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Research Article

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Phylogenetic Analysis Reveals the Conservation of micro RNA 171 genes in Diverse Fruit Species

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Abstract

The MicroRNAs are endogenous non-coding small RNAs about 21 nucleotides in length, play an important role in physiological and biochemical processes. The evolutionary conservation or functional diversification of miRNA171 family members remains indescribable. In this study we used bioinformatics approach to show the phylogenetic relationship among miR171 precursor and mature sequences from three diverse fruit crops *viz*., papaya, citrus, grapes and model plant Arabidopsis. The phylogenetic analysis revealed that mature miR171 has the high conservation in their nucleotide sequences, whereas the precursor miR171 sequences evolved in a lineage specific manner. Mature miR171 are derived from multiple members of miR171 genes and regulate many aspects of plant development by modulating their target genes. Results of this study showed a lead for further in depth investigation of the functional characteristics of miRNAs in fruit crops.

Keywords: miRNA171; Bioinformatics; Evolution; Phylogenetic analysis; Diverse

Introduction

Micro RNAs are the small non-coding RNAs that play a pivotal role in gene expression, specifically at the post transcriptional level during growth and development of plants and animals [1]. In higher plants around 21 miRNA families are conserved [2]. Identification of micro RNAs is important to understand the evolution of miRNAs and miRNA targeted gene regulations. Publically available databases (NCBI, DDBJ, TIGR etc) play a central role in sharing genomic information, as well as *in-silico* biology miR171 represent one of the important and conserved miRNA families mainly involved in regulating flowering and apical meristem development. It also regulates the expression of scarecrow-like (SCL) transcription factor family. In *Arabidopsis*, there are three miR171 genes (a, b and c) which are predicted to regulate three SCL6 genes. MiR171a is most highly expressed in the inflorescence. Over-expression of miR171 affects expression of meristem identity genes suggesting a conservation of the role identified in *Arabidopsis*.

The basis for computational identification of miRNAs is the conserved, mature miRNA sequence coupled with the predictable secondary structure of miRNA surrounding sequences. Previously, based on nucleotide sequence similarity an evolutionary relationship was reported among mature and precursor sequences of different miRNA (miR156, miR 159, miR 160, miR 164, miR 169, miR 172, miR 395) genes from diverse groups of plant species (*Zea maize*), *Oryza sativa* and *Arabidopsis thaliana* [3,4]. In this study gene sequences for miR171 (mature miRNA, precursor miRNA) from Arabidopsis, Papaya, Citrus and grapes were retrieved from the Mir Base and analysed for evolutionary relationship among them by phylogenetic analysis.

Materials and Methods

Mature and precursor sequences

The mature and the precursor sequences of miRNA171 from *Arabidopsis thaliana* (ath), *Carica papaya (cpa), Citrus clementine (ccl), Citrus reticulate (crt), Citrus sinensis (csi), Citrus trifoliata (ctr) and Vitis vinifera (vvi)* were obtained from miR base registry database [5]. MiRNA171 was queried against miRBase for the identification of number of mature and precursor sequence of each plant species. The upstream intergenic sequence was carefully identified after a BLAST search of miRNA precursor sequences against different databases like Arabidopsis Database, Citrus Genome Database *Citrus sinensis* annotation project [6].

Sequences alignment

Sequence alignment of mature and stem-loop miRNA of, *Arabidopsis thaliana* (ath), *Carica papaya, Citrus clementine, Citrus reticulata, Citrus sinensis, Citrus trifoliata and Vitis vinifera* were performed using software “MUSCLE” (multiple sequence alignment (MSA) application tool,). We used MUSCLE aligned sequences for reconstructing the phylogenetic tree (Figure 1). The maximum likelihood (ML) phylogenetic analysis was performed with PHYML, software tool using the web version software package of Phylogeny. The phylogenetic trees were viewed using TreeDyn (198.3 doc) tree viewer software package [7]. In order to compare the similarity and differences of PHYML tree. The whole part of the alignment was used for reconstructing the phylogenetic tree in case of both mature and precursor sequences. An unrooted phylogenetic tree reconstructed with precursor miRNA species of *Arabidopsis thaliana* (ath), *Carica papaya, Citrus clementine, Citrus reticulata, Citrus sinensis,Citrus trifoliata and Vitis vinifera.* MUSCLE alignment and Maximum likelihood (PhyML) method were used for tree generation.

