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IN SILICO EST DATA ANALYSIS OF CURCUMA LONGA: A CROSS KINGDOM APPROACH

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ABSTRACT

MicroRNAs are a newly discovered class of non-coding small RNAs with 22–24nucleotides. They play multiple roles in biological processes including development, cell proliferation, apoptosis, stress responses and many other cell functions. In this research, several approaches were combined to make a computational prediction of potential miRNAs and their targets in Curcuma longa.The already available information of the plant miRNAs present in miRbase v21 was used against expressed sequence tags (ESTs). A total of 89 uniq miRNA weredetected following a range of strict filtering criteria. The target prediction was carried out for this miRNA having various targets. These targets were functionallyannotated and GO terms were assigned. To study the conserved nature of the miRNAs, predicted phylogeny analysis was carried out. These findings will significantly provide the broader picture for understanding the functions of Curcuma longa in Anti-cancer and Anti-Immunosuppressor activity.

INTRODUCTION

Plant parts that are food for animals including human contains significant quantities of siRNAs. Arecent breakthrough research paper has suggested that some plant miRNAs can pass through the animal gastro-intestinal track and enter the circulatory system [1]. Certain recent studies have explored a way of plant miR into the animal body and its tissues. Epithelial cells in the intestine might take up miRNAs in food, package them into microvesicles and release them into the circulatory system [2]. The secreted microvesicle could then deliver exogenous plant miRNA to target organs where they could regulate cognate mRNAs [3]. Presence of 2′-O-methyl modificationat their 3′ end which is absent on the animal miR renders identification of plant miR in the animaltissues and body fluid. The abundance of plant miRNA found in human serum is also quite high. miR168 was 3.2×10^{-6} fmol (1920 copies) per 100 pg of total RNA in human serum, equivalent to 850 copies per cell, which is equivalent to the average amount of a human [4]. As an example of miR acting in the human body we like to discuss three examples. MIR168a is abundant in rice and is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese human. MIR168a could bind to the human/mouse LDLRAP1 mRNA, inhibit its expression in liver, and consequently decrease LDL removal from mouse plasma .A more recent finding shows that a plantmiRNA from honeysuckle is able to inhibit Influenza A replication, indicating that plant miRNAsmay be useful for treating human diseases [5]. miR159 is found in various plants particularly from broccoli and could be detected in human sera and its levels were inversely correlated with breast cancer incidence and progression. *TCF7 i*s a target for miR159 and reduces proliferation of breastcancer cells.

Indians have known the health benefits of turmeric for thousands of years. More recent studies have proven that the people of India were right in their use of turmeric for medicinal purposes. The active ingredient in turmeric, curcumin, has anti-inflammatory and disinfecting properties [6].This explains its healing powers when applied to cuts and abrasions. Taken orally as an anti- inflammatory, turmeric does not have the side-effects commonly associated with some modern pharmaceutical medicines. Studies have shown numerous benefits of using turmeric. Scientific claims prove the benefits of the herb in the relief of digestive complications, cancer, anti- inflammatory and detoxification [7][8]. Turmeric also reduces the build-up of plaque associated with cardiovascular disease. Turmeric reduces blood sugar levels, a property valuable for helpingthose suffering from diabetes [9]. Other foods combined with turmeric boost its healing capabilities. When combined with cauliflower, turmeric may help prevent prostate cancer. Onionsmay enhance the effects of turmeric on preventing colon cancer. Turmeric also helps prevent melanoma and stops the growth of new blood vessels developing in tumors. It is also thought to reduce the risk of childhood leukemia. Turmeric prevents and slows the progression of Alzheimerdisease by removing amyloid plaque from the brain [10]. However, all beneficial effects of turmeric cannot be explained only by turmeric. Besides curcumin there are several other alkaloidsand other phytochemicals which may also responsible or enhance effect of turmeric. At this juncture we would like to emphasize that some effects of turmeric may also be mediated by its miR. As far as the Cross-Kingdom gene expression is concerned, very few reviews and articles have been published. And only two miRNAs, one is miR168 and other is miR159, were experimentally proven their activity and presence in human tissues and plasma. Based on these studies, we have used bioinformatics tools and identified the targets of plant miRNAs in human, which may involve in metabolic pathways or diseased regulations.

MATERIAL AND METHOD:

1. Reference set of *Curcuma longa*:

To identify the potential miRNAs a total of 427 mature miRNAs of *Arabdopsis thaliana* sequencewere retrived from the miRBase version 21[11]. A total of 12,678 *Curcuma longa* ESTs were downloaded from the publicly available database dbEST of Genbank nucleotide database and all these ESTs were subjected to bioinformatics tools against reference set of miRNAs to find the turmeric miRNA homologs.

