

## COMPUTATIONAL IDENTIFICATION OF CITRUS RETICULATA L. microRNAs AND THE CIS-ACTING REGULATORY ELEMENTS TO PREDICT THE EXPRESSION PROBABILITY OF THEIR RESPECTIVE MIR GENES

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*MicroRNA(miRNA), a small non-coding class of RNA that regulates the gene expression, is conserved among several plant species. In the present study, an in-silico approach was adopted to identify miRNA from the known expressed sequences tags (ESTs) of Citrus reticulata L. A total of 17 miRNAs from 23 different ESTs along with their secondary structures and targets were predicted. The identified 63 targets include several transcription factors, proteins that regulate plant growth, development, flowering, and seed development together with stress response. The cis-regulatory element present at the promoter region of the MIR genes of C. reticulata showed relevance towards light responsiveness, auxin, gibberellins, abscisic acid (ABA), anthocyanin responsiveness, salicylic acid responsiveness, anaerobic induction, circadian control, nitrate dependent regulation of the cell cycle and DNA replication, defense, and stress responsiveness. The present study identifies the miRNAs along with their regulatory elements in C. reticulata. The study will also support the research on miRNAs identification from the genomic data of different plants and prediction of the possibilities of expression of identified MIR genes based on the presence of upstream promoter and other regulatory elements.*

**Key words:** miRNA, *Citrus reticulata*, Expressed Sequence Tag, Cis-regulatory element, psRNATarget.

### ОБЧИСЛЮВАЛЬНА ІДЕНТИФІКАЦІЯ мікроРНК І ЦИС-ДІЮЧИХ РЕГУЛЯТОРНИХ ЕЛЕМЕНТІВ CITRUS RETICULATA L. ДЛЯ ПЕРЕДБАЧЕННЯ ВІРОГІДНОСТІ ЕКСПРЕСІЇ ЇХНІХ ВІДПОВІДНИХ ГЕНІВ MIR

МікроРНК (miRNA), невеликий клас некодуючих РНК, який регулює експресію генів, обмежені декількома видами рослин. У цьому дослідженні було застосовано підхід in-silico з метою визначення

мікроРНК у відомих маркерних експресованих послідовностях (EST) *Citrus reticulata* L. Загалом було передбачено 17 мікроРНК із 23 різних EST, а також їхні вторинні структури та цілі. До ідентифікованих 63 цілей входять декілька транскрипційних факторів, білки, що регулюють ріст, розвиток, цвітіння рослин та розвиток насіння, а також реакцію на стрес. Цис-регуляторний елемент, присутній у промоторній ділянці MIR генів *C. reticulata*, продемонстрував свою актуальність щодо реакції на світло, ауксин, гібберелліни, абсцизову кислоту (ABA), антоцианін, саліцилову кислоту, щодо анаеробної індукції, контролю циркадного ритму, нітратозалежного регулювання клітинного циклу та реплікації ДНК, захисту та реакції на стрес. У цьому дослідженні було встановлено мікроРНК та їхні регуляторні елементи в *C. reticulata*. Наше дослідження також буде корисним для вивчення можливості ідентифікації мікроРНК на основі геномних даних різних рослин та передбачення можливості експресії ідентифікованих генів мікроРНК на основі наявності висхідного промотера та інших регуляторних елементів.

**Ключові слова:** мікроРНК, *Citrus reticulata*, маркерна експресована послідовність, цис-регуляторний елемент, psRNATarget.

### REFERENCES

- Baghban KB, Bastami M, Nayeri S (2018) In silico identification of novel microRNAs and targets using EST analysis in *Allium cepa* L. Interdisciplinary Sciences: Computational Life Sciences 10:771–780  
Barrett H, Rhodes A (1976) A numerical taxonomic study of affinity relationships in cultivated Citrus and its close relatives. Systematic Botany 105–136 p  
Baumberger N, Baulcombe D (2005) Arabidopsis ARGONAUTE1 is an RNA Slicer that selectively recruits microRNAs and short interfering RNAs. Proc Nat Acad Sci 102:11928–11933  
Bologna NG, Schapire AL, Zhai J, Chorostecki U, Boisbouvier J, Meyers BC, Palatnik JF (2013) Multiple RNA recognition patterns during microRNA biogenesis in plants. Genome Res 23:1675–1689  
Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56:317–333  
Brivanlou AH, Darnell Jr JE (2002) Signal transduction and the control of gene expression. Science 295:813–818  
Chen X, Li Q, Wang J, Guo X, Jiang X, Ren Z, Weng C, Sun G, Wang X, Liu Y (2009) Identification and characterization of novel amphioxus microRNAs by Solexa sequencing. Genome Biol 10:1–13  
Chowdhury MR, Basak J, Bahadur RP (2020) Elucidating the functional role of predicted miRNAs in

