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miRNA and their putative targets in *Andrographis paniculata*

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miRNAs are non-coding RNAs participate in post transcriptional and translational regulation of genes involved in various biological activities. Besides, miRNAs are also involved in plant protection against invading viruses, bacteria, fungi etc. In plants, RNA polymerase II transcribes primary miRNAs (pri-miRNAs) of heterogenous length. These molecules are first sequentially cleaved by DICER LIKE 1 (DCL1) in the nucleus where a processing complex is formed. A double stranded miRNA/miRNA* duplex is emerged by processing complex. This is O-methylated by Hua-Enhancer1 (HEN1) and further exported to the cytoplasm by exporting 5 homolog, HASTY. Mature or pre-miRNA are formed after degradation of miRNA* by 3′–5′ exoribonucleases. Mature miRNAs are exported to cytoplasm by HASTY (HST) and loaded on major player of pathway, ARGONAUTE (AGO) forming RNA-induced silencing complex (RISC) facilitating interaction with target RNAs. These complex actions negatively regulate target gene expression by either translation inhibition or a target transcript cleavage. Recently, miRNA regulation has been reported in secondary metabolite production and abiotic or biotic stresses.

Andrographis paniculata is a plant species among 40 different species of *Andrographis* genus (family Acanthaceae) native to India / Srilanka which has enormous medicinal values. It is mainly cultivated in southeastern and southern Asia. *A. paniculata* has been shown anti-microbial, anticarcinogenic, anti-inflammatory, immunostimulatory, hepatoprotective, health-promoting and anti-cold properties. Low yield of bioactive compounds in *A. paniculata* is a major constraint for medicinal industries. Understanding of biosynthesis and regulation of bioactive metabolites can provide a way to enhance its production by manipulating at molecular levels. Recently, various biotic stresses have been reported in *A. paniculata* causing loss in yield. In this, begomovirus member, phytoplasma (16SrII-D) and *Sclerotinia sclerotiorum* have been found to be disease causing agents in *A. paniculata.* Various miRNAs and their putative targets were identified using transcriptome data of *A. paniculata.*

AP miRNAs and their characteristics

Fifty-one families of miRNAs derived from different transcripts were reported in *A. paniculata.* Pre-miRNAs of *A. paniculata* show heterogenicity in their nucleotide length, which ranges between 32 to 2431 for miR1168 and miR5021 respectively. MFEI for predicting Ap-miRNAs varies from -11.9 to -0.25 kcal/mol with an average value -0.792

kcal/mol. Stability of secondary structures of miRNAs is represented by MFE. This ranges between -2.2 to -696.5 kcal/mol for Ap-miRNAs. Another feature for miRNA is AU content and uracil is a predominant base of such RNAs. Ap-miRNAs show the comparative high AU contents. Different miRNA families show variable number of predicted sequences (Fig. 1).

Putative targets of Ap-miRNA

Ap-miRNAs supposed to have 435 targets in *A. paniculata* and single target in virus. The MFE (kcal/mol) for miRNA-target folding ranges between -48.4 to -3.7 indicating stable folding. Further, various individual AP-miRNAs are shown to regulate more than one target. These targets belong to various biological molecules such as enzymes, signalling molecules, transcription factors, ribosomal proteins, storage proteins and structural proteins. A single target was identified in a viral genome, which is predicted to be regulated by AP-miR5021. This single target identified in viral genome is AC1 protein, which is a replication associated protein.

Secondary metabolites and viral target associated miRNAs and targets

Since long time, plants are known for their nutritional, medicinal and aromatic uses which is evidenced by several texts. Medicinal properties of plants are due to the presence of secondary metabolites. These metabolites are large groups of phytochemicals rendering protection against various biotic and abiotic stresses as well as responsible for specific aroma and color. Such metabolites are categorized broadly into three categories viz. flavonoids, terpenoids and nitrogen containing compounds. Regulation of the biosynthesis and accumulation of these secondary metabolites in plants is regulated in various aspects and metabolic engineering may provide a concrete way to enhance the level of particular metabolites. Among such mechanisms regulating by miRNA is one of the ways modulating expression levels of genes involved in secondary metabolites biosynthesis. *A. paniculata* possesses several classes of important metabolites such as flavonoids, terpenes etc. Various miRNAs along with their molecular targets involved in secondary metabolites biosynthesis and transcription factors were identified (Table. 1). In *A. paniculata* various enzymes participating in diverse secondary metabolite biosynthesis pathways were found to be targets of miRNAs. Similarly, enzymes participating in biosynthesis of some metabolites like brassinosteroid, aromatic amino acid, carotenoids which share some common precursors as a carbon skeleton with secondary metabolites were also found to be targets of miRNAs. Studies indicated that all these biological targets may be regulated by 7 miRNAs (Table. 1). Interacting different members belonging to same family have some differences in nucleotide sequences in mature miRNA, which result in binding to targets with different affinities. Two Web based psRNAtarget programs validated the target identification involved in secondary metabolite production and viral invasion in Kalmegh. Andrographolides are labdane related diterpenes which are derived from ent-copalyl diphosphate. This metabolite is formed from GGPP by the action of ent-CPS. Earlier studies showed that the transcription factor SPL9 activating promoter of terpene synthase 21 gene and positively regulating its transcription, was shown to be regulated by miR156. Similarly, in *A. paniculata* SPL9 was identified as target for miR156 as well as miR157. In *A. paniculata* flavonoid, phenylpropanoid, sesquiterpene and flavonoid biosynthesis may also under the regulation of different miRNAs (Table. 1). Metabolites derived by these all pathways are responsible for the high medicinal values of *A. paniculata*. Similarly, molecular targets against yellow vein leaf curl and yellow vein leaf curl betasatellite viruses were reported. However, no protein coding sequence was

