

Current Botanical and Agriculture Aspects in Plants and Physiological Mechanism

Shahid Fareed¹, Azka Saleem¹, Hafsa Farooq^{2*}, Sana Razzaq¹, Muzamil Shabir¹, Hakim Zamir¹, Zoima Tariq¹, Messum Ali³, Ghulam Murtaza³

¹Department of Botany, University of Agriculture Faisalabad, Pakistan

²Department of Chemistry, University of Agriculture Faisalabad, Pakistan

³Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan

DOI: [10.36348/sjls.2023.v08i10.005](https://doi.org/10.36348/sjls.2023.v08i10.005)

| Received: 21.09.2023 | Accepted: 27.10.2023 | Published: 20.11.2023

*Corresponding author: Hafsa Farooq

Department of Chemistry, University of Agriculture Faisalabad, Pakistan

Abstract

The molecular epigenetics study in the plants plays a vital role in plant gene regulations, since the early descriptions of the non-Mendelians plant-based gene activities to pivotal detections of chromatin amending proteins necessary for plant growth and the RNAs which facilitates the silencing of genes in human and in the eukaryotes. Different factors playing important role in gene regulation in plants through cellular signaling pathways. Different genes show over expression and repression in response to different conditions in plants as a result of environmental stresses. While on the other hand, plants have haploid (gametophyte) development stage which occurs after meiosis and before fertilization. Genomic screening in *Arabidopsis thaliana*, the model plant, have been mainly worthwhile, yielding more than one hundred and thirty epigenetic regulators so far. The major contribution of plant science to current global hot problems like sustainability and climate change makes the expansion of plant science research capacity crucial. The objective is to highlight that reflect these developments in the creation of new biotechnological tools (NBTs) and the creative uses of plant genetic engineering. Studies that concentrate on the creation of NBT for resistant or previously non-transformable species to enable the unlocking of these species' biology are of great relevance to this collection. In addition, the use of cutting-edge genetic engineering techniques such as genome/gene editing and protein-domain specific technology (such as K-Domain technology).

Keywords: Molecular epigenetics, Epigenetic regulation, chromatin, Plant metabolism, plant developmental.

Copyright © 2023 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

The newest advancement in plant genetic engineering, artificial mini chromosomes serve as independent platforms for the expression of foreign genes and as instruments for the study of chromosome structure and function. Stacking several foreign genes without gene segregation has been successfully accomplished using this telomere-associated chromosomal truncation method in both plants and mammals. In *Brassica napus*, the shortened mini chromosome was used as a platform to receive foreign genes. When comparing and contrasting the differences and similarities between animals and plants, it is critical to recognize the unique life cycles of the plants. Plants have haploid (gametophyte) development stage which occurs after meiosis and before fertilization [1-3]. Female and male gametophytes are embryo and pollen-

sacs, correspondingly, and are made up of numerous cells formed by the mitotic divisions of first haploid meiotic outputs. Loss of genetics or molecular epigenetics information in the haploid gametophytes, that are metabolically and genetically active, cannot be accounted for by data on the homologous-chromosomes; thus, detrimental mutations in important gene are chosen in contradiction [4-7]. Unlike humans, there is no indication of widespread molecular epigenetics mark deletion during the plant's gametogenesis. Conversely, particular trans-silencing RNAs produced in nearby nucleus appear to enhance suppressive molecular epigenetics mark in the plant reproductive cells. This could explain why epigenetic alterations are frequently transmitted by meiosis in the plants [8-12].

