The Use of RNA Interference in Plant Breeding

Abstract

RNA interference (RNAi) has emerged as a powerful tool in crop improvement, revolutionizing the field of plant biotechnology. By leveraging the natural process of gene silencing, RNAi enables precise and targeted regulation of gene expression in crops. This technology has significant implications for crop improvement, offering the potential to enhance traits related to disease resistance, stress tolerance, yield, and nutritional content. Through RNAi, specific genes involved in plant development, pathogen susceptibility, and stress response can be selectively silenced, resulting in improved crop performance. The application of RNAi in crop improvement holds promise for reducing crop losses due to pests and diseases, mitigating the effects of adverse environmental conditions, and developing nutritionally enhanced crops. This abstract highlight the potential of RNAi as a transformative technology in crop improvement and its role in addressing the challenges faced by agriculture. Harnessing the power of RNAi opens up new avenues for sustainable agriculture, contributing to increased food production and improved crop quality.

Keywords: *RNAi, gene silencing, siRNA, miRNA*

Authors

Raiza Christina G
Ph.D scholar,
Department of Plant Breeding and Genetics,
Agricultural college and Research Institute,
TNAU, Madurai, Tamil Nadu, India.

Sumaiya Sulthana J
Ph.D scholar,
Department of Genetics and Plant Breeding,
School of Post Graduate Studies,
TNAU, Coimbatore, Tamil Nadu, India.

I. INTRODUCTION

RNA interference (RNAi) has revolutionized the field of crop improvement by providing a powerful tool to manipulate gene expression and enhance desirable traits in crops (Fire *et al.,* 1998). RNAi is a natural biological process that regulates gene activity through the introduction of small interfering RNAs (siRNAs) that target specific genes for silencing (Waterhouse *et al.* 2001). This technology enables breeders to precisely control gene expression, resulting in improved crop characteristics such as enhanced resistance to pests and diseases, increased tolerance to environmental stresses, and improved nutritional profiles. Traditional breeding methods have been effective in crop improvement, but they often rely on lengthy and labor- intensive processes (Baulcombe, 2004). RNAi offers a more targeted and efficient approach by directly silencing specific genes responsible for undesirable traits or by enhancing the expression of genes associated with desirable traits. By introducing siRNAs that match the target gene sequence, the RNAi machinery can degrade the target RNA, leading to reduced gene expression and subsequent changes in the phenotype of the crop plant. One of the key advantages of RNAi in crop improvement is its ability to enhance resistance to pests and diseases (Waterhouse *et al.,* 2001). By targeting essential genes in pests or pathogens, breeders can develop crops that produce siRNAs specifically designed to silence those genes. When pests or pathogens interact with these RNAi-expressing crops, the ingested siRNAs can trigger gene silencing in the pest or pathogen, leading to reduced damage or infection. Furthermore, RNAi technology can be utilized to enhance crop tolerance to environmental stresses such as drought, heat, and salinity (Koch *et al.,* 2006). By silencing genes that are responsible for sensitivity to these stresses or by activating genes associated with stress tolerance, breeders can develop crops that exhibit improved resilience in challenging environments. This holds significant promise for ensuring food security and sustainable agriculture in the face of climate change. RNAi also enables the enhancement of crop nutritional profiles (Borges *et al.,* 2015). By targeting genes involved in nutrient metabolism or synthesis pathways, breeders can modulate their expression to increase the content of specific nutrients in crops. This approach, known as biofortification, holds great potential for addressing malnutrition and improving the nutritional value of staple crops. While RNAi has immense potential for crop improvement, careful consideration must be given to potential off-target effects and the regulatory aspects surrounding its use (Dubrovina *et al.,* 2019). Rigorous evaluation of the target genes, efficient delivery systems, and stable expression of RNAi constructs in crop plants are crucial for successful implementation.