identified in case of yellow vein leaf curl betasatellite genes for Ap-miRNAs. Whereas, only one target gene was identified in yellow vein leaf curl virus encoding AC1 protein. AC1 is a crucial for virus replication hence for its multiplication in the host. Ap-miR5021 probably regulates AC1 of the virus. In conclusion, present report may be helpful in understanding the role of miRNA in various metabolism in *A. paniculata*.

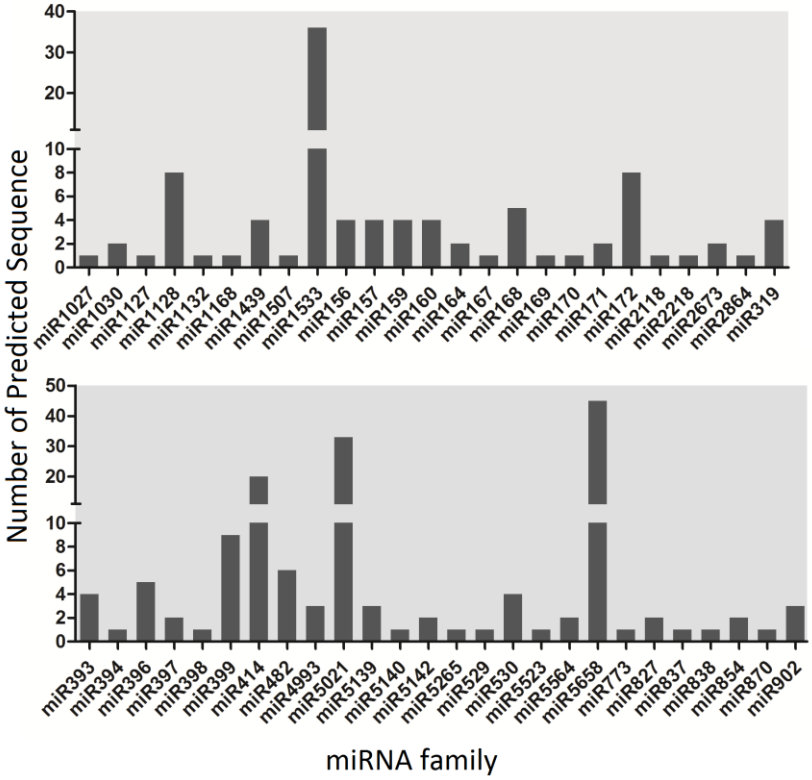

Fig. 1. miRNAs families and number of members sequences in A. paniculata

Table. 1 Ap-miRNAs involved in secondary metabolites biosynthesis in Kalmegh and Viral invasion

Target	Pathway/molecular role	C-mii	psRNAtarget
GDSL esterase/lipase	Multifunctional	miR1533	miR1533
3-epi-6-deoxocathasterone 23-monooxygenase	Brassinosteroid biosynthesis	miR1533	miR1533
Flavanoid 3,5-hydroxylase	Flavanoid biosynthesios	miR1533	miR1533
SPL9	Sesquiterpene biosynthesis	miR156, miR157	miR156, miR157
Anthocyanin 5-aromatic acyltransferase	Anthocyanin biosynthesis	miR414	*
Trans-cinnamate 4-monooxygenase	Flavanoid and Phenylpropanoid biosynthesis	miR5021	miR5021, miR1533
Flavanoid 3-monooxygenase	Flavanoid and Phenylpropanoid	miR5021	*

	biosynthesis		
Isoflavon 2-hydroxylase	Isoflavonoid biosynthesis	miR5658	miR5658, miR414
Zeta carotenoid desaturase	Carotenoid biosynthesis	miR902	*
Viral AC1	Viral replication associated protein	miR5021	miR5021

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