Conventional breeding is a different method that makes use of advantageous traits found in natural variants and incorporates them into commercial lines. However, the typical breeding approach takes a long period and has a limited amount of genetic resources [2, 4]. As an alternative, genetic engineering and biotechnology are strong tools that can be utilized to directly alter the genetic code of particular crop types to change metabolic processes or boost mineral intake [4]. Plants have laterally meristems in addition to the apical meristems, which are clusters of stem cells at shoot and root apex. They are comprised of stem cells in perennials which forms the fresh xylem and phloem annually, leading to distinctive growth-rings of tree trunks, and also buds at each leaf base which can grow to become vegetative organ or flower. Most plants have special organs like underground-rhizome, bulbs or tubers which contain stem cells having ability to produce shoots that create independent, new plant. These clonal or vegetative propagation processes are ubiquitous in plants and are frequently more efficient than seed dispersal as a technique for occupying a good area. Furthermore, mitotically transmittable epigenetic states can be passed down by clones formed through propagation of plants [1, 8, 9].

The main adaptive strategies are the induction of high-affinity Pi transporters, reconstruction of root morphology and architecture, accumulation of anthocyanins in shoots and leaves, substitution of phospholipids with glycolipids and sulfolipids in biomembranes, increased root exudates (e.g., organic acids and acid phosphatases), and symbiotic associations with beneficial microbes [13, 14]. A reworking of the root morphology and architecture in response to low-P stress is frequently seen in most plant species, including increases in lateral root length and root hair density as well as the development of shallow root architectures [15-17]. Plants also have plasmodesmata that are the cytoplasmic connections among cells that allow viruses, RNAs, metabolites, and even proteins to move across. Plant branches, known as scions, can be cut and grafted onto genetically distinct root-stocks. As a result, chimaeras are formed in which the shoots and roots are genetically distinct. Diffusible molecular epigenetics signals travel via the vascular system and plasmodesmata, and in grafted plants, they can be transported from roots to shoots. In this approach, inputs from the distant parts of plant body have the capacity to modify the state of epigenetic of the stem cells and the gametes [13, 14].

Genetic Features in Plant Molecular Epigenetics

Sexual reproduction involves the union of a male sperm with a female egg cell or ovary. This process is called fertilization. There are two types of pollination: self-pollination and cross-pollination. When the pollen of a plant pollinates a flower on the same plant the process is called self-pollination. Palindromic repeats are separated by short (32 to 36 bp) sequences derived from

the DNA of viruses that have previously infected the cell or its predecessors. These virus-derived sequences integrated into the bacterial genome provide a memory system of previous virus infection [18-20]. Binary complexes formed by guide RNA-Cas9 recognize and cleave DNA of incoming viruses with sequence similarity to the guide RNA. Plant breeding requires the retirement of traditional theories that assume random mating, such as the average effect of an allele and additive variance, because the entirety of the germplasm available in a breeding program is not in Hardy-Weinberg equilibrium. This is possible because molecular markers allow for the employment of mixed-model techniques for best linear unbiased estimate (BLUE) and prediction that only require a few genetic assumptions. It would be advantageous for plant breeding to use techniques that have been successful in other fields. Examples include operations research and simulation methods for developing breeding programs as well as reliability as a new indicator of the relative importance of genetic versus nongenetic impacts. Physical or chemical treatments, randomized transgenes insertion, or mobility of transposons can all be used to effectively mutagenize plants. Furthermore, in self-pollinating plants like *Arabidopsis*, homozygous mutants could be quickly discovered amid thousands of progenies from single plant which is mutagenized, eliminating the necessity for time-consuming out-crossing or backcrossing processes. Generally, molecular epigenetics regulator mutations screening is based on expression recovery of silent marker genes, that are usually created transgenes. Thus, the capacity to simply create transgenic plants has considerably aided molecular epigenetics study. Aside from these forward genetic methods, reversed genetic procedures that disrupt the functioning of genes are also available. This procedure is eased by the mutant's insertion or by the use of transgenes induced RNAi to delete or suppress the expression of potential genes, such as the genes homologous to molecular epigenetics regulators acknowledged in the other creatures [15,16].