Results and Discussion

MiR171is a well-studied plant miRNA implicated in various aspects of plant development. To understand how a specific class of miRNA derived from multiple genes plays diverse developmental roles in different plants, it is necessary to dissect out the commonalities and divergence among different miRNAs and their genes. For this purpose, we used miR171, an important regulator of multiple developmental processes in our study. We compared the paralogous as well as orthologous sequences of gene structures of miR171 family members from *A. thaliana*, *Carica papaya, Vitis vinifera, Citrus reticulate, Citrus clementine, Citrus trifoliata and Citrus sinensis*. The availability of well annotated complete genome sequences of *A. thaliana* and genomic

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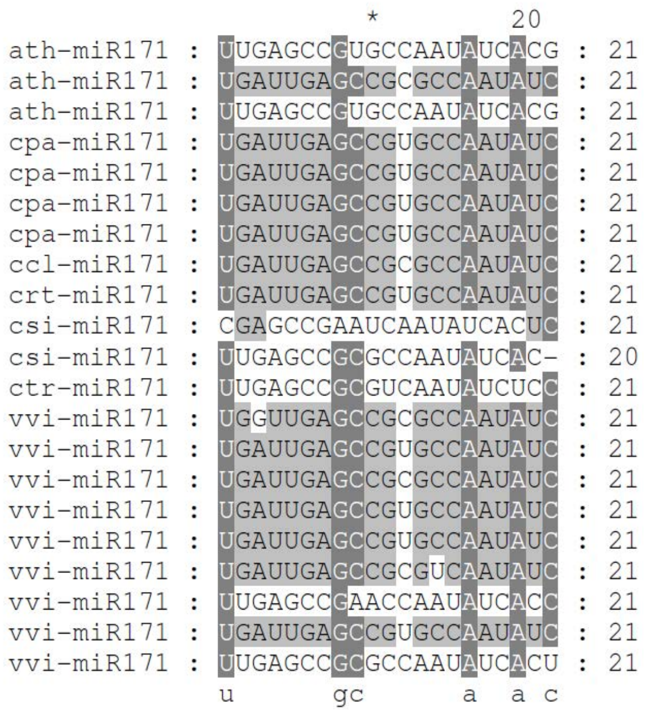

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                           *        20
ath-miR171 : UUGAGCCGUGCCAAUAUCACG : 21
ath-miR171 : UGAUUGAGCCGCGCCAAUAUC : 21
ath-miR171 : UUGAGCCGUGCCAAUAUCACG : 21
cpa-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
cpa-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
cpa-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
cpa-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
ccl-miR171 : UGAUUGAGCCGCGCCAAUAUC : 21
crt-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
csi-miR171 : CGAGCCGAAUCAAUAUCACUC : 21
csi-miR171 : UUGAGCCGCGCCAAUAUCAC- : 20
ctr-miR171 : UUGAGCCGCGUCAAUAUCUCC : 21
vvi-miR171 : UGGUUGAGCCGCGCCAAUAUC : 21
vvi-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
vvi-miR171 : UGAUUGAGCCGCGCCAAUAUC : 21
vvi-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
vvi-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
vvi-miR171 : UGAUUGAGCCGCGUCAAUAUC : 21
vvi-miR171 : UUGAGCCGAACCAAUAUCACC : 21
vvi-miR171 : UGAUUGAGCCGUGCCAAUAUC : 21
vvi-miR171 : UUGAGCCGCGCCAAUAUCACU : 21
             u       gc       a  a c
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Figure 1: The mature sequence comparison of miR171 in *Arabidopsis thaliana* (ath), Carica papaya (cpa), Citrus clementine (ccl), Citrus reticulate (crt), Citrus sinensis (csi), Citrus trifoliata (ctr) and Vitis vinifera (vvi).Alignment of phylogenetic tree and target homology of mature miR171; In MUSCLE alignment of the mature miR171 sequence, the black shaded blocks indicate the highly conserved residues by the alignment in the species.

information available in miRNAs and PUBMED for *Carica papaya, Vitis vinifera, Citrus reticulate, Citrus clementine, Citrus trifoliata and Citrus sinensis* have enabled the comparative genomics to explore the evolutionary relationship of miR171 gene family among these diverse plant species.