- post-transcriptional gene regulation along with Symbiosis in *Medicago truncatula*. *Cur Bioinform* 15: 108–120

Consortium GO (2015) Gene ontology consortium: going forward. *Nucl Acid Res* 43:D1049–D1056

Devi KJ, Chakraborty S, Deb B, Rajwanshi R (2016) Computational identification and functional annotation of microRNAs and their targets from expressed sequence tags (ESTs) and genome survey sequences (GSSs) of coffee (*Coffea arabica* L.). *Plant Gene* 6:30–42

Dezulian T, Remmert M, Palatnik JF, Weigel D, Huhson DH (2006) Identification of plant microRNA homologs. *Bioinform* 22:359–360

Ding Y, Chen Z, Zhu C (2011) Microarray-based analysis of cadmium-responsive microRNAs in rice (*Oryza sativa*). *J Exper Bot* 62:3563–3573

Elhiti M, Stasolla C (2009) Structure and function of homodomain-leucine zipper (HD-Zip) proteins. *Plant Signal Behav* 4:86–88

Fan F, Yang X, Cheng Y, Kang Y, Chai X (2017) The DnaJ gene family in pepper (*Capsicum annuum* L.): comprehensive identification, characterization and expression profiles. *Front Plant Sci* 8:689

FAO (2019). Citrus fruit fresh and processed Statistical bulletin 2020. <https://www.fao.org/3/cb6492en/cb6492en.pdf>. Accessed 16 May 2023

Frazier TP, Xie F, Freistaedter A, Burkew CE, Zhang B (2010) Identification and characterization of micro RNAs and their target genes in tobacco (*Nicotiana tabacum*). *Planta* 232:1289–1308

Gbjaj MA, Sadawe IA, Meiqal NM, Bensaber SM, Maamar MS, Hermann A, Gbjaj AM (2019) Evaluation of neuropharmacological activities of methanolic and aqueous extracts of *Citrus reticulata* (Rutaceae) fruit peels. *Am J Biomed Sci Res* 2:131–135

Griffiths-Jones S, Grocock RJ, Van Dongen S, Bateman A, Enright AJ (2006) miRBase: microRNA sequences, targets and gene nomenclature. *Nucl Acid Res* 34:D140–D144

Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2007) miRBase: tools for microRNA genomics. *Nucl Acid Res* 36:D154–D158

Hall T, Biosciences I, Carlsbad C (2011) BioEdit: an important software for molecular biology. *GERF Bull Biosci* 2:60–61

Han Y-Q, Hu Z, Zheng D-F, Gao Y-M (2014) Analysis of promoters of microRNAs from a *Glycine max* degradome library. *J Zhejiang Univer Sci B* 15:125–132

Hao X, Wang B, Wang L, Zeng J, Yang Y, Wang X (2018) Comprehensive transcriptome analysis reveals common and specific genes and pathways involved in cold acclimation and cold stress in tea plant leaves. *Scientia Horticult* 240:354–368

Hassan AZ, Ahmed KM, Abu-Gabal NS, Mahrous KF, Shalaby NM (2017) Phytochemical and genotoxicity studies of *Citrus reticulata* aerial part in mice. *Egyptian Pharmaceut J* 16:87

Hindi NKK, Chabuck ZAG (2013) Antimicrobial activity of different aqueous lemon extracts. *J Appl Pharmaceut Sci* 3:074–078

Jagadeeswaran G, Zheng Y, Li YF, Shukla LI, Matts J, Hoyt P, Macmil SL, Wiley GB, Roe BA, Zhang W (2009) Cloning and characterization of small RNAs from *Medicago truncatula* reveals four novel legume-specific microRNA families. *New Phytologist* 184: 85–98

Jones-Rhoades MW, Bartel DP, Bartel B (2006) Micro RNAs and their regulatory roles in plants. *Ann Rev Plant Biol* 57:19–53

Kangarkar V, Gavimath C, Vijapur V, Gowri B, Hooli V, Mathapati P (2010) Protective effect of essential oil of *Citrus reticulata* on isoniazid induced hepatotoxicity in wistar rats. *Inter J Pharmaceut Appl* 1