Molecular Biology Tools and Machineries in Plants

In plants, methyl cytosine (5mC) is a marker of heterochromatin and molecular epigenetics genes silencing. While 5mC is almost found mainly in differentiated human cells nuclei at CG loci (also known as Gene loci), plant methylate cytosines within CHG, CHH, or CG, patterns (where H is A, T, or C) [11, 13, 16]. Mammal regulators are frequently found in methylation-free CG rich areas designated as CpG-islands; however, CpG-islands are difficult to recognize in the plants. Nonetheless, methylation of cytosine occurs non-randomly in the plants, especially in repeated areas of genome abundant in transposons, centromeric repeats, or array of silenced 5-S or 45-S rRNA genes repeats. Some differential expression promoters and protein coding regions of overexpressed genes are additionally methylated. Another type of gene body methylation has been found in animals as different as

mammals and honeybee. The importance of CG methylation in gene bodies is unknown, however its abundance inside exons implies a possible function in pre-mRNA splicing [17, 18].

Cross-pollination is often observed in crop species that show protogyny (i.e., the pistils/stigmas of a plant mature and become receptive before the anthers of that plant) and protandry (i.e., stamens/anthers of a plant develop and the pollen grains mature and are shed before the pistils/stigma of that plant mature and become receptive) [4, 8, 9]. The enzyme CHROMOMETHYLASE 3 (CMT3) is mainly responsible for the CHG maintenance-methylation. The chromodomain of CMT3 binds the histone H3 that has been demethylated on lysine-9 (H3K9me2). CHG-methylation, in response, offers a binding domain for the H3K9 methyltransferase. As a result, SUVH4 and CMT3 form a self-reinforcing loop in which restrictive DNA methylation and the histone alteration marks identify one another in order to sustain an epigenetic position. CHROMOMETHYLASE 2 (CMT2) maintains CHH methylation in certain genomic locations, like the core regions of major transposons, likely by cross interaction with histone alterations like CMT3 [19, 20].

Male sterility is caused by the formation of non-functional pollen grains, which prevents self-pollination and promotes cross-pollination. Two types of male sterility are: cytoplasmic male sterility (CMS), which is caused by mitochondrial genes interacting with nuclear genes; and genic male sterility (GMS), which is caused by nuclear genes alone. These phenomena can be exploited for hybrid seed production. In spite of processes permitting the methylation of DNA to be continued, cytosine methylation can be lost. Passive damage happens when the methylation is lost during replication or after repair of DNA. Activity of enzymes can also result in active demethylation. Active demethylation in the animals was demonstrated many years ago, but the precise mechanisms and players are still unknown [2, 19]. ROS1 nicks methylated DNA, which results in the replacement and removal of methylated cytosines through a mechanism similar to nucleotide excision repair. ROS-1 is predominantly expressed, which may contribute to DNA methylation loss in the non-dividing cells at all development stages. ROS-1 is assumed to be directed to its action areas by its connection with the ROS-3, an RNA binding protein, implying that RNA may be involved in both guiding demethylation and directing de novo methylation in RNA directed DNA methylation pathways [20, 21].

Crops are capable of both self- and cross-pollination. These crops are classified as either autogamous (self-pollinated) or allogamous (cross-pollinated) depending on the relative frequency of self- or cross-pollination that is observed in the species. Mutation in Arabidopsis gene that encodes this enzyme

led to reduced methylation of DNA and transcriptional silencing release, particularly from the pericentromeric heterochromatin genes. The mutations also have an effect on histone-methylation, which further influences molecular epigenetics regulation [22, 23].

Histone-Modifying Enzymes and Histone Variants

Plants, like the other species, have many enzymes and histone variants which changes the histones post-translationally and affect regulation of gene expression. The use of chromatin immunoprecipitation coupled by deep-sequencing reveals the genome wide prevalence of histones and histone variants with various post-translational changes [24-26].

In plants, big gene groups frequently encode the histone modification enzymes. Histone Demethylases and Methyltransferases are the enzymes which methylate histones. Methylation of histones, like the acetylation, is a hypothetically an alterable mark. HKMTs can either hinder or promote transcription based on the particular histone lysine which is methylated. Some SET domains proteins belong to the Tri-thorax group (TrxG) and Polycomb group (PcG), which sustain transcriptionally restricted or activated states of genetic markers all through the development of animals and plants, accordingly. Other Su(var)3-9 group SET domains proteins are involved in regulating compacted heterochromatin, repressing transposons, and regulating replication of DNA [24-26].