II. RNAi TECHNIQUES

RNA interference (RNAi) techniques involve the use of small RNA molecules to suppress or silence the expression of specific genes. These techniques have revolutionized the field of molecular biology and have various applications in both research and practical settings. Here are some commonly used RNAi techniques:

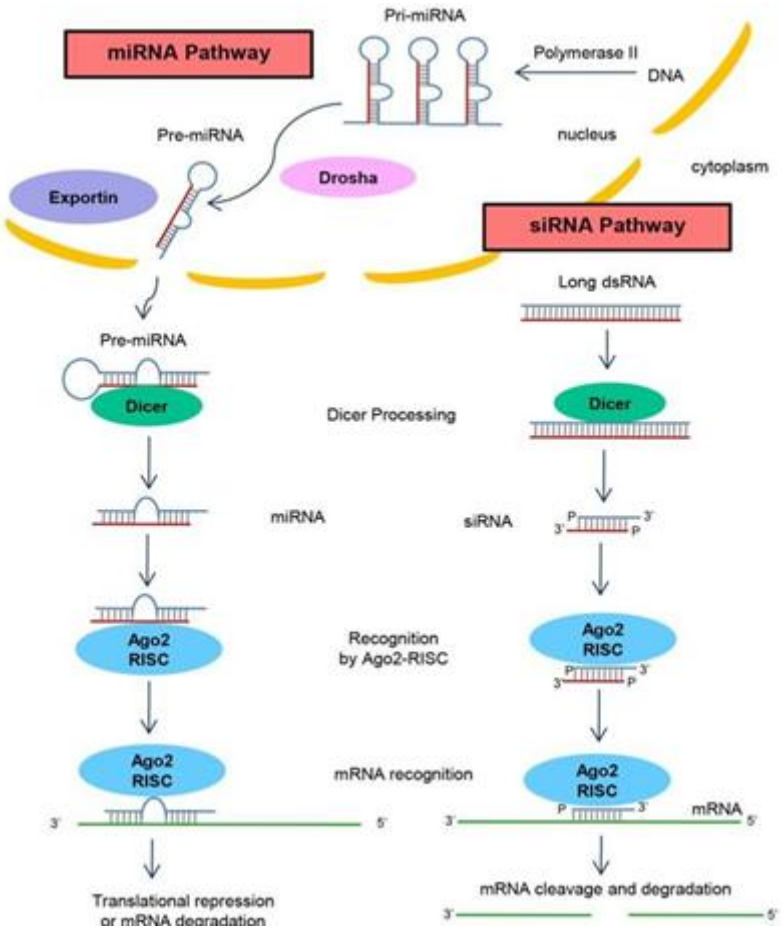

Figure 1: Process of miRNA and siRNA synthesis and function

A. Small Interfering RNA (siRNA): Small interfering RNA (siRNA) is a class of small RNA molecules that play a crucial role in the RNA interference (RNAi) pathway (Hannon, 2002). siRNAs are typically 20-25 nucleotides in length and are double-stranded RNA molecules with a characteristic structure (Elbashir *et al.*, 2001). They are designed to be complementary to specific target mRNA sequences and trigger gene silencing through sequence-specific degradation or translational repression of the target mRNA (Fire *et al.*, 1998). The process of siRNA-mediated gene silencing involves several steps (Fig 1.):

- **siRNA Synthesis or Introduction:** siRNAs can be synthesized chemically in the laboratory or introduced into cells through various delivery methods. Synthetic siRNAs can be designed to precisely match the target gene's mRNA sequence, ensuring specific gene silencing (Amarzguioui *et al.*, 2003).
- **Incorporation into RNA-Induced Silencing Complex (RISC):** Once inside the cell, siRNAs are loaded onto the RISC, a multi-protein complex that facilitates the recognition and cleavage of target mRNA. One strand of the siRNA duplex is selected as the guide strand (antisense strand) and is retained in the RISC, while the other strand (sense strand) is typically discarded (Liu and Paroo, 2010).
- **Target mRNA Recognition and Cleavage:** The RISC, with the guide siRNA strand bound, searches for complementary target mRNA sequences (Meister *et al.*, 2004). When the guide siRNA encounters a complementary target mRNA, it promotes base pairing between the siRNA and mRNA, resulting in mRNA cleavage by the endonuclease activity of the RISC-associated protein, Argonaute (Song *et al.*, 2004). This cleavage prevents the translation of the mRNA into protein and leads to gene silencing.
- **Amplification of the RNAi Effect:** The mRNA cleavage event initiated by the siRNA can lead to the release of secondary siRNAs known as short interfering RNA amplification (or secondary siRNAs). These secondary siRNAs can further contribute to gene silencing by targeting additional copies of the mRNA transcript (Pak and Fire, 2007).

