(2) the spring ecosystem is complex and dynamic in nature, and is dependent on variable conditions and gradients of different component, and (3) presently, a descriptive knowledge of the spring ecosystem is available; but a quantitative knowledge is lacking.

The following recommendations were made at the end of the meeting, which has paved the way for future research on spring ecosystem: (1) The data deficiency must be considered to create a database in order to facilitate research on the spring ecosystem across the Himalaya. (2) The spring ecosystem definition must cover all bio-geo-physical aspects of spring and its services; whereas boundary should coincide with the area of (its) influence. (3) Spring ecosystem research could cover (a) quantitative geomorphology, (b) quantitative ecosystem services, (c) quantitative hydrology, and (d) quantitative socio-economics. (4) The spring ecosystem assessment protocol must cover all the possible parameters that connect the physiology of the spring ecosystems. (5) The concept, methodology or modelling techniques developed or used in other contemporary scientific field may be explored for its possible application to understand, monitor and assess the spring ecosystem health.

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Vaibhav E. Gosavi*, Sandipan Mukherjee, Ashutosh Tiwari, Kireet Kumar, Vasudha Agnihotri and **Ranjan Joshi,** G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora 263 643, India.
*e-mail: vaibhavgosavi8@gmail.com

OPINION

CRISPR/Cas9 as a dexterous tool to mend plant *MIR* genes for agronomic trait improvement

Swati Verma

As quoted by Thomas E. Lovejoy, 'Natural species are the library from which genetic engineers can work. Genetic engineers don't make new genes, they rearrange existing ones'. The CRISPR/Cas9 genetic scissor has proved to be a wonder tool for carrying out these gene rearrangements.

Being sessile, plants have developed an intricate signal transduction system to respond to different environmental cues. On perceiving environmental stresses, signal transduction in plants commences into activation or repression of genes by regulatory molecules to generate an appropriate physiological response[1]. MicroRNAs (miRNAs) are small, 20–24 nucleotide length, non-coding RNAs involved in post-transcriptional regulation of gene expression through mRNA cleavage or translational repression of targets. These small regulatory molecules are known to play big roles in influencing gene expression during plant development and stress responses[2].

Till date, plant miRNA-based research mainly emphasized on analysing miRNA expression using high-throughput sequencing and miRNA-target prediction. These studies have revealed unique and conserved expression profiles of plant miRNAs under normal and stressed conditions and have led to the identification of a large number of novel miRNAs in plants[3]. The increasing amount of miRNA-related systems biology data needs more comprehensive exploration. In the recent past, use of

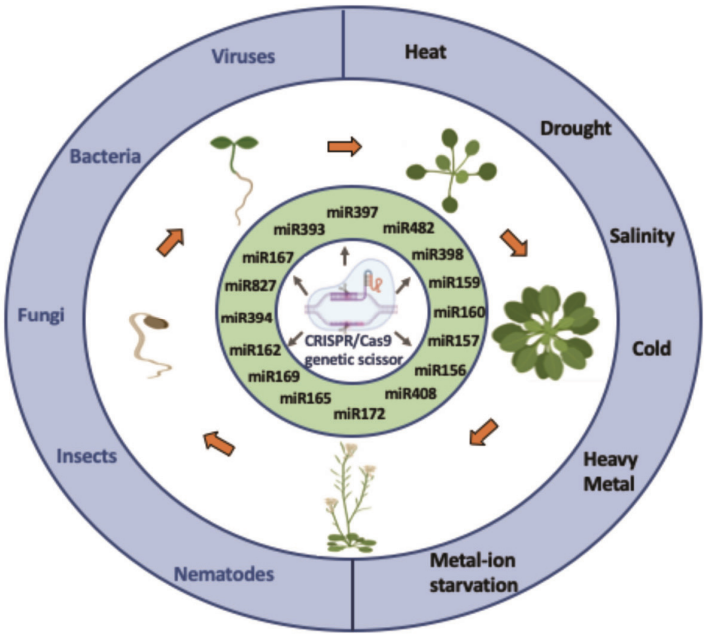

Figure 1. Potential candidate miRNAs involved in regulating various aspects of growth and development, and stress responses in plants, as targets for CRISPR/Cas9 genome editing. (Image created on BioRender.com.)

mutant, overexpression and short tandem target mimic (STTM) lines have geared up for characterization of miRNA functions. This research is slowly gaining pace in revealing various nodes of the miRNA-mediated gene regulatory networks for generation of plants with enhanced productivity and stress tolerance[4–8]. The genome editing of *MIR* genes could prove to be a turning point in these plant trait improvement programmes.