SNF/SWI and the other transformation complexes acts on the nucleosomes which are already linked with the DNA, many other actions are obligatory for the core histones assembly into a new nucleosome after the replication, regenerating chromatin after repairing or the recombination linked DNA formation, or for histone exchange in assembly with the transcription procedures. These activities are employed by the histone's chaperones that are typically acidic nature proteins which interacts with one another and certain variant or canonical, histones. This shows that the right deposition of nucleosome is crucial for the development, genomic stability, and for control of epigenetic [25-27].

The inclusion of short RNAs, particularly siRNAs and miRNAs, is a typical aspect of plant post-transcriptional and transcriptional silencing processes. The synthesis of these short RNAs in the plants is identical to that of the other eukaryotes, implying that RNA based suppressing processes involving si and miRNA share an evolutionary basis. Furthermore, in plants, the sub-functionalization and the duplication of genes engaged in siRNA or miRNA mediated activities has resulted in the creation of different routes that are specialized to perform certain functions. These processes, when combined, give plants an arsenal of RNA mediated suppressing capability that no other eukaryote has [28, 29].

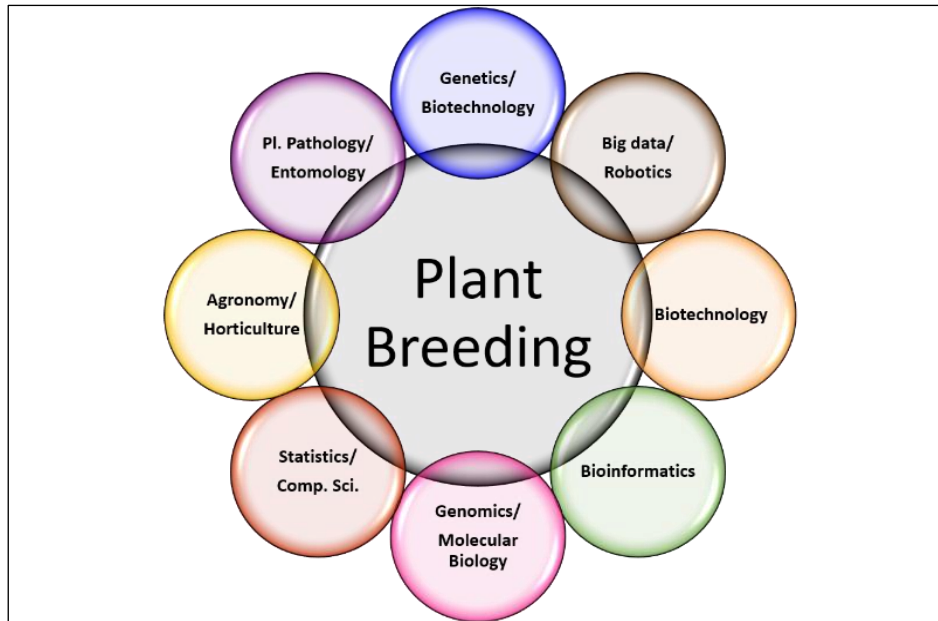


Fig-1: Shows the molecular biology and plant-based principles

siRNAs or miRNAs shared many common structures in plants, like in other eukaryotes. Both are generated from dsRNA forerunners by RNase-III-related-Dicer (DCL) endonucleases [30, 31]. The resulting short RNAs are then subsequently integrated into a multi-protein RNA induced suppression complex (RISC) that contains a member of Argonaut protein family at its core. The Argonaut protein binds the short RNA's 3' end through its PAZ-domain and utilizes it to base pair with the complementary target RNAs. As a result, the Argonaut proteins PIWI-domain can cleave targeted RNA, or translations could be stopped without destruction of the linked RNA, or chromatin modification components can be activated to transcriptionally mute the locus [32, 33].

