimutation is more or less stable, will passes through next generation. DNA methylation, Histone modification and RNAi technology can modify the transcriptomes in such a precise manner that plant can buffer or adjust under any adverse climate and also maintain their different agronomic traits such as yield, and yield related parameter. Scientists and breeders are rapidly increasing interest on this new science and technology.

Keywords: *Epigenetics, Histone, RNAi, Transcriptome, Variation*

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I. INTRODUCTION

Epigenetics means ‘above’ or ‘on top of genetics. Any heritable changes that occur in the organisms which does not involve any alterations in the DNA sequences but results in changes in gene expression, leads phenotypic diversity. In the organisms the cells will be genetically identical but after epigenetic modifications there will be silencing effect in genes, create epigenetic variations. These variations will pass through one generation to next generation.

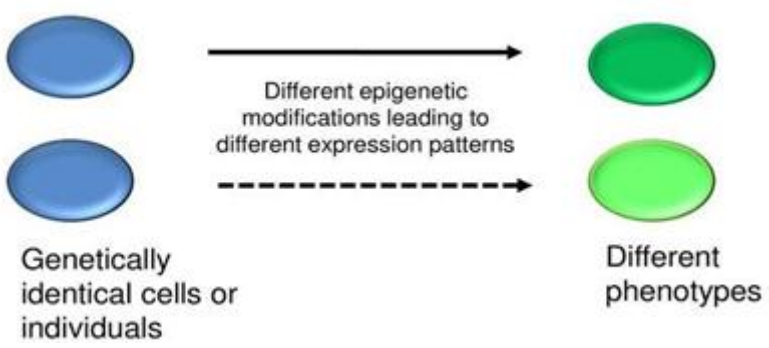


Figure 1: Expression of different phenotypic after modification

In modern day’s involvement of intense breeding programmes and over exploitation of crops, there will be accidental disappearance of many desirable traits (loss of germplasm). In that case, epigenetics can drive plant breeding strategies in a new direction to and is rapidly gaining interest in modern crop breeding approaches for the breeders. For our evergreen population, the increasing demands of food and changes in diet preferences require new strategies to meet. Subsequently, breeders are obliged to consider new characteristics for selecting elite crop variation for breeding, which intensifies the demand for a wider source of variation. Epigenetic modification like DNA methylations, histone modification and RNAi technology can buffer the effect of adverse environment and maintain the stability in case of yield and other agronomic traits. Also, these strategies are time and cost effective as there is no involvement of enforcement of gene from any outsources. According to Lamarck (1801), acquired traits will passes through its next generation although this theory has been discredited later, but the overall concept raises some possibility of ‘Epimutations’ which can play an important role in evolution.

1. Molecular Basis of Epigenetics

The molecular basis behind epigenetics is a complex phenomenon which involves several modifications like DNA methylation, Histone modification or

RNA interference. All these modifications are results in changes in gene expression, leads to phenotypic variations without alterations of DNA sequences.

A. DNA Methylation

This is process where a methyl group (-CH3) will be added covalently to the cytosine (C-5 positioning) presents in DNA sequences with the help the enzyme called DNMT (DNA methyltransferase). There are 3 DNMTs, classified 2 classes – i) maintenance class and ii) De novo class. Maintenance class are helps to maintain the methylation from parent DNA to daughter DNA and also binds with hemi-methylated DNA to work with (DNMT1) whereas De novo class will add methyl group in a new place of a DNA sequences and they do not need any hemi- methylated DNA (DNMT3a, DNMT3b).

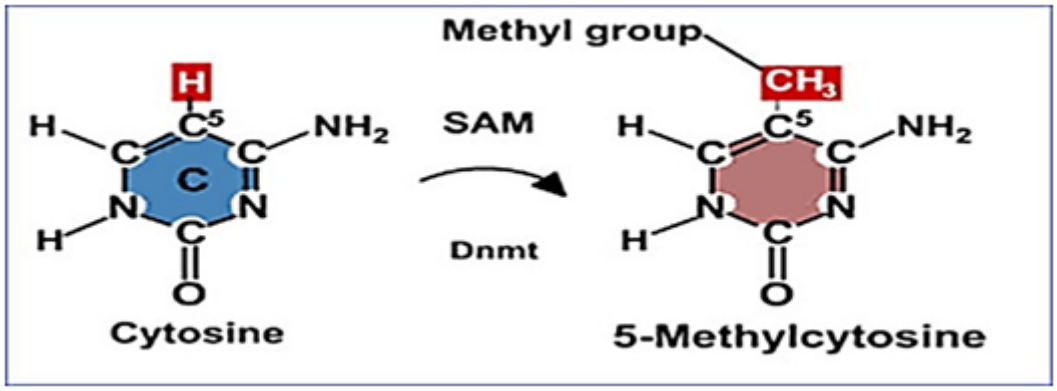


Figure 2: DNA methylation (SAM, SAH)