Phylogenetic analysis of mature and stem-loop miRNA171 genes

In this study, we observed some difference in nucleotide sequence of mature miRNA171 within and across the species (Table 1). Among different miR171 genes in the *Arabidopsis, Papaya, citrus*, the mature miR171 sequences were same but they differ from each other in 1-2 nucleotide. However, primary transcripts of miR171 genes differ in sequences within a single species as well as among different plant species. The topology of mature miRNA171 analysed through maximum likelihood (ML) tree method, were found to be quite similar (Figure 2). Tree can be divided into two clades (clade I, clade II). Clade I contains vvi-mir171g (21nt) and csi-mir171b (20nt) differing in single base at 3'position. The clade II is again divided into three subclades as a, b and c. Subclade 'a' is represent vvi-miR171a, vvi miR-171c, vvi-miR171d, crt-miR171, cpa-miR171c, cpa-miR171b, cpa-miR171a, cpa-miR171d, ath-miR171b, ath-miR171c and vvi-miR171i with maximum number of species in close relationship, subclade 'b' contains ctr-miR171 and vvi-miR171b with variation in nucleotide at 5' region, while subclade 'C' contains ath miR-171a, ccl-miR171, csi-miR171a, vvi-miR171h, vvi-miR171e and vvi-miR171f. The two subclades 'b' and 'c' suggests that it has undergone sequence variation during course of plant evolution. The variation may be due to mutation occurred during evolutionary period. In general, we observed a very low polymorphism of mature sequences of these members, which strongly suggested the high conservation of miR171 across different plant species.

Based on the precursor pre-sequence homology, a neighbour

Sl.No.	miRNA	Mature Sequence	LM (nt)
1	ath-miR171 a	UGAUUGAGCCGCGCCAAUAUC	21
2	ath-miR 171b	UUGAGCCGUGCCAAUAUCACG	21
3	ath-miR171c	UUGAGCCGUGCCAAUAUCACG	21
4	ccl-miR171	UGAUUGAGCCGCGCCAAUAUC	21
5	crt-miR171	UGAUUGAGCCGUGCCAAUAUC	21
6	csi-miR171a	UUGAGCCGCGCCAAUAUCAC	20
7	csi-miR171b	CGAGCCGAAUCAAUAUCACUC	21
8	ctr-miR171	UUGAGCCGCGUCAAUAUCUCC	21
9	vvi-miR171a	UGAUUGAGCCGUGCCAAUAUC	21
10	vvi-miR171b	UGAUUGAGCCGCGUCAAUAUC	21
11	vvi-miR171c	UGAUUGAGCCGUGCCAAUAUC	21
12	vvi-miR171d	UGAUUGAGCCGUGCCAAUAUC	21
13	vvi-miR171e	UGAUUGAGCCGCGCCAAUAUC	21
14	vvi-miR171f	UUGAGCCGCGCCAAUAUCACU	21
15	vvi-miR171g	UUGAGCCGAACCAAUAUCACC	21
16	vvi-miR171h	UGGUUGAGCCGCGCCAAUAUC	21
17	vvi-miR171i	UGAUUGAGCCGUGCCAAUAUC	21
18	cpa-miR 171a	UGAUUGAGCCGUGCCAAUAUC	21
19	cpa-miR 171b	UGAUUGAGCCGUGCCAAUAUC	21
20	cpa-miR 171c	UGAUUGAGCCGUGCCAAUAUC	21
21	cpa-miR 171d	UGAUUGAGCCGUGCCAAUAUC	21

Table 1: Sequence of mature miR171 family in Arabidopsis, Papaya, Citrus and grapes.