There are various techniques to create the double stranded siRNAs or miRNAs precursors. In

miRNAs case, DNA dependent RNA polymerase II transcriptions with the wide self-complementarity fold back on themselves to generate stem loop forms with the defective double stranded stems which can be diced. In siRNAs case, double stranded predecessors can be produced through convergent, bi-directional transcriptions through a DNA dependent RNA polymerase like RNA Pol II, in that way making transcripts which overlaps and base pair. RNA transcripts can also be utilized for templates for RNA dependent RNA polymerase to produce a complementary strand [13, 19, 20, 21].

Stem cell differentiation and maintenance, floral patterning, vascular development, organ polarity characterization, hormone transmission, and reactivity to environmental perturbations are among developmental processes that require miRNAs [21, 22, 28, 29].

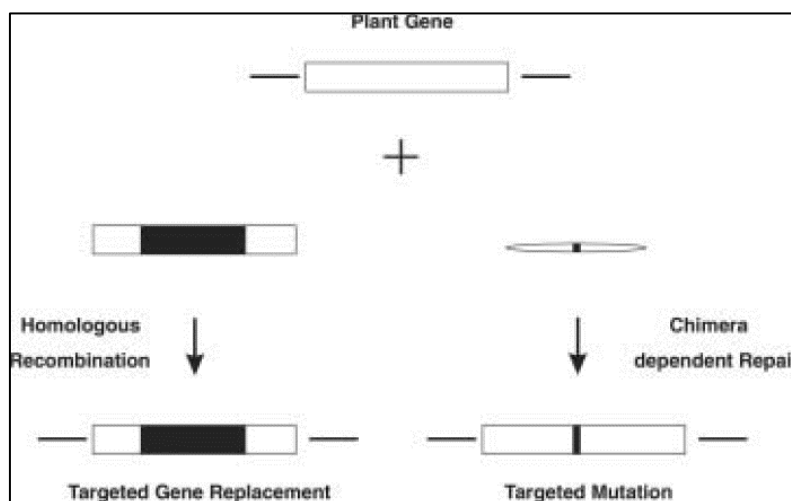


Fig-2: Shows the predecessors can be produced through convergent molecular biology

Current Aspects of Plant Genetics in Molecular Biology

However, some environmental conditions induce altered chromatin and gene expression states that persist even after a return to the original environmental condition, as in the case of vernalization in which plants “remember” their experience of winter to flower the following spring. There is also the possibility that environmentally or pathogeninduced epigenetic states might be transmitted to progeny if the changes occur in meristems and can be maintained through meiosis. So far, there is only rudimentary evidence for transmission and inheritance of adaptive epigenetic states, as opposed to DNA sequence-based inheritance. Nevertheless, with our growing insight into epigenetic regulation and the transmission of mobile small RNAs influencing chromatin states, such neo-Lamarckian possibilities warrant careful consideration [33-36].

CONCLUSION

There is the chance that pathogens or environmentally induced states of epigenetic may be introduced in progeny if the variations occurred in the meristems and can be sustained through meiosis. So yet, there is only simple indication for the inheritance and transmission of adaptive states of epigenetic, as divergent to DNA sequencing-based inheritance. Nonetheless, with our rising vision into the molecular epigenetics regulations and transmission of portable smaller RNAs influencing the states of chromatin.