B. Short Hairpin RNA (shRNA): shRNAs are artificial RNA molecules that can be expressed within cells from DNA constructs or viral vectors (Paddison and Hannon, 2002). Similar to siRNAs, shRNAs are processed by the cellular machinery into siRNA-like molecules, leading to gene silencing (Brummelkamp *et al.*,2002). The advantage of shRNAs is that they can provide a sustained silencing effect as they are continuously produced within the cell. Short hairpin RNA (shRNA) is a type of RNA molecule commonly used in molecular biology and genetic research to induce RNA interference (RNAi) and achieve gene silencing (Yu *et al.*, 2002). It is an artificial RNA molecule that mimics the structure of naturally occurring hairpin-shaped RNA molecules, such as precursor microRNAs (pre-miRNAs). Here are the key features and steps involved in utilizing shRNA for gene silencing:

- **Design and Construction:** The design of shRNA involves creating a DNA template that encodes a short hairpin structure. This template typically consists of a stem- loop structure with a sequence complementary to the target gene. The stem of the hairpin is formed by

complementary sequences that allow the hairpin to fold back on itself, while the loop region contains the sequence that matches the target gene (Paddison and Hannon,2002).

- **Vector Incorporation:** The shRNA sequence is usually incorporated into a suitable vector, such as a plasmid or a viral vector. The vector serves as a carrier to deliver the shRNA into the target cells or organisms (Rubinson *et al.,* 2003).
- **Transfection or Viral Delivery:** The shRNA-containing vector is introduced into the target cells through transfection (in vitro) or viral delivery (in vivo). This allows the shRNA to enter the cells and interact with the cellular machinery responsible for gene silencing.
- **Processing and Silencing:** Once inside the cells, the shRNA is recognized and processed by the cellular machinery involved in the RNAi pathway. The shRNA is cleaved by an enzyme called Dicer, producing a small interfering RNA (siRNA) duplex with a guide strand and a passenger strand. The guide strand is incorporated into the RNA-induced silencing complex (RISC), while the passenger strand is typically degraded. The guide strand within the RISC then binds to the target mRNA molecule, resulting in mRNA degradation or translational repression, leading to gene silencing (Schwarz *et al.,* 2003).
- **Phenotypic Analysis:** The silencing of the target gene can result in observable changes in the cells or organisms, allowing researchers to study the function of the gene and its impact on cellular processes or organismal phenotypes (Mohr *et al.*, 2014).

C. **MicroRNA (miRNA):** MicroRNAs (miRNAs) are a class of small, endogenous RNA molecules that play a critical role in the regulation of gene expression. They are typically 21-23 nucleotides in length and are found in plants, animals, and some viruses. miRNAs function by binding to complementary sequences in messenger RNA (mRNA) molecules, resulting in post-transcriptional gene silencing or translational repression. (Bartel and David, 2004). The process of miRNA-mediated gene regulation involves several steps (Fig 1.)

- **Biogenesis:** miRNAs are initially transcribed from DNA in the cell nucleus by RNA polymerase II, resulting in primary miRNA (pri-miRNA) transcripts. These pri-miRNAs are processed by a complex called the Microprocessor, which consists of the enzyme Drosha and its cofactor DGCR8. The Microprocessor cleaves the pri-miRNA to generate precursor miRNAs (pre-miRNAs), which are typically hairpin-shaped structures.

- **Export to Cytoplasm:** Pre-miRNAs are then exported from the nucleus to the cytoplasm by Exportin-5, a transport protein.
- **Processing to Mature miRNA:** In the cytoplasm, the pre-miRNAs are further processed by an enzyme called Dicer, resulting in the formation of a miRNA duplex. One strand of the duplex, known as the mature miRNA, is preferentially incorporated into the RNA-induced silencing complex (RISC).
- **Target Recognition and Gene Regulation:** The mature miRNA within the RISC guides the complex to target mRNA molecules. The miRNA recognizes target mRNAs through base pairing between the miRNA and the mRNA's complementary sequences, primarily within the 3' untranslated region (UTR). Binding of the miRNA to the mRNA can lead to mRNA degradation or translational repression, resulting in reduced protein production from the target gene.

D. Virus-Induced Gene Silencing (VIGS): Virus-induced gene silencing (VIGS) is a technique used in plant research to study gene function and investigate the role of specific plant physiology and development (Baulcombe,1999). VIGS takes advantage of plant viruses' ability to deliver RNA molecules into plant cells and induce gene silencing (Ruiz *et al.* ,1998: Kumar *et al.*, 2020). The virus is engineered to carry a fragment of the target gene's sequence, which is then expressed within the infected plant (Ratcliff and Harrison, 1987). The expression of this viral RNA triggers gene silencing and allows researchers to study the effects of gene knockdown in plants (Senthil and Mysore, 2011 Kumar *et al.*, 2023). Here's an overview of the VIGS process:

