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# COMPUTATIONAL IDENTIFICATION OF MICRORNAS AND THEIR PUTATIVE TARGETS FROM WHOLE GENOME SEQUENCE OF CUCUMBER MOSAIC VIRUS (CMV)

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## ABSTRACT

Cucumber Mosaic Virus (CMV) is a plant pathogenic virus in the family Bromoviridae. It is a linear positive-sense, single-stranded RNA virus. This virus has a worldwide distribution and very wide host range such as pepper, tomato, squash, celery, cucumber, melons, spinach and petunia. MicroRNAs are conserved small non-coding endogenous RNAs which are approximately 21-22 Nucleotide in length. They play important role in post transcriptional gene regulation. miRNAs are negative regulators that function as specificity determinants or guides within complexes that inhibit protein synthesis (animals) or promote degradation (plants) of mRNA targets. Computational prediction of miRNAs and their targets from whole genome sequenceNC_002034.1of Cucumber mosaic virus which was taken from NCBI. By following the criteria, a total of 42 potential miRNAs (both 5' stem and 3' stem) were identified through probabilistic algorithm search. The psRNA target server predicted 103 targets for 32 miRNA sequences and their functions were illustrated. Most of the target genes encoded translation and cleavage.

**Keywords: ssRNA, pre-miRNA, MFE, psRNATarget, NCBI, Mipred**.

## 1. INTRODUCTION

Mature microRNAs (miRNAs) are a class of naturally occurring, small noncoding RNA molecules, about 21–25 nucleotides in length. MicroRNAs are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to downregulate gene

expression in a variety of manners, including translational repression, mRNA cleavage, and deadenylation. They were first described in 1993 by Lee and colleagues, and the term microRNA was coined in 2001 [1, 2, 3] Thousands of miRNAs have since been identified in various organisms through randomcloning and sequencing or computational prediction. MiRNAs in plants were discovered few years late than in animals. MiRNAs in plant were first reported from *Arabidopsis thaliana* in early 2002 [4].

The mature miRNA sequences are highly conserved across the plant kingdom [5]. This conservation of sequences has provided a powerful tool for the identification of novel miRNA genes through comparative genomics based approach.Identification of target genes is important in determining the biological functions of miRNAs. Multiple miRNAs may control the expression of single gene or single miRNA may involve in regulating the expressions of multiple genes. Plant miRNAs have implicated in various development processes such as leaf morphogenesis, flowering time, floral organ development, root development, seed development etc. Plant development is a highly regulated process that is controlled at many levels. Plant miRNAs are highly complementary to conserved target mRNAs, which allows fast and confident bioinformatics identification of plant miRNA targets. A large number of miRNAs have been identified in plants by computational and experimental approaches which include high throughput techniques.

miRNA biogenesis requires multiple steps in order to form mature miRNAs from miRNA genes [6 , 7] .First, a miRNA gene is transcribed to a primary miRNA (pri-miRNA), which is usually a long sequence of more than several hundred nucleotides . This step is controlled by Pol II enzymes [6, 7, 8]. Second, thepri-miRNA is cleaved to a stem loop intermediate called miRNA precursor or pre-miRNA. This step is controlled by the Drosha RNase III endonuclease in animals [6, 9] or by Dicer-like 1 enzyme (DCL1) in plants [7 , 10]. Loss-of-function dcl1 mutants have low levels of miRNA synthesis [11]. In animals, pre-miRNAs are then transported by exportin 5 from the nucleus into the cytoplasm [12, 13, 14], followed by formation of miRNA:miRNA* duplex and mature miRNAs by another RNase III-like enzyme called Dicer (6,15). In this step, however, plant miRNAs differ from animals. Plant miRNAs are cleaved into miRNA: miRNA* duplex possibly by dicer like enzyme 1 (DCL1) in the nucleus rather than in the cytoplasm [16, 6 ] then the duplex is translocated into the cytoplasm by HASTY, the plant orthologue of exportin 5

[17]. In the cytoplasm, both plant and animal miRNAs are unwound into single strand mature miRNAs by helicase. Finally, the mature miRNAs enter a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC) [18, 19, 20]. This suggests that miRNA biogenesis is complicated; several enzymes are required for processing long pri-miRNA to ¨20–24 nt mature miRNAs.