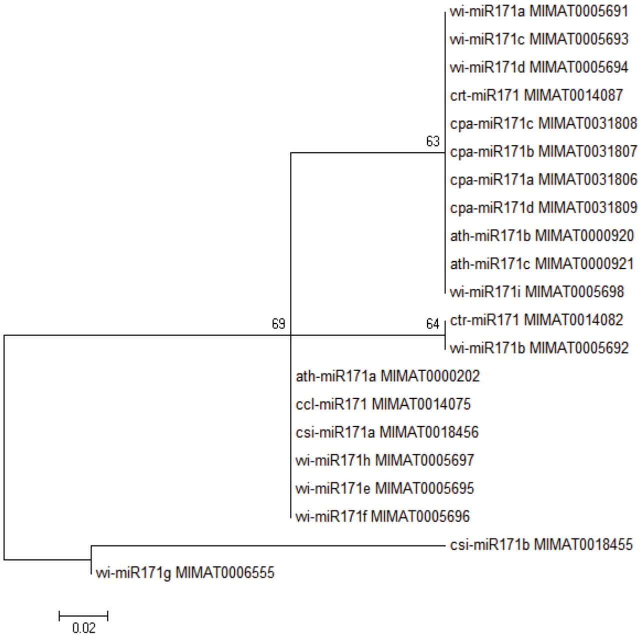

Figure 2: Phylogenetic tree generated based on the mature pre-sequence homology, a neighbour joining tree for 17 homologous members of miR171 family to display the homology between them. ath- Arabidopsis thaliana; cpa- Carica papaya; ccl- Citrus Clementine; crt-Citrus reticulate; csi-Citrus sinensis; ctr-Citrus trifoliata; vvi- Vitis vinifera. haliana; cpa- Carica papaya; ccl- Citrus Clementine; crt-Citrus reticulate; csi-Citrus sinensis; ctr-Citrus trifoliata; vvi- Vitis vinifera.

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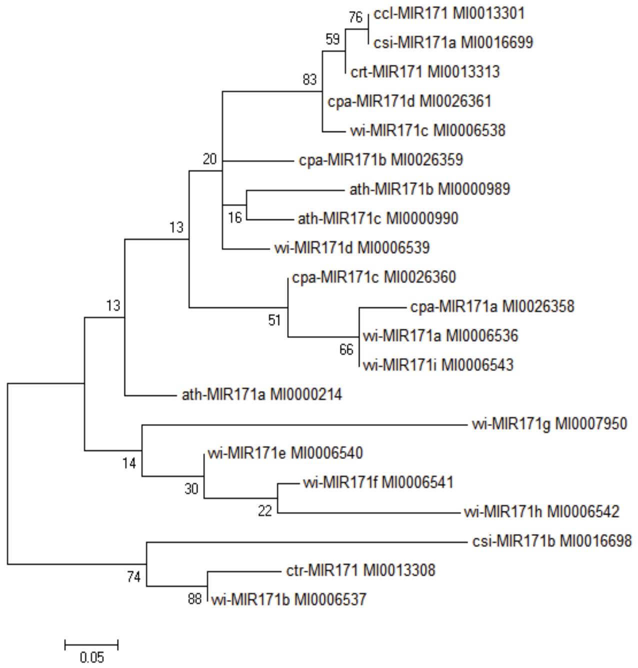

Figure 3: An unrooted phylogenetic tree reconstructed with precursor miR171- by MUSCLE alignment and maximum likelihood method. ath- Arabidopsis thaliana; cpa- Carica papaya; ccl- Citrus Clementine; crt-Citrus reticulate; csi-Citrus sinensis; ctr-Citrus trifoliata; vvi- Vitis vinifera.

joining tree was also constructed for 17 homologous members of miR171 family to display the homology between them. As shown in Figure 3, the phylogenetic tree was classified into two categories, *csi* miR171b (20nt), *ctr*-miR171 (21nt) and *vvi*- miR171b (21 nt) with variation near 5' region for some nucleotides, they are closely related and located in a single clade. On the second clade, again it is divided into two subclades 'a' and 'b', in which subclades 'b' shows close evolutionary relationship within the *Vitis vinifera* genera *vvi* miR-171h/f/e/g. All members except for *ath*-miR171a which pre-sequence had low homology compared with others situated on the same subclades- a (Figure 3). Moreover, this *cpa* miR171a and *vvi*171 a/i shows that they might be originate from the common ancestral genes. *ccl* miR171 and *csi* miR171a shows the close relationship between them. This suggests that although gene (pri-miR171) might have originated by probable duplication of individual pre-miR171 gene and diversified in course of evolution through extensive changes in the sequence. Suvukanta et al. reported conservation of miR166 gene family among *P. patens*, *O. sativa*, and *A. thaliana.* Several other studies also reported that miRNA166 and their target genes are conserved among different plant species [8,9]. These conserved mir171 regulates various families of transcription factors such as *NAC* domain containing transcription factors [10], *basic-leucine Zipper* genes [11], *CCAAT binding*1transcription factors [12], *Scarecrow like* genes [13], and *Growth Regulation Factor* genes [14,15] which were studied in different crop plants. These transcription factors play a crucial role in plant developmental mechanism. It appears that, despite some similarity, miR171 precursors show more diversification than mature miR171 sequences in the phylogenetic trees (Figures 2 and 3). Analysis of sequence identity among mature and precursors of miR171s indicates that precursor sequence have undergone most sequence diversification within each species, followed by mature precursor [16,17].