SAM: S- Adenosyl Methionine (donor of methyl group) after donating methyl group it will convert into SAH (S-Adenosyl Homocysteine).

Whether there is methylation or not to determine or for detection there are several methods such as-

- High-performance capillary electrophoresis (HPCE)
- Bi-sulfite treatment method
- Methylation-sensitive representational difference analysis (MS-RDA).

B. Histone Modification

Histones are the chief proteins that tightly bind with DNA molecules. There are 4 histone proteins - H2A,H2B,H3,H4. As DNA is negatively charged and on the other hand Histones are positively charged, results in strong interactions

between them. So, there are several modification taking places to loosen the interaction between them such Histone methylation, Acetylation, Phosphorylation, Ubiquitination, SUMO ylating.

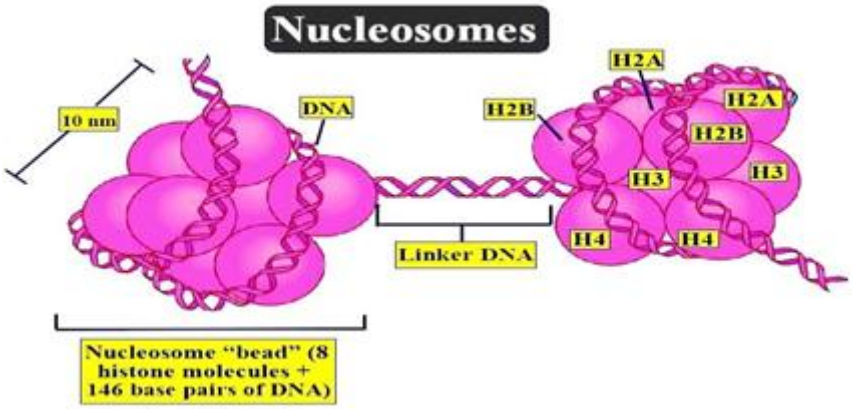


Figure 3: Different Histone Proteins (Forming Octamer)

- **Histone Methylation:** This process is involving a methyl group (-CH3) group to the amino acid (lysine or arginine) presents in histone tails with the help of enzyme Histone Methyltransferase (HMT) and Lysine Methyltransferase (LMT). Here H3 and H4 will actively participate. In Histone methylation there will be gene activation as well as gene repression. If methyl group will add to the 4th or 36th positioning lysine it will lead gene activation whereas gene repression will occur if methyl group will add to 27th or 9th position lysine in the histone tails.

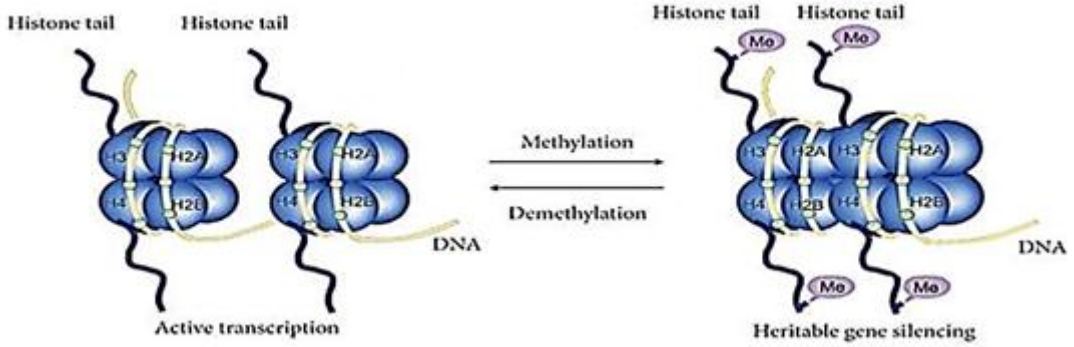


Figure 4: Histone Methylation

- **Histone Acetylation:** Here, additional of an acetyl group to the amino acid (lysine) present on the histone tails with the help of HAT (Histone Acetyltransferase). Acetylation reduces the positive charges in histone protein and weaken the interaction between DNA molecules and histone proteins and then RNAPII (RNA polymerase II) starts transcription.