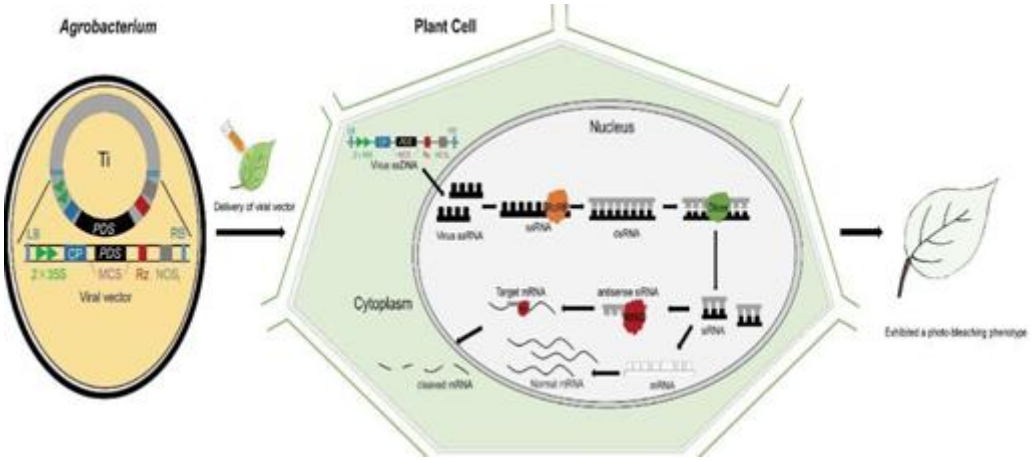

Figure 2: Mechanism of VIGS

- **Selection of Viral Vector:** A plant virus is chosen as the vector for VIGS. The virus should have the ability to infect the target plant species and be engineered to carry a portion of the target gene's sequence (Smith *et al.,* 2004).
- **Construction of the Viral Vector:** The viral vector is modified by inserting a fragment of the target gene's sequence into the virus genome. This fragment is typically chosen to be highly conserved among gene family members, ensuring efficient gene silencing across related genes (Liu *et al.,* 2002).
- **Infection of Target Plants:** The modified viral vector is introduced into the target plant using various methods, such as agroinfiltration (injection of viral DNA into plant tissue) or mechanical inoculation (rubbing the virus onto plant leaves). The virus infects the plant cells and spreads throughout the plant.
- **Gene Silencing:** Once inside the plant cells, the viral RNA is transcribed, and the viral genome is replicated. The inserted fragment of the target gene's sequence is transcribed as well. The resulting viral RNA molecule, along with the fragment of the target gene's sequence, triggers gene silencing through a phenomenon known as RNA silencing or RNA interference (RNAi). The plant's endogenous gene silencing machinery recognizes the viral RNA and the complementary target gene sequence, leading to the degradation or suppression of both viral RNA and target gene mRNA (Dalmay *et al.* 2000).
- **Phenotypic Analysis:** The silencing of the target gene can result in observable changes in the plant's phenotype, such as altered growth, development, or specific physiological traits. By comparing the VIGS-treated plants with control plants, researchers can infer the function of the target gene and its impact on plant biology (Lu *et al.,* 2003)

E. CRISPR-Cas9-Mediated Gene Silencing: While CRISPR-Cas9 is mainly associated with gene editing, it can also be used for gene silencing. In this approach, the Cas9 protein is fused with a transcriptional repressor domain. The guide RNA directs Cas9 to the target gene's promoter region, where it disrupts transcriptional activity and suppresses gene expression (Jones and Smith, 2020).

III. APPLICATIONS OF RNAi CROP BREEDING

The use of RNA interference (RNAi) in crop breeding has emerged as a valuable tool for improving crop traits and enhancing agricultural productivity. RNAi techniques can be applied to manipulate gene expression and target

specific genes involved in traits of interest. Here are some key applications of RNAi in crop breeding:

A. Pest and Disease Resistance

RNAi can be employed to enhance crop resistance against pests and diseases. By targeting essential genes in pests or pathogens, RNAi technology can be used to develop crops that produce small interfering RNAs (siRNAs) specifically designed to silence those genes. When pests or pathogens interact with these RNAi- expressing crops, the ingested siRNAs can trigger gene silencing in the pest or pathogen, resulting in reduced damage or infection.