2. Identification of potential miRNAs of *Curcuma longa*

For the data analysis the sequences of the known plants miRNAs were used as query sequences for Blast against the EST Database. The similarity search was carried out using Blast - 2.6.0+ program downloaded from the NCBI fttp site (ftp://ftp.ncbi.nih.gov/); where the parameters obtained as e-value being 10, wordsize 7, max- target sequence 3 and format size 6 and mismatcheswas 1 [12]. Mature miRNA sequences should not be less then 18nt and should be a maximum of 24nt. The sequences were filtered and clustered which were coordinated from BED tools version BED 2.3.0+ and the conserved miRNAs were identified where the criteria considered was p- values.

3. Target prediction of identified miRNAs:

Those conserved miRNAs were carried out for the target prediction psRNATarget: a plant small RNA target analysis server. The miRNA sequence mapped against homo sapiens and the hsp sizeis 18 [13].

4. Functional Annotation *Curcuma longa.*

The targeted genes were annotated using PANTHER classification system. PANTHER was used for gene ontology analysis including Biological process, Molecular Process, and the protein family. Pathway analysis was carried out using PANTHER Classification system [14].

5. Protein – protein interaction:

As one miRNA can regulate a multiple number of genes, it can be a challenging task to identify key miRNA target, so understand genes and their function, predicted genes target wasused for protein - protein interaction through STRING web services [15].

RESULTS & DISCUSSION

Many recent studies have been demonstrated that plant miRNA are involved in the crucial metabolicactivities [1]. The second important feature is that they are conserved in nature. Therefore, we have used the previously known plant mature miRNAs of *Arabidopsis thaliana* from the publicly available database miRBase to search for homologs of miRNAs of *Curcuma longa* from the EST database. Atotal of 427 mature miRNAs was aligned against the 12,678 ESTs of turmeric through BLAST program with e-value 10, wordsize 7, max-target sequence 3, format size 6 and mismatches was 1. The BLAST alignment detected 151 miRNAS with the repetitive sequence, to avoid the repeat sequence and to remove the redundant of the sequences the filtration has been carried out using bedtools where the quantified 89 matures miRNAs were retrived from the total of 151 miRNAs. To get insights of the key role played by miRNAs in development and other activities, the target prediction has been done using computational approach where 37 targeted genes were the genes coding for transcription factors, cleavage and the regulatory proteins are involved in the transcriptorfactors (Table 1). Employing computational approach to find the plant miRNA that bind their targets by complete or nearly complete complemantarity where the primary targets of miRNA s the 3'UTR.The predicted miRNAs were seen to have a complemantarity relation with more than one regulatorytarget. These UTRs have already been recognized as an important regulatory region even before thediscovery of miRNAs, due to the presence of numerous regulatory signals involved in the control ofnuclear export, subcellular localization, and transcript stability amongst other processes whichcontains multiple target sites more than one miRNA to interact .It is well known that animal miRNAtargets are difficult to predict, unlike plant targets since miRNA: mRNA duplexes often contain several mis-matches, gaps and G+U base pairs in many position. In present studies we have conductedthese potential targets involved in developmental process, molecular process, protein involved in transcriptor factor and the pathway analysis are involved in the signalling pathway and in the metabolism activity (figure 1 ,figure 2 & figure 3). Gene like FLT1, PDGFRA, RETN are highly interacted and are involved in the immuno factors, receptor kinase, growth factors (figure 4). In (figure 1) shows that the HRH4 are involved in biological process which functions as stress response,regulation of catalytic activity and in several process. During gene ontology, we have observed that some of targets were found repetitively in multiple functions like HRH4 involved in 8 biological functions regulation of catalytic activity and in matabolic process and MICB and CLEC2d are involved in the response to stimulates which include response to immune and response to stress.