REFERENCES

- Akomeah, B., Quain, M. D., Ramesh, S. A., Anand, L., & Rodríguez López, C. M. (2019). Common garden experiment reveals altered nutritional values and DNA methylation profiles in micropropagated three elite Ghanaian sweet potato genotypes. *PLoS one*, 14(4), e0208214.
- Ashapkin, V. V., Kutueva, L. I., Aleksandrushkina, N. I., & Vanyushin, B. F. (2019). Epigenetic regulation of plant gametophyte development. *International Journal of Molecular Sciences*, 20(12), 3051.
- Batista, R. A., & Köhler, C. (2020). Genomic imprinting in plants—revisiting existing models. *Genes & development*, 34(1-2), 24-36.
- Böhmdorfer, G., & Wierzbicki, A. T. (2015). Control of chromatin structure by long noncoding RNA. *Trends in cell biology*, 25(10), 623-632.
- Cedillo-Jiménez, C. A., Hernández-Salazar, M., Escobar-Feregrino, T., Caballero-Pérez, J., Arteaga-Vázquez, M., Cruz-Ramírez, A., & Cruz-Hernández, A. (2016). MicroRNAs sequencing for understanding the genetic regulation of plant genomes. *In Plant Genomics*, (pp. 137-144).
- Chen, Z., & Pongs, N. (2020). H2A. Z and chromatin remodelling complexes: a focus on fungi. *Critical reviews in microbiology*, 46(3), 321-337.
- Dalakouras, A., & Ganopoulos, I. (2021). Induction of promoter DNA methylation upon high-pressure spraying of double-stranded RNA in plants. *Agronomy*, 11(4), 789.
- Davarinejad, H., Huang, Y. C., Mermaz, B., LeBlanc, C., Poulet, A., Thomson, G., ... & Jacob, Y. (2022). The histone H3. 1 variant regulates TONSOKU-mediated DNA repair during replication. *Science*, 375(6586), 1281-1286.
- Feng, S., Zhong, Z., Wang, M., & Jacobsen, S. E. (2020). Efficient and accurate determination of genome-wide DNA methylation patterns in *Arabidopsis thaliana* with enzymatic methyl sequencing. *Molecular epigenetics & chromatin*, 13(1), 1-17.
- Frost, J. M., Kim, M. Y., Park, G. T., Hsieh, P. H., Nakamura, M., Lin, S. J., & Fischer, R. L. (2018). FACT complex is required for DNA demethylation at heterochromatin during reproduction in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, 115(20), E4720-E4729.
- Gallusci, P., Agius, D. R., Moschou, P. N., Dobránszki, J., Kaiserli, E., & Martinelli, F. (2022). Deep inside the epigenetic memories of stressed plants. *Trends in Plant Science*.
- Gui, X., Liu, C., Qi, Y., & Zhou, X. (2022). Geminiviruses employ host DNA glycosylases to subvert DNA methylation-mediated defense. *Nature communications*, 13(1), 1-11.
- Habu, Y. (2010). Epigenetic silencing of endogenous repetitive sequences by MORPHEUS/MOLECULE1 in *Arabidopsis thaliana*. *Molecular epigenetics*, 5(7), 562-565.
- Hoekstra, M. J. (2022). Characterization of lysine demethylase KDM5 family: Substrate specificity and identification of potential novel non-histone substrates (Doctoral dissertation, Carleton University).
- Hoffer, P., Ivashuta, S., Pontes, O., Vitins, A., Pikaard, C., Mroczka, A., ... & Voelker, T. (2011). Posttranscriptional gene silencing in nuclei. *Proceedings of the National Academy of Sciences*, 108(1), 409-414.
- Huang, J., Lynn, J. S., Schulte, L., Vendramin, S., & McGinnis, K. (2017). Epigenetic control of gene expression in maize. *International review of cell and molecular biology*, 328, 25-48.
- Jiang, L., Li, D., Jin, L., Ruan, Y., Shen, W. H., & Liu, C. (2018). Histone lysine methyltransferases Bna SDG 8. A and Bna SDG 8. C are involved in the floral transition in *Brassica napus*. *The Plant Journal*, 95(4), 672-685.
- Kagale, S., & Rozwadowski, K. (2011). EAR motif-mediated transcriptional repression in plants: an underlying mechanism for epigenetic regulation of gene expression. *Molecular epigenetics*, 6(2), 141-146.
- Kim, J. M., To, T. K., & Seki, M. (2012). An epigenetic integrator: New insights into genome regulation, environmental stress responses and developmental