For example,

- **Insect Resistance in Maize:** RNAi has been utilized to enhance insect resistance in maize crops. By targeting specific genes essential for insect development or survival, researchers have developed maize plants that produce siRNAs that can silence those genes in insect pests. This approach has shown promise in reducing the damage caused by pests such as corn rootworm and European corn borer.

- **Disease Resistance in Potatoes:** RNAi has been employed to improve disease resistance in potatoes. For instance, late blight is a devastating disease that affects potatoes worldwide. By introducing siRNAs that target genes involved in the pathogenicity of the late blight-causing pathogen, Phytophthora infestans, researchers have successfully reduced disease severity in potato plants.

B. Abiotic Stress Tolerance

RNAi techniques can be used to enhance crop tolerance to abiotic stresses such as drought, heat, salinity, and cold. By suppressing the expression of genes that are responsible for sensitivity to these stresses or by activating genes associated with stress tolerance, breeders can develop crops that exhibit improved resilience in challenging environments. This can lead to increased yield stability and sustainability in the face of climate change.

For example,

- **Drought Tolerance in Rice:** RNAi techniques have been used to enhance drought tolerance in rice, a critical staple crop (Kumar et al, 2019). By silencing genes that are involved in water loss through transpiration or that negatively regulate drought response pathways,

researchers have developed rice varieties that exhibit improved drought tolerance and can maintain productivity under water-limited conditions.

C. Nutritional Enhancement

RNAi can also be utilized to enhance the nutritional quality of crops. By targeting genes involved in nutrient metabolism or synthesis pathways, breeders can modulate their expression to increase the content of specific nutrients in crops. This approach, known as biofortification, has been applied to develop crops with higher levels of vitamins, minerals, and other beneficial compounds, addressing malnutrition and improving human health.

D. Weed Control

RNAi-based approaches have shown potential for weed control in crops. By targeting essential genes in weeds, breeders can develop crops that produce siRNAs specifically designed to silence those genes. When weeds consume these crops, the siRNAs can enter the weed cells and induce gene silencing, resulting in stunted growth or lethality. This approach offers an environmentally friendly alternative to conventional weed control methods.

For example, RNAi techniques have been employed to develop crops with enhanced tolerance to herbicides. By targeting genes involved in herbicide sensitivity or metabolism, researchers have developed crop varieties that can withstand specific herbicides, allowing more effective weed control without harming the crops.

E. Quality Improvement

RNAi can be used to modify genes involved in crop quality traits such as taste, texture, color, or post-harvest characteristics. By modulating the expression of these genes, breeders can develop crops with improved sensory attributes or enhanced shelf life, meeting consumer preferences and market demands.

For example, ‘Fruit quality in tomatoes: RNAi has been utilized to improve fruit quality traits in tomatoes. By targeting genes involved in fruit ripening processes, such as genes associated with the production of enzymes responsible for softening and flavor development, researchers have developed tomatoes with delayed ripening, improved shelf life, and enhanced nutritional profiles.

The application of RNAi in crop breeding offers great potential for developing crops with improved traits, including enhanced pest and disease resistance, abiotic stress tolerance, nutritional quality, weed control, and overall crop performance. However, careful consideration of potential off-target effects, regulatory aspects, and the stability of RNAi constructs within crop plants is crucial for successful implementation. Continued research and advancements in RNAi technology hold promise for further enhancing crop breeding efforts and addressing the challenges faced in agriculture.

IV. CONCLUSION

The utilization of RNA interference (RNAi) in crop improvement represents a transformative advancement with far-reaching implications for agriculture. By harnessing the natural mechanism of gene silencing, RNAi offers precise control over gene expression, facilitating the development of crops with enhanced traits and resilience to environmental challenges. While challenges such as efficient delivery mechanisms and regulatory considerations persist, collaborative efforts and ongoing research hold promise for overcoming these obstacles. As RNAi technology continues to evolve, its integration into breeding programs stands to revolutionize crop production, enhancing food security, sustainability, and resilience in the face of climate change and growing global demands. Moreover, RNAi presents an opportunity to reduce reliance on chemical inputs, optimize resource utilization, and address nutritional deficiencies through biofortification. Ultimately, the widespread adoption of RNAi in crop improvement holds immense potential to meet the demands of a rapidly evolving agricultural landscape and ensure a more secure and sustainable food future.