In conclusion, this study reports a comprehensive analysis of the phylogenetic relationship of miRNA171 family members belonging to three diverse fruit species *viz.*, Citrus, Grapes, Papaya with Arabidopsis. Our phylogenetic analysis revealed that mature miR171 has the high conservation in their nucleotide sequences, whereas the precursor miR171 sequences evolved in a lineage specific manner. Mature miR171 are derived from multiple members of miR171 genes and regulate many aspects of plant development by modulating their target genes. This study provides a ground for exploring the evolution and functional divergence of plant miRNA.

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References

1. Arazi T, Talmor M, Neiman Stav R, Riese M, Huijser P, et al. (2005) Cloning and characterization of micro-RNAs from moss. Plant J 43: 837-848.
2. Axtell MJ, Bartel DP (2005) Antiquity of microRNAs and their targets in land plants. Plant Cell 17: 1658-1673.
3. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism and function. Cell 116: 281-297.
4. Chevenet F, Brun C, Banuls AL, Jacq B, Christen R (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinforma 7: 439.
5. Guo HS, Xie Q, Fei JF, Chua NH (2005) MicroRNA directs mRNA cleavage of the transcription factor nacl to down regulate auxin signals for *Arabidopsis* lateral root development. Plant Cell 17: 1376-1386.
6. Lee MH, Kim B, Song SK, Heo JO, Yu NI, et al. (2008) Large-scale analysis of the gras gene family in *Arabidopsis thaliana*. Plant Mol Biol 67: 659-670.
7. Li WX, Ono Y, Zhu J, He XJ, Wu JM, et al. (2008) The *Arabidopsis NFYA5* transcription factor is regulated transcriptionally and post-transcriptionally to promote drought resistance. Plant Cell 20: 2238-2251.
8. Llave C, Xie Z, Kasschau KD, Carrington JC (2002) Cleavage of s*carecrow-like* mRNA targets directed by a class of *arabidopsis* miRNA. Science 297: 2053-2056.
9. Liu D, Song Y, Chen Z, Yu D (2009) Ectopic expression of miR396 suppresses *GRF* target gene expression and alters leaf growth in *arabidopsis*. Physiol Plant 136: 223-236.
10. Sunkar R, Zhou X, Zheng Y, Zhang W, Zhu JK (2008) Identification of novel and candidate miRNAs in rice by high throughput sequencing. BMC Plant Biol 8: 25.
11. Sunkar R, Li YF, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. Trends Plant Sci 17: 196-203.
12. Barik S, Sarkar Das SS, Singh A, Gautam V, Kumar P, et al. (2013) Phylogenetic analysis reveals conservation and diversification of micro RNA166 genes among diverse plant species. Genomics 103: 114–121.
13. Voinnet O (2009) Origin, biogenesis, and activity of plant microRNAs. Cell 136: 669-687.
14. Williams L, Grigg SP, Xie M, Christensen S, Fletcher JC (2005) Regulation of *Arabidopsis* shoot apical meristem and lateral organ formation by microRNA miR166g and its *AtHD-ZIP* target genes. Development 132: 3657-3668.
15. Yang F, Liang G, Liu D, Yu D (2009) *Arabidopsis* miR396 mediates the development of leaves and flowers in transgenic tobacco. J Plant Biol 52: 475-481.
16. http://microrna.sanger.ac.uk/cgi-bin/sequences/
17. http://citrus.hzau.edu.cn/cgi-bin/orange/blastPlant

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