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Turmeric (*Curcuma longa*): miRNAs and their regulating targets are involved in development and secondary metabolite pathways

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ABSTRACT

Turmeric has been used as a therapeutic herb over centuries in traditional medicinal systems due to the presence of several secondary metabolite compounds. microRNAs are known to regulate gene expression at the post-transcriptional level by transcriptional cleavage or translation repression. miRNAs have been demonstrated to play an active role in secondary metabolism regulation. The present work was focused on the identification of the miRNAs involved in the regulation of secondary metabolite and development process of turmeric. Eighteen miRNA families were identified for turmeric. Sixteen miRNA families were observed to regulate 238 target transcripts. LncRNAs targets of the putative miRNA candidates were also predicted. Our results indicated their role in binding, reproduction, stress, and other developmental processes. Gene annotation and pathway analysis illustrated the biological function of the targets regulated by the putative miRNAs. The miRNA-mediated gene regulatory network also revealed co-regulated targets that were regulated by two or more miRNA families. miR156 and miR5015 were observed to be involved in rhizome development. miR5021 showed regulation for terpenoid backbone biosynthesis and isoquinoline alkaloid biosynthesis pathways. The flavonoid biosynthesis pathway was observed to be regulated by miR2919. The analysis revealed the probable involvement of three miRNAs (miR1168.2, miR156b and miR1858) in curcumin biosynthesis. Other miRNAs were found to be involved in the growth and developmental process of turmeric. Phylogenetic analysis of selective miRNAs was also performed.

1. Introduction

Turmeric (*Curcuma longa*), a highly important plant in the family *Zingiberaceae*, comprises about 100 accepted species [1]. Its rhizome has been widely used in traditional Asian medicine for numerous hepatic disorders [2]. *C. longa* has been used as a therapeutic herb over centuries in traditional medicinal systems. Several pharmacological effects of turmeric as anti-ulcerogenic, anti-inflammatory, anti-tumour and anti-cancerous ones, have been reported earlier [3–6]. In Ayurveda, *C. longa* is used to strengthen the overall energy of the body, relieve gas, dispel worms, improve digestion, regulate menstruation, dissolve gallstones, and relieve arthritis [1]. microRNAs (miRNAs) are approximately 20–22 nucleotide long and endogenous small RNA molecules. Post-transcriptional regulation of the gene expression made by miRNAs take part in almost all biological and metabolic processes of plants and animals [7–10]. Growing evidences suggests their role in signal transduction, leaf, and root development, reproduction and in response to environmental biotic and abiotic

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stress [11–14]. Computational screening of potential miRNAs is utilized frequently to avoid the high cost, lower expression and time consuming process of experimental techniques. It has also been successful for the discovery of conserved miRNAs as well as new miRNAs [15–19].

Plants produce a large number of secondary metabolites that possess a lot of biological activities. These metabolites protect the host plant against competitors, predators, pathogens, and ultraviolet light, helping them to adapt to the environmental niche, and some of those biologically active metabolites have medicinal properties [20]. In the case of turmeric, curcumin is the most active bioactive compound. It has been reported to alter the expression profiles of microRNAs in human pancreatic cancer cells [21]. Except for curcumin, numerous other secondary metabolites are reported to have several therapeutic properties. A total of 720 compounds, including 102 diphenylalkanoids, 19 phenylpropene derivatives, 529 terpenoids, 15 flavonoids, 7 steroids, and 3 alkaloids were already reported in Curcuma [1]. Its diterpenoid compound C fought against gastric cancer [22]. The role of sesquiterpenes from its essential oil was reported to have inhibitory effects on nitric oxide production [23,24]. A large amount of flavonoid and alkaloid reported from turmeric suggests that they contribute to its antioxidant activities [25].

miRNAs have been demonstrated to play an active role in secondary metabolism regulation [26]. The regulation of secondary metabolites by an endogenous target mimic [eTM] of particular miRNAs has been reported as a new approach. This technique has been used to enhance the production of nicotine in *Nicotiana tabacum* [27]. This was the first successful as well as initial story for the regulation of secondary metabolite biosynthesis by a miRNA-eTM regulatory module in plants [27]. More efforts are needed to identify a significant role of miRNAs in other plants.

Despite the immense importance of turmeric as a culinary and medicinal plant, no miRNAs have been reported in miRBase [28–30]. In addition to other work related to miRNAs and their activity in *Curcuma* [31–34], we have identified miRNAs from turmeric showing regulation for different secondary metabolic pathways as well as their involvement in growth and development process.

2. Materials and methods

2.1. miRNA and target prediction

ESTs, nucleotide and SRA (SRX627351) data were downloaded from the public database NCBI (http://www.ncbi.nlm.nih.gov/). For the identification of putative miRNA and target candidates, C-mii [35] was used. Filtering criteria were also applied to narrow down the false predictions. For miRNA, the prediction criteria were as follows: (a) the length of mature miRNA should be in range of 19–25 nucleotides; (b) more than four mismatches were not allowed between candidate mature miRNAs and known mature miRNA; (c) the number of flank bases should not be more than 3; (d) the AU content should be higher than the GC content; (e) a smaller number (≤ 1) of bulges and their size; (f) MFEI values should be highly negative (≥ 0.90 (–kcal/mol)). To enhance the prediction probability, miRNA-dis [36] and imiRNA-PseDPC [37] were used. Thus, the results were again verified at the sequential and structural levels, as miRNA-dis used structural-order information into the prediction, while imiRNA-PseDPC used a formulated novel feature vector based on pre-miRNA sequence.

The target prediction was performed by two channels using turmeric data. The first one was for turmeric miRNAs, and the second one was for all miRNAs reported in miRBase. The targets were predicted using the following criteria: (a) more than four mismatches were not allowed in complementary sites between miRNA and mRNA levels; (b) no mismatches were allowed at positions 10 and 11.

The lncRNAs of *Arabidopsis thaliana* reported in CANTATAdb [38] was used as a reference data set for the prediction of lncRNA targets of the predicted miRNAs by psRNATarget [39]. A biological network was constructed by Cytoscape [40].

2.2. Pathway and phylogenetic analysis of the identified miRNA

Selected target transcripts were considered for functional annotation. Annotation and pathway analyses were performed by Blast2GO [41] and KASS sever (http://www.genome.jp/tools/kaas/), respectively. The phylogenetic tree was constructed using maximum likelihood method based on Kimura2-parameter substitution model from MEGA 6.0 [42].

3. Results

3.1. miRNA identification and characterization

The prediction results showed 145 miRNA families by C-mii using default parameters. The number of predictions was reduced to 50 miRNA candidates by applying the above-mentioned filtering criterion. To further improve the accuracy, filtered miRNA precursors were again cross validated at sequence and structure level by using imiRNA-PseDPC and miRNA-dis. After removing the redundancy and false positive values, the results were optimized with eighteen miRNAs, which were finally considered for the study.

3.1.1. Length variation

A variation from 20 to 22 nucleotides was observed for mature miRNA sequences. On the other hand, precursor miRNA candidates showed a large variation within the range of 38 to 574 nucleotides (Table 1). The finding was in accordance with earlier reports [36,43,44].

3.1.2. AU and GC contents

Higher AU content is a feature to distinguish miRNA from other non-coding RNAs. We observed a high AU content, which was in the range from 32.00 to 73.22 (Table 1), with an average of 59.84, as reported in earlier studies [19,43,45].

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