REFERENCES

[1] Amarzguioui, M., Holen, T., Babaie, E., and Prydz, H. (2003). Tolerance for mutations and chemical modifications in a siRNA. Nucleic Acids Research, 31(2), 589-595.
[2] Bartel, David P. (2004). MicroRNAs: Biogenesis, Function, and Role in Cancer Authors. Cell, 116(2), 281-297. DOI: 10.1016/S0092-8674(04)00045-5
[3] Baulcombe, D. C. (1999). Fast forward genetics based on virus-induced gene silencing. Current Opinion in Plant Biology, 2(2), 109-113.
[4] Baulcombe, D. C. (2004). RNA silencing in plants. Nature, 431(7006), 356-363.
[5] Borges, F., and Martienssen, R. A. (2015). The expanding world of small RNAs in plants. Nature Reviews Molecular Cell Biology, 16(12), 727-741.
[6] Brummelkamp, T. R., Bernards, R., and Agami, R. (2002). A system for stable expression of short interfering RNAs in mammalian cells. Science, 296(5567), 550-553.
[7] Burch-Smith, T. M., Anderson, J. C., Martin, G. B., and Dinesh-Kumar, S. P. (2004). Applications and advantages of virus-induced gene silencing for gene function studies in plants. The Plant Journal, 39(5), 734-746.

[8] Dalmay, T., Hamilton, A., Rudd, S., Angell, S., and Baulcombe, D. C. (2000). An RNA-dependent RNA polymerase gene in Arabidopsis is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell, 101(5), 543-553.
[9] Dias, N., Stein, P. C., and Cohen, A. (2018). RNAi in agriculture: Current status and perspectives. Pest Management Science,4(4), 827–840. DOI: 10.1002/ps.4716.
[10] Dubey, A., Farmer, A., Schlueter, J., *et al.* (2021). Application of RNAi technology for crop improvement in the era of genome editing. Current Opinion in Biotechnology, 70, 109–116. DOI: 10.1016/j.copbio.2021.05.006.
[11] Dubrovina, A. S., and Kiselev, K. V. (2019). Exogenous RNAs for Gene Regulation and Plant Resistance. International Journal of Molecular Sciences, 20(9), 2282.
[12] Elbashir, S. M., Lendeckel, W., and Tuschl, T. (2001). RNA interference is mediated by 21- and 22- nucleotide RNAs. Genes and Development, 15(2), 188-200.
[13] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature, 391(6669), 806-811.
[14] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature, 391(6669), 806-811.
[15] Hannon, G. J. (2002). RNA interference. Nature, 418(6894), 244-251.
[16] Hu, H., and Gelvin, S. B. (2016). RNAi-mediated gene silencing in plants: Approaches, mechanisms, and applications. Plant Cell Reports, 35(5), 933–952. DOI: 10.1007/s00299-016-1943-y.
[17] Islam, W., Ali, I., Khan, M. A., *et al.* (2021). RNA interference: A promising tool for crop improvement and food security. Biotechnology Letters, 43(6), 1133–1154. DOI: 10.1007/s10529-021-03131-7.
[18] Jin, S., Singh, N. D., and Li, L. (2017). Transgenic plants: Progress and challenges. Journal of Integrative Plant Biology, 59(11), 729–731. DOI: 10.1111/jipb.12598.
[19] Jin, S., Zhang, X., Nie, Y., *et al.* (2019). RNA interference: A powerful tool for crop improvement.
[20] International Journal of Molecular Sciences, 20(4), 971. DOI: 10.3390/ijms20040971.
[21] Jones, A., and Smith, B. (2020). CRISPR-Cas9-mediated gene silencing: Mechanisms and applications.
[22] Molecular Biology Reports. 47(8), 2105-2115. https://doi.org/10.1007/s11033-020-05467-8 Koch, A., Kogel, K. H., and van Themaat, E. V. L. (2006). RNA silencing mechanisms in fungi. In
[23] Advances in Genetics (Vol. 57, pp. 1-29). Academic Press.
[24] Kumar, V., & Suman, S. K. (2019). Brassinosteroids and its Implication in Agriculture. Plant Stress Bioloy, 328.
[25] Kumar, P., Kirti, S. Kiran, Gangwar, R. P. C. (2023). In vitro studies in Gymnema sylvestre (Gudmar)-A high value medicinal plant. In vitro, 54(04).
[26] Kumar, P., Kriti S., Kiran and Chourasia, S.K. (2020) AGROMICROBES POTENTIAL TO ENHANCE SOILS FERTILITY AND YIELD, Agriallis, 2(8); 22-31
[27] Liu, Q., and Paroo, Z. (2010). Biochemical principles of small RNA pathways. Annual Review of Biochemistry, 79, 295-319.
[28] Liu, Y., Schiff, M., and Dinesh-Kumar, S. P. (2002). Virus-induced gene silencing in tomato. The Plant Journal, 31(6), 777-786.
[29] Lu, R., Martin-Hernandez, A. M., Peart, J. R., Malcuit, I., and Baulcombe, D. C. (2003). Virus-induced gene silencing in plants. Methods, 30(4), 296-303.