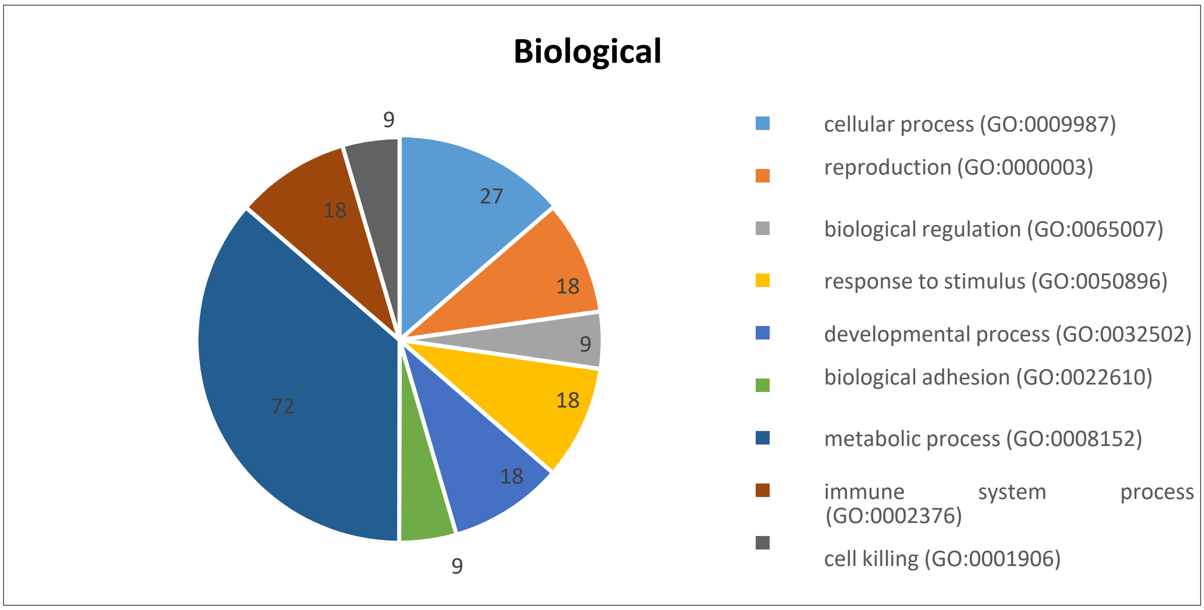

Figure 1: Functional analysis of target with respect to Biological process.

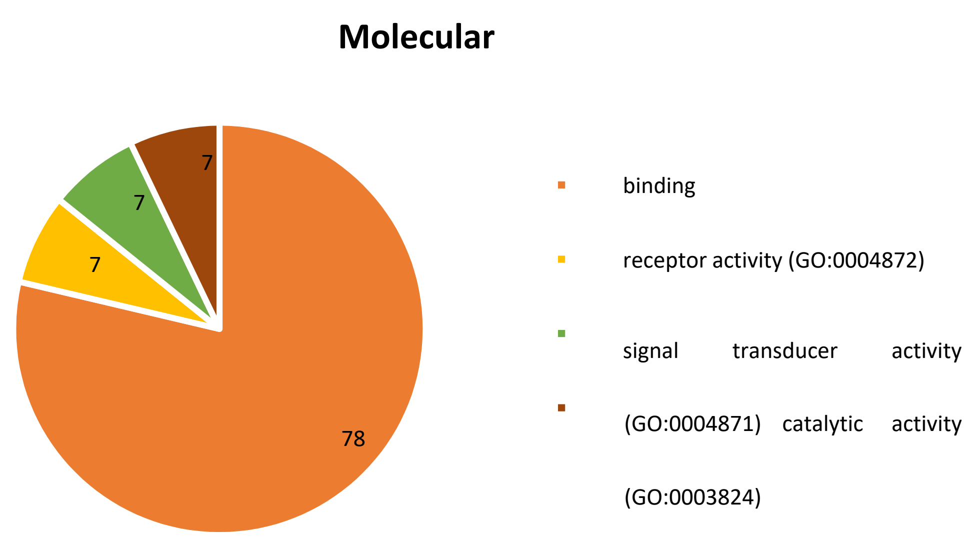

Figure 2: Functional analysis of target with respect to Molecular Function.