**MATERIALS AND METHODS**

Whole genome sequence of Cucumber Mosaic Virus (NC_002034.1) of size 3.36kb was extracted from NCBI (National Centre for Biotechnology Information) (https://www.ncbi.nlm.nih.gov/).Sequence was taken in FASTA format. Precursor miRNA for genome sequence of CMV was predicted using online tool miREeval 2.0 (http://mimirna.centenary.org.au/mireval/). Classification of real and pseudo microRNA was done using online web server Mipred (http://server.malab.cn/MiPred/). Given a sequence, MiPred decides whether it is a pre-miRNA hairpin sequence or not. Secondary structure of pre-miRNA and its minimum free energy (MFE) was predicted using online webserver RNAfold webserver (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). After finding secondary structure and MFE, mature miRNA prediction was done using online tool Mature Bayes (http://mirna.imbb.forth.gr/MatureBayes.html). MatureBayes tool is used for finding mature miRNA with in a pre-miRNA sequence using a Native Bays classifier. Target prediction was done using online webserver psRNA Target: A Plant Small RNA Target Analysis Server (http://plantgrn.noble.org/psRNATarget/).

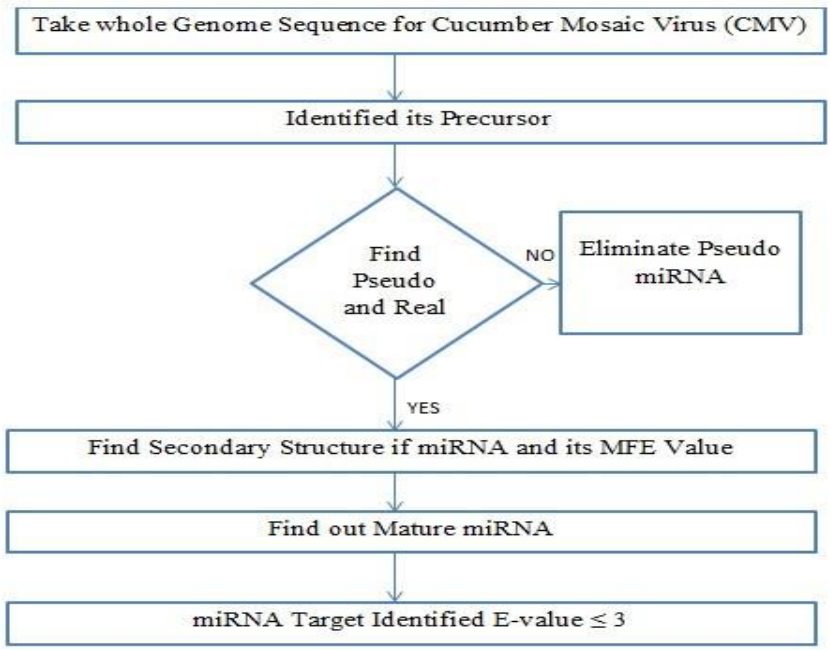


**Fig: 1 Showing Methodology [21,22,23)]**

## 2. RESULT

Whole Genome sequence of CMV (Cucumber mosaic virus RNA 1, complete sequence) was taken from NCBI in FASTA format. The size of the sequence was 3.36kb. MiREval online tool was used to predict the 39 precursor miRNA (Supplementary Table 1). MiPred was to distinguish between real and pseudo precursor microRNA. It was used to predict whether the precursor miRNA has hairpin structure or not. Among 39 precursor miRNAs, 21 precursor miRNAs were predicted having hairpin structure (Supplementary Table 2). RNAfold webserver was used to predict secondary structure and minimum free energy (MFE). These 21 precursor miRNAs were now used for prediction of their secondary structure and minimum free energy (Supplementary table 3)

MatureBayes was then used for prediction of mature miRNAs from pre-miRNAs. In total 42 mature miRNAs was predicted from 21 precursor miRNAs. Both 3'stem and 5'stem was included in mature miRNAs. (Supplementary Table 4). For target prediction, psRNATarget: A Plant Small RNA Target Analysis server was used. In total 103 targets were predicted from both 3' stem and 5' stem. Those targets having expectation value less than or equal to 3 were selected and also among those the targets which were similar or repeated were not taken into an account. There were total 90 targets. These targets genes inhibited either translation or cleavage. Mature miRNA **>Csa-miR-24-1** showed maximum targets. (Supplementary table 5).

## 3. CONCLUSION

Into this, total 39 precursors were predicted. These 39 precursors were distinguished between pseudo and real pre-miRNA. 21 pre-miRNAs were taken forward to predict secondary structures and minimum free energy, as they were real pre-miRNAs. Two mature microRNA having 3' stem and 5' stem were predicted from each pre-miRNA therefore, there were 42 mature miRNAs in total. psRNAtarget: an online web server was used. 103 targets were predicted from 42 mature miRNAs

We can find out 42 mature miRNA that target to those mRNA whose synthesis disease causing protein the main role of these miRNA targeted to mRNA and block the process of translation of disease causing protein. In our work we predict that Mature miRNA >Csa-miR-24-1 showed maximum targets of total 17 in number. Their expression inhibited by either translation or cleavage repression of targeted mRNA.

The biological network for further analysis of gene interaction may use in future analysis.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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