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evolut* 16:111–120

Kong F, Deng Y, Zhou B, Wang G, Wang Y, Meng Q (2014) A chloroplast-targeted DnaJ protein contributes to maintenance of photosystem II under chilling stress. *J Exper Bot* 65:143–158

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evolut* 35: 1547–1549

Lin Y, Lai Z (2013) Comparative analysis reveals dynamic changes in miRNAs and their targets and expression during somatic embryogenesis in longan (*Dimocarpus longan* Lour.). *PLoS One* 8:e60337

Liu Y, Heying E, Tanumihardjo SA (2012) History, global distribution, and nutritional importance of citrus fruits. *Compr Rev Food Sci Food Safety* 11:530–545

Lu Y-B, Qi Y-P, Yang L-T, Guo P, Li Y, Chen L-S (2015) Boron-deficiency-responsive microRNAs and their targets in *Citrus sinensis* leaves. *BMC plant biology* 15:1–15

Ma C-L, Qi Y-P, Liang W-W, Yang L-T, Lu Y-B, Guo P, Ye X, Chen L-S (2016) MicroRNA regulatory mechanisms on *Citrus sinensis* leaves to magnesium deficiency. *Front Plant Sci* 7:201

Mallory AC, Vaucheret H (2006) Functions of micro RNAs and related small RNAs in plants. *Nat Genet* 38:S31

Megraw M, Baev V, Rusinov V, Jensen ST, Kalantidis K, Hatzigeorgiou AG (2006) MicroRNA promoter element discovery in *Arabidopsis*. *RNA* 12:1612–1619

Mishra AK, Duraisamy GS, Týcová A, Matoušek J

- (2015) Computational exploration of microRNAs from expressed sequence tags of *Humulus lupulus*, target predictions and expression analysis. *Computat Biol Chem* 59:131–141

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. *Nucl Acid Res* 35:W182–W185

Motameny S, Wolters S, Nürnberg P, Schumacher B (2010) Next generation sequencing of miRNAs—strategies, resources and methods. *Genes* 1:70–84

Pandey B, Gupta OP, Pandey DM, Sharma I, Sharma P (2013) Identification of new stress-induced microRNA and their targets in wheat using computational approach. *Plant Signal Behav* 8:e23932

Qiu X-B, Shao Y-M, Miao S, Wang L (2006) The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones. *Cell Mol Life Sci CMLS* 63:2560–2570

Rajwanshi R, Chakraborty S, Jayanandi K, Deb B, Lightfoot DA (2014) Orthologous plant microRNAs: microregulators with great potential for improving stress tolerance in plants. *Theor Appl Genet* 127: 2525–2543

Ravichandran S, Ragupathy R, Edwards T, Domaratzki M, Cloutier S (2019) MicroRNA-guided regulation of heat stress response in wheat. *BMC Genom* 20:1–16

Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. *Genes Develop* 16:1616–1626

Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP (2002) Prediction of plant microRNA targets. *Cell* 110:513–520

Rogers K, Chen X (2013) Biogenesis, turnover, and mode of action of plant microRNAs. *Plant Cell* 25:2383–2399

Rombauts S, Déhais P, Van Montagu M, Rouzé P (1999) PlantCARE, a plant cis-acting regulatory element database. *Nucl Acid Res* 27:295–296

Shikata M, Matsuda Y, Ando K, Nishii A, Takemura M, Yokota A, Kohchi T (2004) Characterization of *Arabidopsis* ZIM, a member of a novel plant-specific GATA factor gene family. *J Exper Bot* 55:631–639

Singh A, Singh S, Panigrahi KC, Reski R, Sarkar AK (2014) Balanced activity of microRNA166/165 and its target transcripts from the class III homeodomain-leucine zipper family regulates root growth in *Arabidopsis thaliana*. *Plant Cell Rep* 33:945–953

Singh R, Tiwari JK, Rawat S, Sharma V, Singh BP (2016) ‘In silico’ identification of candidate micro RNAs and their targets in potato somatic hybrid ‘*Solanum tuberosum*(+)’S. *pinnatisectum*’ for late blight resistance. *Plant Omics* 9:159–164

Solovyev VV, Shahmuradov IA, Salamov AA (2010) Identification of promoter regions and regulatory sites. *Computat Biol Transcrip Factor Bind* 57–83 p