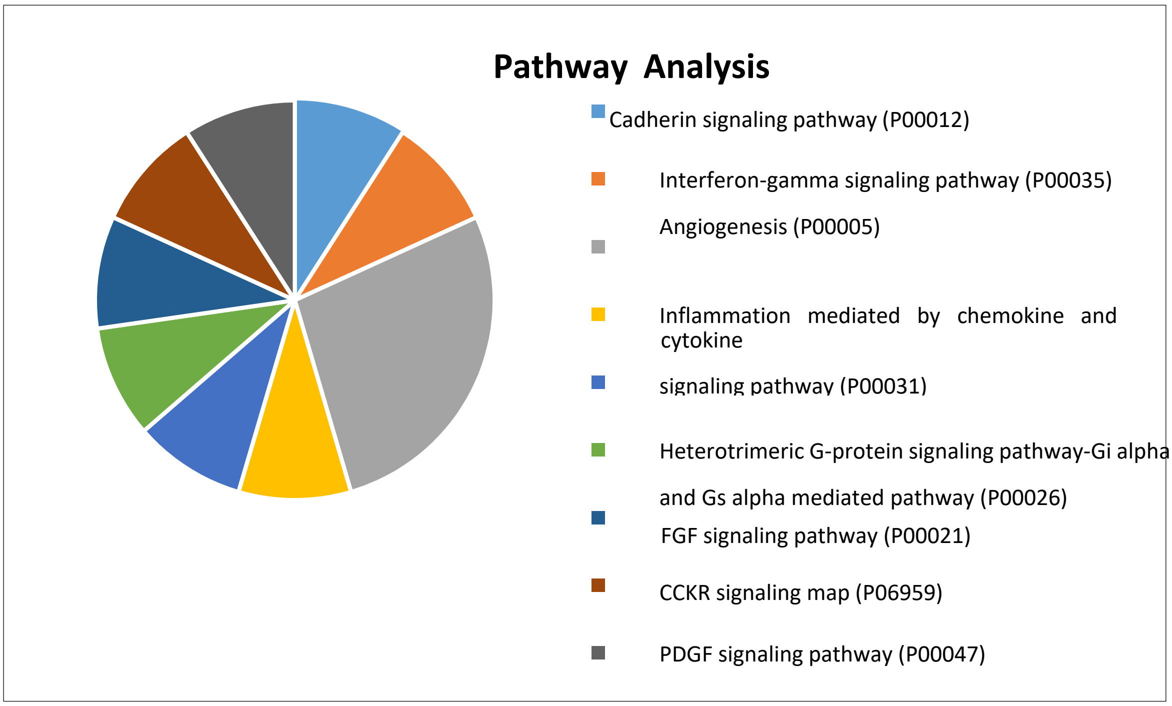

Figure 3: Pathway analysis of the miR- targets of *Curcuma longa.*

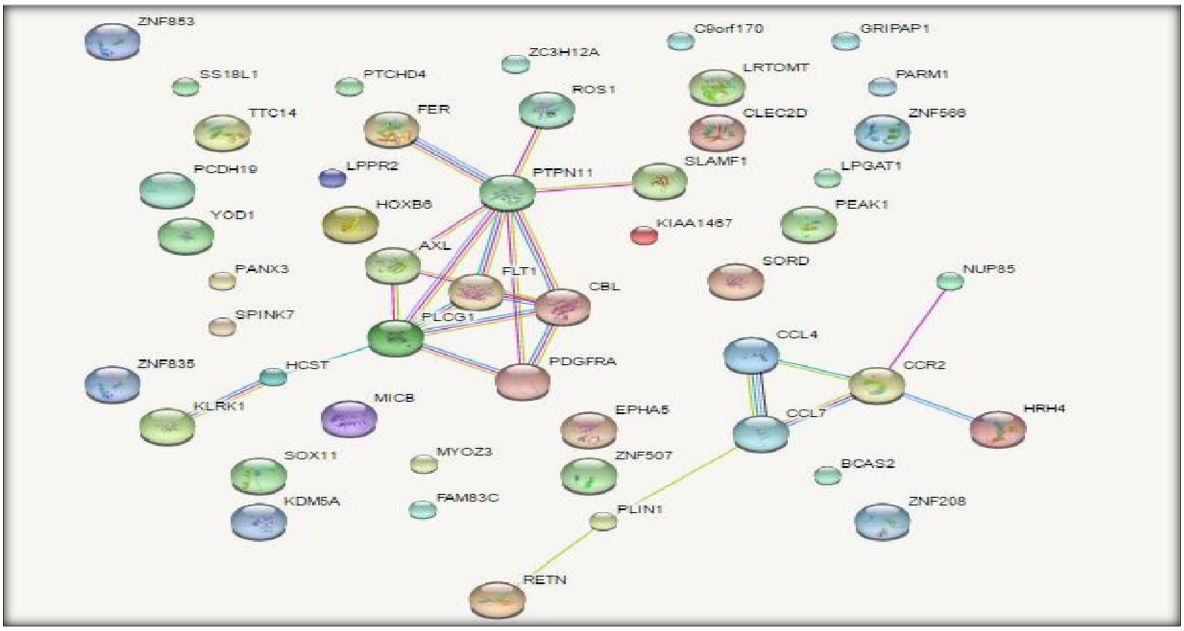

Figure 4: Protein -protein interaction of 38 gene targets.

CONCLUSION

In this work, a new approach to identify potential miRNA EST data of *Curcuma longa* with the medicinal properties. The present study shows the predicted miRNAs of *Curcuma longa* were found to target genes which involved in biological process. Predicted miRNAs of might be useful as potential agents for treatment of diseases associated with respective targets. Predicted miRNA targets were involved in DNA repairing agent, tumour suppressor, anti-cancer and anti- Immunosuppressor activity.

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