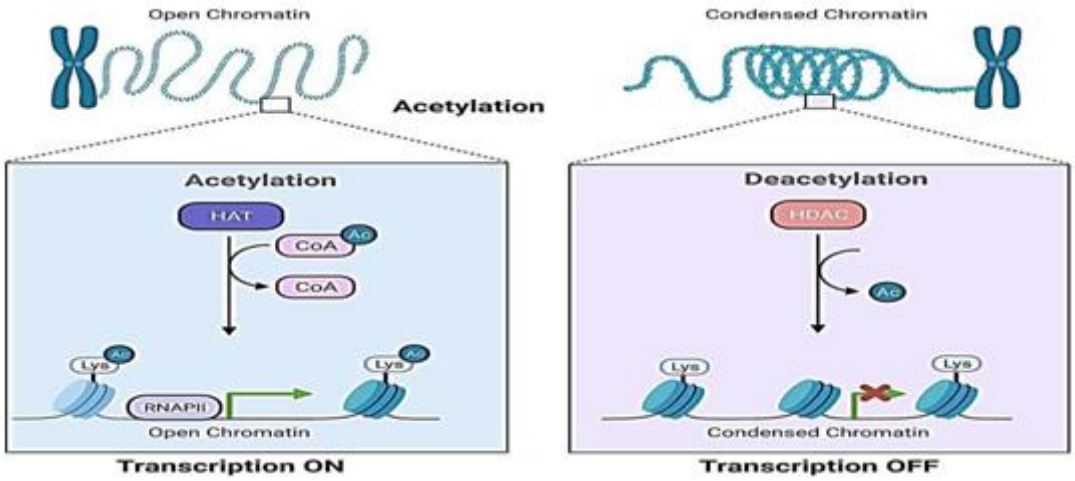


Figure 5: Histone Acetylation

Whereas, in Histone deacetylation, HADC (Histone acetyltransferase) acts exactly opposite to the HAT. It will remove the acetyl group from the histone tail and increase the interaction between the histone and DNA molecules, results in gene suppression.

- **Histone Phosphorylation:** It is one of the epigenetic modifications where Protein Kinase (enzyme) add a phosphate group to the histone tail and increases the negative charges in histone, as a results in less interaction between DNA molecules and histone proteins, then it will go for transcription. Where in enzyme phosphatase increases positive charges on histone by removing the phosphate group, results in condensation of the chromatin.

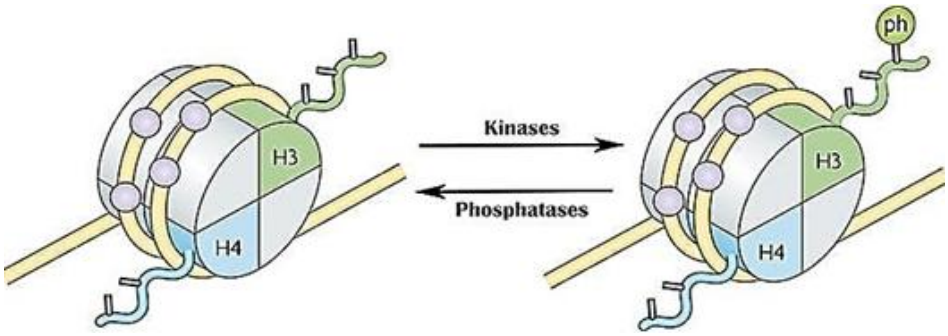


Figure 6: Histone Phosphorylation

- **Ubiquitination:** Ubiquitin is a small protein which regulates various cellular process, by masking some gene, or by changing cellular location. Ubiquitination mostly takes places in H2a and H2B. If H2b is added with ubiquitin, leads to gene activation where in H2a is modified with ubiquitin then it will lead to gene repression.

- **SUMOylation:** SUMO (Small ubiquitin-related modifier), it will covalently be binding to lysine in the histone proteins (H2A and H2B). It is a site-specific protein which acts via cascade analogous to but distinct from the Ubiquitination pathway.