[30] Meister, G., Landthaler, M., Patkaniowska, A., Dorsett, Y., Teng, G., and Tuschl, T. (2004). Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Molecular Cell, 15(2), 185-197.
[31] Mohr, S. E., Smith, J. A., Shamu, C. E., Neumüller, R. A., and Perrimon, N. (2014). RNAi screening comes of age: improved techniques and complementary approaches. Nature Reviews Molecular Cell Biology, 15(9), 591-600.
[32] Paddison, P. J., and Hannon, G. J. (2002). RNA interference: the new somatic cell genetics? Cancer Cell, 2(1), 17-23.
[33] Pak, J.,and Fire, A., Distinct populations of primary and secondary effectors during RNAi in C. elegans (2007). Science, 315(5809), 241-244.
[34] Ratcliff, F., and Harrison, B. D. (1987). A similarity between viral defense and gene silencing in plants.
[35] Science, 276(5318), 1558-1560.
[36] Rubinson, D. A., Dillon, C. P., Kwiatkowski, A. V., Sievers, C., Yang, L., Kopinja, J., ... and Hannon, G. J. (2003). A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. Nature Genetics, 33(3), 401-406.
[37] Ruiz, M. T., Voinnet, O., and Baulcombe, D. C. (1998). Initiation and maintenance of virus-induced gene silencing. The Plant Cell, 10(6), 937-946.
[38] Saha, S., and Bariana, H. S. (2018). RNAi technology in crop improvement: Current challenges and prospects. GM Crops and Food, 9(3), 159–171. DOI: 10.1080/21645698.2018.1509205.
[39] Schwarz, D. S., Hutvágner, G., Du, T., Xu, Z., Aronin, N., and Zamore, P. D. (2003). Asymmetry in the assembly of the RNAi enzyme complex. Cell, 115(2), 199-208.
[40] Senthil-Kumar, M., and Mysore, K. S. (2011). New dimensions for VIGS in plant functional genomics. Trends in Plant Science, 16(12), 656-665.
[41] Song, J. J., Smith, S. K., Hannon, G. J., and Joshua-Tor, L. (2004). Crystal structure of Argonaute and its implications for RISC slicer activity. Science, 305(5689), 1434-1437.
[42] Wang, M.-B., and Smith, N. A. (2020). RNAi for crop improvement: Placing the spotlight on off-target effects and other unintended effects. Annual Review of Phytopathology, 58, 161–183. DOI: 10.1146/annurev-phyto-080615-095903.
[43] Waterhouse, P. M., Wang, M. B., and Lough, T. (2001). Gene silencing as an adaptive defence against viruses. Nature, 411(6839), 834-842.
[44] Yu, J. Y., DeRuiter, S. L., and Turner, D. L. (2002). RNA interference by expression of short-interfering RNAs and hairpin RNAs in mammalian cells. Proceedings of the National Academy of Sciences, 99(9), 6047-6052.
[45] Zhu, Q., Chen, Y., and Liu, L. (2020). RNAi-mediated crop protection against insects. Trends in Biotechnology, 38(7), 757–766. DOI: 10.1016/j.tibtech.2020.01.002.

Short Answers

1. Define RNA interference
2. Give the steps involved in small RNA synthesis
3. How micro-RNA functions in gene silencing
4. Discuss the applications of RNAi in crop improvement
5. Explain VIGS in detail

Self Assesment

1. What is the primary mechanism of RNA interference (RNAi)?
   a. Inhibition of DNA replication
   b. Inhibition of protein translation
   c. Activation of gene expression
   d. Promotion of DNA mutation

2. Which enzyme is responsible for cleaving double-stranded RNA molecules into small RNA fragments during RNAi?
   a. Polymerase
   b. Ligase
   c. Dicer
   d. Helicase

3. Which type of RNA molecules are typically used as mediators of RNAi?
   a. Messenger RNA (mRNA)
   b. Transfer RNA (tRNA)
   c. Small interfering RNA (siRNA)
   d. Ribosomal RNA (rRNA)