Song C, Fang J, Li X, Liu H, Chao CT (2009) Identification and characterization of 27 conserved microRNAs in citrus. *Planta* 230:671–685

Stark A, Bushati N, Jan CH, Kheradpour P, Hodges E, Brennecke J, Bartel DP, Cohen SM, Kellis M (2008) A single Hox locus in *Drosophila* produces functional microRNAs from opposite DNA strands. *Genes Develop* 22:8–13

Sultana HS, Ali M, Panda BP (2012) Influence of volatile constituents of fruit peels of *Citrus reti-culata* Blanco on clinically isolated pathogenic micro-organisms under In-vitro. *Asian Pacif J Trop Biomed* 2:S1299–S1302

Tatematsu K, Nakabayashi K, Kamiya Y, Nambara E (2008) Transcription factor AtTCP14 regulates embryonic growth potential during seed germination in *Arabidopsis thaliana*. *Plant J* 53:42–52

Tumbas VT, Ćetković GS, Đilas SM, Čanadanović-Brunet JM, Vulić JJ, Knez Ž, Škerget M (2010) Antioxidant activity of mandarin (*Citrus reticulata*) peel. *Acta Period Technol* 195–203 p

Viveka A, Moossab F (2016) Identification of novel micro RNAs and their targets in *Cocos nucifera* – a bioinformatics approach. *Biosci Biotechnol Res Com* 9:481–488

Wang J, Chen Z, Zhang Q, Meng S, Wei C (2020) The NAC transcription factors OsNAC20 and OsNAC26 regulate starch and storage protein synthesis. *Plant Physiol* 184:1775–1791

Wang T, Chen L, Zhao M, Tian Q, Zhang W-H (2011) Identification of drought-responsive microRNAs in *Medicago truncatula* by genome-wide high-throughput sequencing. *BMC Genom* 12:1–11

Xia Z, Zhang X, Li J, Su X, Liu J (2014) Overexpression of a tobacco J-domain protein enhances drought tolerance in transgenic *Arabidopsis*. *Plant Physiol Biochem* 83:100–106

Xie F, Frazier TP, Zhang B (2010) Identification and characterization of microRNAs and their targets in the bioenergy plant switchgrass (*Panicum virgatum*). *Planta* 232:417–434

Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC (2005) Expression of *Arabidopsis* MIRNA genes. *Plant Physiol* 138:2145–2154

Yang C, Liu T, Bai F, Wang N, Pan Z, Yan X, Peng S (2015) miRNAome analysis associated with anatomical and transcriptomic investigations reveal the polar exhibition of corky split vein in boron deficient *Citrus sinensis*. *Mol Gen Genom* 290:1639–1657

Yu B, Bi L, Zheng B, Ji L, Chevalier D, Agarwal M,

- Ramachandran V, Li W, Lagrange T, Walker JC (2008) The FHA domain proteins DAWDLE in Arabidopsis and SNIP1 in humans act in small RNA biogenesis. *Proc Nat Acad Sci* 105:10073–10078
- Zhang B, Pan X, Cannon CH, Cobb GP, Anderson TA (2006) Conservation and divergence of plant micro RNA genes. *Plant J* 46:243–259
- Zhang C, Zhang B, Ma R, Yu M, Guo S, Guo L (2015) Isolation and expression analysis of four HD-ZIP III family genes targeted by microRNA166 in peach. *Genet Mol Res: GMR* 14:14151–14161
- Zhang W, Luo Y, Gong X, Zeng W, Li S (2009) Computational identification of 48 potato micro RNAs and their targets. *Computational Biol Chem* 33:84–93
- Zhang W, Ruan J, Ho T-hD, You Y, Yu T, Quatrano RS (2005) Cis-regulatory element based targeted gene finding: genome-wide identification of abscisic acid- and abiotic stress-responsive genes in *Arabidopsis thaliana*. *Bioinform* 21:3074–3081
- Zhang Y, Zhu X, Chen X, Song C, Zou Z, Wang Y, Wang M, Fang W, Li X (2014) Identification and characterization of cold-responsive microRNAs in tea plant (*Camellia sinensis*) and their targets using high-throughput sequencing and degradome analysis. *BMC Plant Biol* 14:1–18
- Zhu Q-H, Fan L, Liu Y, Xu H, Llewellyn D, Wilson I (2013) miR482 regulation of NBS-LRR defense genes during fungal pathogen infection in cotton. *PLoS One* 8:e84390
- Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucl Acid Res* 31:3406–3415.

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