C. RNA Interference

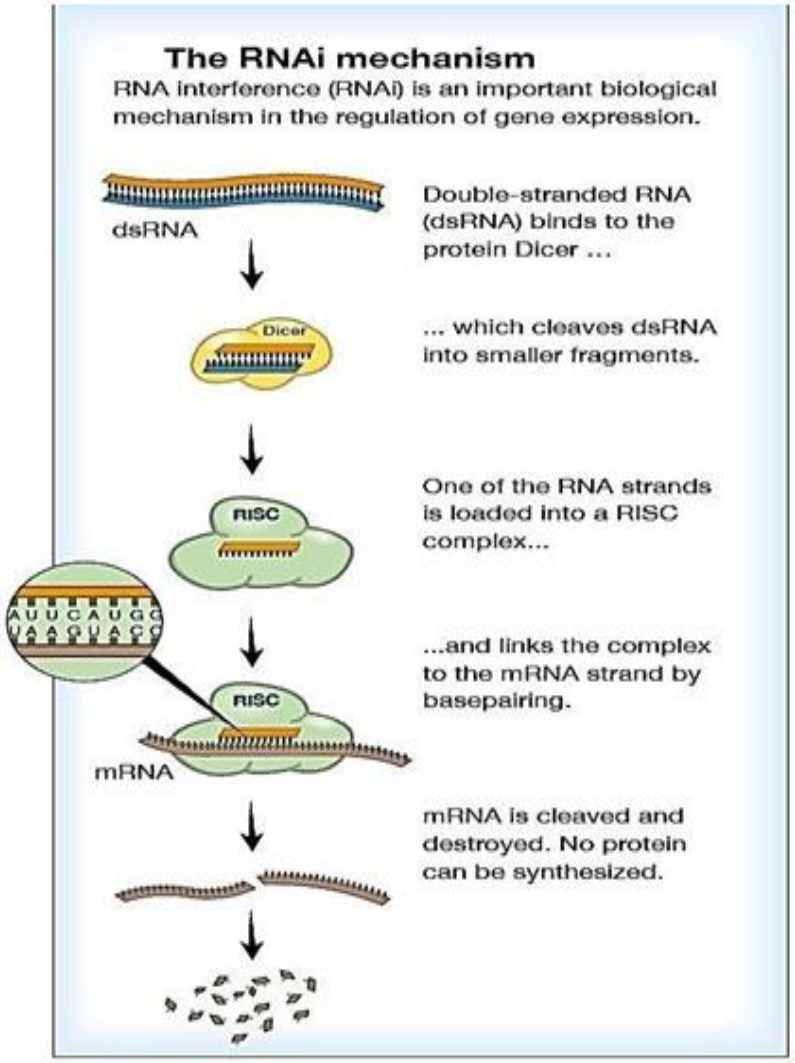


Figure 7: RNA Interference Technology

When transcription (synthesis of protein from RNA) is hampered with some miRNA (micro-RNA) and siRNA (small interference RNA), results in no synthesis of protein, refers as RNA interferences. These molecules are 21-28 base pairs long and produces from duplex RNA with involvement of restriction endonuclease enzyme called Dicer. siRNA are exogenous by origin and mostly having 100% complementary base pair to the target mRNA where in, miRNA is

endogenously originated and often not posses 100% complementary base pairs to the target mRNA. miRNA only regulates the post transgenerational gene expression and siRNA commonly response to the foreign RNA (usually viral, transposons).The RNAi mechanism firstly starts in the nuclease, there the hp-RNA (hairpin RNA) cut in to short fragments hp-RNA (contains 70 base pairs). Short ds RNAs are exported to the cytoplasm with enzyme called Exportin 5. There the hp-RNAs are cut into dsRNAs, restriction endonuclease enzyme involved called Dicer. In the duplex RNA, one strand is guiding strand and another is passenger strand. Then RISC (RNA -silencing complex) destroy the passenger strand and release the miRNA which has thecomplementary base pairs to the target mRNA. Then it will go and binds to the target mRNA and cleave the target or else it will block ribosome to enters in, in both the cases no protein will synthesis, results in no gene expression.