The game changer genetic scissor, CRISPR/Cas9, has been widely adopted as a genome editing tool in various biological systems. CRISPR/Cas9 genome editing is based on the type II CRISPR/Cas defence system of bacteria *Streptococcus pyogenes*. The CRISPR/Cas9 tool kit majorly comprises Cas9 protein and guide RNA (gRNA). The gRNA recruits Cas9 to work as a genome editor complex. The gRNA sequence recognizes the target loci and the endonuclease activity of Cas9 cleaves the target DNA forming DNA double-strand breaks (DSBs). After the formation of DSBs, a cascade of *in vivo* DNA repair events is initiated which results in gene mutation via insertion, deletion or replacement of nucleotides[9]. Unlike other genome editing systems, CRISPR/Cas9 has a simple experimental design and is capable of carrying out multiple mutations simultaneously. Since the discovery of CRISPR/Cas9, several modifications have been introduced to increase target specificity by minimizing offsite targeting[10,11]. Therefore, this genetic scissor has become a method of choice for genome editing in plants. Several studies discuss the success stories of CRISPR/Cas9 in genome editing of protein coding genes for improving plant health[9,11,12]; however, the genome editing of *MIR* genes using CRISPR/Cas9 is still in its infancy.

The miRNA-mediated gene regulation seems more complex than our current understanding. Most often, the miRNA targets are paramount regulators of gene expression like the mRNAs coding for transcription factors. Therefore, modulation of miRNA expression can lead to changes in the expression of downstream non-target genes[13]. A miRNA target can also be a transcription factor which regulates the expression of other miRNAs. Further adding to the complexity, the members of a miRNA family can have both conserved and non-conserved targets. Therefore, large miRNA families have been found to regulate multiple aspects of growth and development, nutrient uptake and stress responses[14]. Till date, miRNA-mediated gene regulation remains a complex and relatively less-explored process.

Initially, an emphasis on genome editing could be laid on miRNAs whose functions are comparatively well understood as compared to other plant miRNA families. These involve plant miRNA families like miR156, miR172, miR169, miR408, miR166, miR167, miR397, miR398, miR394 and miR160. Different roles of these miRNAs have been studied in regulating various aspects of plant growth and development, and stress responses[15,16]. Application of CRISPR/Cas9 for precise genome editing of *MIR* genes could be helpful in the generation of crop varieties with improved yield, nutritional value and stress response. As a case, role of the miR156–miR172 module has been established in the regulation of vegetative to reproductive phase transition in plants[17]. Precise genome editing of *MIR156* and *MIR172* could assist in the generation of early flowering or early maturing crop varieties. *MIR* genes negatively regulating abiotic and biotic stresses could be the genome editing targets for the development of stress-tolerant crop varieties.

Many studies illustrate the involvement of miRNAs in regulating phytohormone responses in plants. MiR164 and miR319-mediated phytohormonal crosstalk regulates plant lateral organ development and senescence. While miR390 has been known to regulate the hormonal control of tissue outgrowth and senescence, MiR393 mediates ABA-to-AUX signals during plant stress response[18]. CRISPR/Cas9-based genome editing of specific phytohormone-responsive miRNAs could be beneficial in the development of crop varieties with improved root and shoot system architecture for better nutrient uptake, and improved yield and nutritional value.

Since the functions of many miRNAs are still unexplored, CRISPR/Cas9 genome editing would also prove a useful tool for this exploration. Elucidation of miRNA functions would reveal many potential miRNA candidates for CRISPR/Cas9 genome editing-based crop improvement programmes. CRISPR/Cas9 could also be deployed for genome editing of *MIR* genes negatively regulating biotic and abiotic stress responses. Further, a more efficient approach could be genome editing of cis-regulatory elements of *MIR* genes to minimize affecting of downstream indirect regulation of non-target genes by miRNAs. Hitherto, most of the CRISPR/Cas9 genome editing studies have been limited to a few crop plants. Our current understanding of gene regulation in plants suggests that miRNAs are important post-transcriptional regulators of gene expression and potential targets for genome editing-based crop improvement programmes. Thus, future research will involve precision genome editing of *MIR* genes as a one-step process for agronomic trait improvement.

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Swati Verma is in the College of Horticulture and Forestry Thunag, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni 173 230, India.
e-mail: swativerma.bio@gmail.com