4. What is the role of siRNA in RNAi?
   a. siRNA serves as a template for DNA synthesis
   b. siRNA binds to target mRNA and degrades it
   c. siRNA enhances protein translation
   d. siRNA promotes gene transcription

5. Which of the following is NOT a potential application of RNAi in crop improvement?
   a. Increasing crop yield
   b. Enhancing resistance to pests
   c. Reducing post-harvest losses
   d. Promoting weed growth

6. How does RNAi contribute to pest resistance in crops?
   a. By directly killing pests upon ingestion
   b. By interfering with pest gene expression
   c. By stimulating pest growth
   d. By promoting pest reproduction

7. What is the term for unintended suppression of genes other than the intended target in RNAi?
   a. On-target effects
   b. Off-target effects
   c. Side effects
   d. Targeted suppression

8. Which of the following is a challenge associated with RNAi technology in crop improvement?
   a. Limited specificity
   b. High cost of implementation
   c. Rapid degradation of RNA molecules
   d. Inability to target specific genes

9. In which part of the plant cell does RNAi typically occur?
   a. Nucleus
   b. Mitochondria
   c. Chloroplast
   d. Cytoplasm

10. Which of the following is an advantage of RNAi technology over traditional breeding methods?
    a. Higher cost-effectiveness
    b. Preservation of genetic diversity
    c. Faster development of new traits
    d. Increased time required for trait modification

11. What is the role of miRNA in RNAi?
    a. It binds to target mRNA and degrades it
    b. It activates gene expression
    c. It cleaves double-stranded RNA into small fragments
    d. It regulates gene expression at the post- transcriptional level

12. Which of the following is NOT a potential environmental benefit of using RNAi in crop improvement?
    a. Reduced use of chemical pesticides
    b. Enhanced soil fertility
    c. Decreased pollution from pesticide runoff
    d. Preservation of beneficial insect populations

13. Which enzyme synthesizes miRNA molecules in plants?
    a. Dicer
    b. Polymerase
    c. Ligase
    d. Helicase

14. What is the primary goal of using RNAi in crop improvement?
    a. To increase genetic diversity
    b. To reduce undesirable traits
    c. To promote mutation
    d. To enhance genetic instability

15. Which of the following is a potential limitation of RNAi technology in crop improvement?
    a. Resistance development in target pests
    b. Decreased crop yield
    c. Increased vulnerability to diseases
    d. Enhanced nutrient uptake

16. Which cellular process does RNAi primarily target?
    a. Transcription
    b. Translation
    c. Replication
    d. Mutation

17. How does RNAi contribute to herbicide tolerance in crops?
    a. By degrading herbicides upon contact
    b. By increasing sensitivity to herbicides
    c. By promoting weed growth
    d. By inhibiting photosynthesis

18. What is the function of the RISC complex in RNAi?
    a. It synthesizes miRNA molecules
    b. It degrades mRNA molecules
    c. It cleaves double- stranded RNA into siRNA
    d. It guides siRNA to target mRNA molecules

19. Which of the following is a potential ethical concern associated with the use of RNAi in crop improvement?
    a. Increased dependence on chemical pesticides
    b. Genetic modification of food crops

c. Loss of biodiversity
d. Environmental pollution

20. Which of the following is NOT a step in the RNAi pathway?
    a. Transcription
    b. Cleavage by Dicer
    c. Assembly of RISC complex
    d. Translation

21. How does RNAi technology contribute to improving crop nutritional quality?
    a. By reducing nutrient uptake
    b. By increasing levels of toxic compounds
    c. By enhancing nutrient absorption
    d. By decreasing levels of anti-nutritional factors

22. Which of the following is a potential economic benefit of using RNAi in crop improvement?
    a. Increased production costs
    b. Decreased market demand
    c. Enhanced crop yield
    d. Reduced farmer income

23. What is the role of AGO proteins in RNAi?
    a. They cleave double-stranded RNA into siRNA
    b. They guide siRNA to target mRNA molecules
    c. They synthesize miRNA molecules
    d. They degrade mRNA molecules

24. Which of the following is a mechanism by which RNAi can confer disease resistance in crops?
    a. By directly killing pathogens upon contact
    b. By enhancing pathogen growth
    c. By inhibiting pathogen gene expression
    d. By promoting pathogen reproduction

25. What is the significance of RNAi technology in sustainable agriculture?
    a. It reduces the need for crop rotation
    b. It promotes monoculture farming
    c. It minimizes the use of chemical inputs
    d. It increases soil erosion