II. IMPLICATIONS OF EPIGENETIC MODIFICATION

A. Paramutation

Paramutation are the epialleles refers identical DNA sequences but display different epigenetic states. Paramutations are transgenerationally inherited and stable and are also expected to contribute to the next generation, results in phenotypic diversity without altering the genomic sequences. Different studies reported that paramutation not only an interesting epigenetic phenomenon but also it is having an economical importance to exploited. Typical inheritance of paramutation firstly observed in studies of the genetics of ‘rogues’ in cultivated pea (Pisum sativum; Bateson and pellew,1915). Crops like tomato and maize, it shows most of similarities and few differences in the biology of paramutation (Gouil *et al.* ,2016).

Table.1: Epigenetic Modification in Various Crops Leading Phenotypic Variations.

Species	Target Gene or Loci	Target Phenotype	Epigenetic Mechanism	Reference
Maize	B1, R1, Pl1, P	Anthocyanin pigment pathway in shoot tissues of maize	Paramutation	Hollick, 2017
Orange	Gene related to ripening	Ripening	Hypermethylation during fruit- ripening	Hung *et al.*, 2019

Tomato	*LeSPL-CNR*	Colourless fruits And abnormal ripening	Silencing of expression by increased DNA methylation in the promoter region	Manning *et al.,* 2006
Cotton	COL2D	Photoperiodicity	Hypomethylation on the 5' region	Song *et al.,* 2017
Rice	D1	Dwarfing	Hypermethylation and repressive histone mark on the promoter region	Miura *et al.,* 2009
Melon	*CmWIP1*	Sex determination	Hypermethylation on promotor leading formation of female flower	Martin *et al.,* 2009
Oil Palm	karma	Mantled trait from somaclonal variant	Hypomethylation on karma	Ong-Abdullah *et al.,* 2015

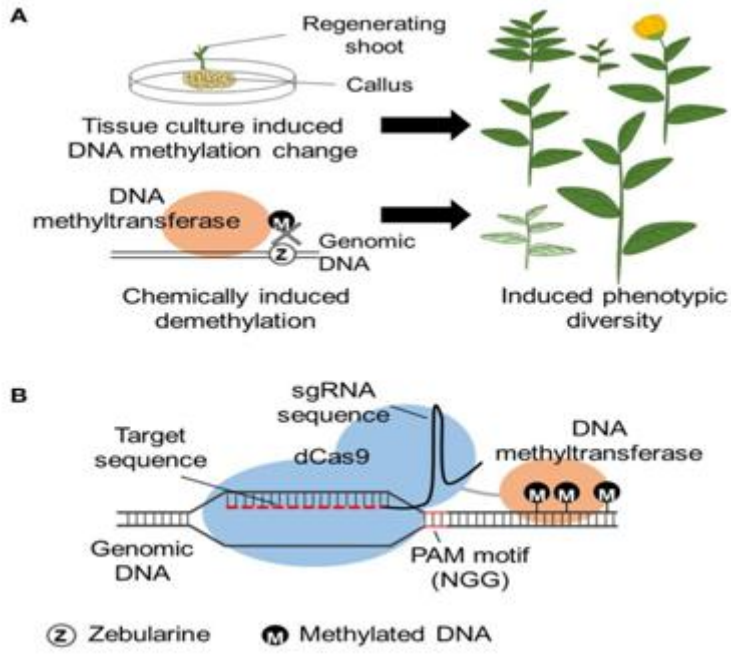
B. Genomic Imprinting

Genomic imprinting refers simply a process of suppression of gene by DNA methylation. In this process some genes are determined whether the gene is inherited from male parent or female parent. In case a gene is preferentially expressed from maternal cell, then it is classified as MEG (Maternally expressed gene) and when from parental allele, known as PEG (Parental expressed gene). In maternal gene different DNA methylation states get reduced by DNA demethylation (Park *et al.,* 2016). Whereas in sperm cell DNA methylation maintained and reinforced via RNA interferences (siRNA) pathway derived from vegetative cell (Kim *et al.,* 2019; Kiran *et al.,* 2019). According to Haig and Westoby, 1989 the most acceptable explanation of imprinted gene is the parental-conflict hypothesis for limited maternal resources which has been passed through next generation. Several crop studies reported that some imprinted gene's function which can control nutrient allocation, example such as, in maize, Meg1 is a maternally imprinted gene and involves establishing and differentiating endosperm nutrient transfer tissue (Costa *et al.,* 2012).