- controls by HISTONE DEACETYLASE 6. *Plant Cell Physiol*, 53, 794–800.
20. Kou, S., Gu, Q., Duan, L., Liu, G., Yuan, P., Li, H., & Liu, L. (2022). Genome-wide bisulphite sequencing uncovered the contribution of DNA methylation to rice short-term drought memory formation. *Journal of Plant Growth Regulation*, 41(7), 2903-2917.
 21. Kumar, S. (2018). Epigenetic memory of stress responses in plants. *J Phytochem Biochem*, 2(1).
 22. Lee, H., Han, S., Kwon, C. S., & Lee, D. (2016). Biogenesis and regulation of the let-7 miRNAs and their functional implications. *Protein & cell*, 7(2), 100-113.
 23. Liu, W., Duttke, S. H., Hetzel, J., Groth, M., Feng, S., Gallego-Bartolome, J., & Jacobsen, S. E. (2018). RNA-directed DNA methylation involves co-transcriptional small-RNA-guided slicing of polymerase V transcripts in Arabidopsis. *Nature plants*, 4(3), 181-188.
 24. Marx, N., Eisenhut, P., Weinguny, M., Klanert, G., & Borth, N. (2022). How to train your cell-Towards controlling phenotypes by harnessing the epigenome of Chinese hamster ovary production cell lines. *Biotechnology Advances*, 107924.
 25. Miryeganeh, M. (2021). Plants' epigenetic mechanisms and abiotic stress. *Genes*, 12(8), 1106.
 26. Parent, J. S., Jauvion, V., Bouché, N., Béclin, C., Hachet, M., Zytnicki, M., & Vaucheret, H. (2015). Post-transcriptional gene silencing triggered by sense transgenes involves uncapped antisense RNA and differs from silencing intentionally triggered by antisense transgenes. *Nucleic acids research*, 43(17), 8464-8475.
 27. Salim, U., Kumar, A., Kulshreshtha, R., & Vivekanandan, P. (2022). Biogenesis, characterization, and functions of mirtrons. *Wiley Interdisciplinary Reviews: RNA*, 13(1), e1680.
 28. Singroha, G., & Sharma, P. (2019). Epigenetic modifications in plants under abiotic stress. *Molecular epigenetics*.
 29. Springer, N. M., & Schmitz, R. J. (2017). Exploiting induced and natural epigenetic variation for crop improvement. *Nature reviews genetics*, 18(9), 563-575.
 30. Subfunctionalization, L. S. Evolutionary History of Plant Multisubunit RNA.
 31. Szucko, I., Kowalska, U., & Skuza, L. (2018). Bioinformatics analysis of the promoter sequence of the 9f-2.8 gene encoding germin. *Acta Biologica*, 25.
 32. Teano, G., Concia, L., Carron, L., Wolff, L., Adamusová, K., Fojtová, M., & Barneche, F. (2021). Histone H1 protects telomeric repeats from H3K27me3 invasion in Arabidopsis. *bioRxiv*, 2020-11.
 33. Tsaballa, A., Xanthopoulou, A., Madesis, P., Tsaftaris, A., & Nianiou-Obeidat, I. (2021). Vegetable grafting from a molecular point of view: the involvement of molecular epigenetics in rootstock-scion interactions. *Frontiers in plant science*, 11, 621999.
 34. Whipple, A. V., & Holeski, L. M. (2016). Epigenetic inheritance across the landscape. *Frontiers in genetics*, 7, 189.
 35. Xu, L., & Jiang, H. (2020). Writing and reading histone H3 lysine 9 methylation in Arabidopsis. *Frontiers in Plant Science*, 11, 452.
 36. Zhang, C., Hung, Y. H., Rim, H. J., Zhang, D., Frost, J. M., Shin, H., & Hsieh, T. F. (2019). The catalytic core of DEMETER guides active DNA demethylation in Arabidopsis. *Proceedings of the National Academy of Sciences*, 116(35), 17563-17571.