C. Chemically Induced DNA Methylation Changes

Chemically induced DNA methylation can develop some useful agronomic traits which can be used for further breeding programme. On the other way it can be used as a tool to understand the biology of the mechanism for better understanding the phenotypic changes. Different base lysine analogs like zebularine and (5-azadC) are incorporated in the genome during DNA replication results in removal of cytosine. When 5'-aza-2'-deoxycytidine is

used, the azacytosine-guanidine dinucleotides bind with DNA methyltransferase but they are unable to accept the methyl group and ultimately leading to suppression of DNA methylation. Zebularine has also a similar mechanism to adduct DNA methylation (Cheng *et al.* ,2003). DNA hypomethylation can result in the activation of mobile TEs that can translocate within the genome by a cut-paste (DNA transposons) or copy-paste (retrotransposons). As a random epigenome modifier, RNA polymerase II inhibitors also can function as DNA methylation inhibitors (Thieme *et al.*, 2017) because Pol II is required for RdDM establishment of some loci (Gao *et al.*, 2009).



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- DNA methylation is altered during tissue culture.
- CRISPR/Cas9 induced DNA methylation by fusion of DNA methyltransferase to the Cas9 protein.

D. CRISPR/Cas9

It is an alternative method for epigenome modification where Cas9 is a DNA endonuclease that associates with a single-guide RNA (sgRNA). sgRNA consists of a 20 nucleotide sgRNA recognition sequence containing a protospacer adjacent motif (PAM). Cas9/sg RNA complex will bind with the recognition sequence on the genomic DNA whereas for genome editing, Dead Cas9 will be used so that Cas9 will still able to bind with the target sequence and cleave the site.

III. NECESSITY OF INCLUDING EPIGENETIC MODIFICATION

Creating variation is one of the most important aspects in every breeding programme. Like that epigenetic modification creates epigenetic variation and it is more or less stable, contribute to the next generation. In case of vegetatively propagated crop (tissue culture) modification of epigenetic patterns may lead to some somaclonal variations.Histon PTMs (post transgenerational modification) are most useful, example like in potato due to the potential erosion during meiosis. For abiotic stress and biotic stress plant can modify their epigenetic states or they can reprogramme their transcriptome in such a way that they can reduce the environmental stress. Epigenetic factors can be played a significant role in inducing broad spectrum resistance or tolerance against abiotic and biotic stress (Kumar *et al.,* 2024). Resistance in Arabidopsis to Pseudomonas syringaepv tomato DC3000 bacteria is also increased due to previous exposure to heat, salinity, or cold stresses driven by an epigenetic-dependent mechanism (Singh *et al.*, 2014; Kumar *et al.,* 2019).

In modern day involvement of intensive breeding programme and over exploitation of any desirable traits there will be some unintentional disappearance of desirable traits, which leads to gene erosion. Addition of epigenetic modification act as a source of phenotypic variations and will enhance the accuracy of the breeding selection process. Also, plant can maintain a good balance between their agronomic traits like they can maintain yield in adverse climatic conditions by buffering the stress condition. Epigenetic modifications are time and cost effective as there is no need for searching new desirable traits in other germplasm.

IV. LIMITATION

But successful implication of this new science and technology for any crop improvement approaches at DNA level it is requires financial and non-financial support from the public, producers, and government. Identifying the heritable epiallele is also very challenging as they are a good source of variation. And modification DNA and histone proteins convert into more complex states to work with. Designing efficient EpiEffectors, and finding the best combinations of the chromatin modification and maintain the stability of the changes are still challenging.

V. FUTURE PROSPECTIVE

Though the research of epigenetic is in its infancy but there are some review papers which reported its potentiality to driven the crop improvement programme to a new direction. As the scientists and breeders are also increasingly attracted to the investigation of epigenetic diversity and its transgenerational behaviour as well as their enhanced inclusion in crop improvement, are expected to promote the application of epigenetic modifications in plant breeding to cope with the direct and indirect climate change effects for sustainable crop production.The right exploration of epigenetics may rapidly develop into one of the most influential areas of scientific research in near future.

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Questions

1. How epigenetics involves in crop improvement?
2. Is it possible to induce artificial epigenetic modification in plant?
3. Is Epigenetic modification stable- yes or no. Give reason
4. What is genomic Imprinting?

Self Assesment

1. The word epigenetic means
   a. On top of genetics
   b. Bottom of Genetics
   c. Both the above
   d. None of the above

2. What are the pillars of Epigenetic modifications
   a. DNA methylation
   b. Histone modification
   c. RNA interference
   d. All the above

3. Which of the following statements is false?
   a. Epigenetics inheritance does not involve a change in DNA sequence
   b. It is highly environmentally influenced.
   c. Both the statements
   d. None of the above

4. Which terms are related to epigenetic phenomena-
   a. Imprinting
   b. Monozygotic twins
   c. Prions
   d. All the above

5. Which are the methods for determining the methylation in DNA?
   a. High-performance capillary Electrophoresis (HPCE)
   b. Bisulfite Treatment method
   c. Methylation-sensitive representational difference analysis (MS-RDA)
   d. All of these

6. Following which enzyme is required for histone methylation?
   a. HMT (Histone Methyltransferases)
   b. LMT(Lysine methyltransferases)
   c. Both (i) and (ii)
   d. None of the above

7. mi RNA stands for -
   a. Mini RNA
   b. Micro RNA
   c. Macro RNA
   d. All the above

8. si RNA stands for-
   a. Short interference RNA
   b. Small interference RNA
   c. Both
   d. none of the above

9. Following which are the Non-coding RNA?
   a. siRNA
   b. miRNA
   c. ln RNA
   d. All the above

10. Which is the nucleotide size of the si RNA?
    a. 21-28 base pair
    b. 30-60 base pair
    c. 60-120 base pair
    d. (iv)100-200 base pair

11. RISC stands for-
    a. RNA- inserted silencing complex
    b. RNA-induced silencing complex
    c. RNA- interference silencing complex
    d. All of the above

12. Who is father of Epigenetics?
    a. Conrad Waddington
    b. Tom Roderick
    c. Bateson
    d. T.H Morgan

13. Which is the first commercial rice variety showing RNAi?
    a. Mutant line low glutelin content-1
    b. DRR Dhan 53
    c. DRR Dhan 55
    d. None of the above

14. The word ‘Epi’ is a-
    a. Latin word
    b. Greek word
    c. Both (i) and (ii)
    d. None of the above

15. MBD stand s for-
    a. Methylcytosine-Binding Domain
    b. Methylthymine-Binding Domain
    c. Methyladenine-Binding Domain
    d. (iv)Methylguanine- Binding Domain

16. CpG islands are rich in which nucleotides?
    a. Cytosine
    b. Guanine
    c. Both (i) and (ii)
    d. All of the above

17. Histone proteins are charged.
    a. negatively
    b. positively
    c. neutral
    d. All of the above

18. Epigenetic modifications are –
    a. more or less Stable
    b. highly environmental influenced
    c. All the above
    d. None of the above

19. Which is/ are the challenges to reach the improvement using epigenetics?
    a. Identification of the new epigenetically regulated traits
    b. Facilitate the selection of elite genotypes for the development of new cultivars/ varieties
    c. Understanding the mechanism that's triggers the resistance/tolerance to multiple stress and evaluate their stability.
    d. All the above

20. Following which method is used successfully in potato to induce heritable transgenerational gene silencing in plants?
    a. RdDM
    b. CRISPR-Cas9
    c. Mega nuclease
    d. TALEN

21. Phased siRNA was reported in which of the following crops?
    a. Maize
    b. Rice
    c. Both (i) and (ii)
    d. None of the above

22. PAM stands for-
    a. Protospacer Active modification
    b. Protospacer Adjacent Motif
    c. Post-transitional Adjacent Motif
    d. None of the above

23. Following which are the cytosine analogs?
    a. 5'-aza-2'-deoxycytidine (5-azadC)
    b. zebularine
    c. Both (i) and (ii)
    d. None of the above

24. Following statements are correct?
    a. Natural epigenome modifications have been acquired through evolution and traditional breeding based on trait and genetic characterization.
    b. Artificial induction of epigenome modification is expected to increase the phenotypic diversity including agronomically useful traits.
    c. Random epigenome modification induces epigenome changes randomly into the genome, whereas targeted epigenome allows epigenome modification of specific regions of the genome.
    d. All the above

25. The phenomenon 'Paramutation' refers-
    a. Artificially occurring epialleles can lead to the epigenetic modification.
    b. Naturally occurring epialleles can lead to the epigenetic modification
    c. Chemically induced epialleles can lead to the epigenetic modification
    d. All of the above