

Short Communication

Volume 2 Issue 1 - April 2017
DOI: 10.19080/IJCSMB.2017.2.555579

Int J cell Sci & mol biol

Copyright © All rights are reserved by Sauren Das

In Silico screening of Putative miRNAs and their Targets from a Common Mangrove *Bruguiera gymnorhiza*



Nirjhar Dasgupta, Anjan Hazra, Sabyasachi Bhattacharya and Sauren Das*

Agricultural and Ecological Research Unit, Indian Statistical Institute, India

Submission: February 21, 2017; **Published:** April 17, 2017

***Corresponding author:** Sauren Das, Agricultural and Ecological Research Unit, Indian Statistical Institute, 203, Barrackpore Trunk Road, Kolkata 700 108, India, Email: sauen@isical.ac.in

Abstract

Micro RNAs, the non-coding single stranded molecules with 18-22 nucleotide sequences, have established its enormous involvement in biological processes including stress response. As the mangrove species restoration program are being conducted world-wide for their protecting and producing nature, investigation on miRNA would be a significant approach towards understanding the small RNA mediated gene regulation leading to plant adaptation. But insufficient molecular data of salt responsive genes and miRNAs have triggered to the present work which might enrich knowledge on adaptability with the rapid salinization of the habitat. The present work elucidate that the target genes for the two miRNAs bg-miR1029 and bg-miR5021 in *Bruguiera gymnorhiza* are involved in major stress response characteristics. Experimental validation and characterization of these two miRNAs and their predicted target genes along the salinity gradient is yet to be validated.

Keywords: *Bruguiera gymnorhiza*; miRNA; miRNA target; RNA secondary structure; Salt response

Introduction

Micro RNAs are small (~22 nt), single-stranded, and non-coding RNA molecules. The RNA Pol II mediates the transcription of capped and polyadenylated miRNA precursors (pri-miRNA). Mature miRNAs regulate a wide array of biological processes like development, metabolism, stress response, pathogen defense etc. [1,2]. Its mode of action involves its integration into the RNA-induced silencing complex (RISC) [3], which controls gene expression by hindering translation or by corrupting coding mRNAs through complementing with the target mRNAs [4,5]. More precisely, miRNAs cleave the target genes to prevent gene expression in plants [6]. Several recent findings are in congruence with the fact that miRNAs have significant role on the plant adaptability to salt stress [7-10].

Mangrove is a specialized group of plant community, growing in the coastal estuarine environments of the tropical and subtropical world, thrive under constant abiotic extremities [11]. They provide the coastal ecology with the first line of defense against the coastal calamity like sea storms, Hurricanes, Tsunamis etc. The enormous productive and protective

ecosystem that mangrove provide to both coastal environment and inhabitants, can be conservatively assessed to be worth about US\$186 million per year [12]. Mangroves have to expose regularly with periodic inundation, physiological stress, high salinity, high temperature and higher UV index [13]. As a result environmental factors (increased salinity level, inundation frequency, sea level rise and global warming) and anthropogenic activities (unplanned poaching, reclamation of mangrove area and siltation of river bed leading to less fresh water discharge in the estuary), mangrove forests all over the world are declining at an alarming level [14]. During the period of 1980-1990 this loss was estimated at 2.0% per year and within 1990-2000, 0.7% per year [12]. Mangrove restoration and conservation programs have been taken up by many countries, but it could be a fruitful effort if the genetic information of the plant species could be amalgamated, which is quiet meager [15].

In the present study, putative miRNAs precursor sequences has been fished out with their potential targets in a mangrove species *Bruguiera gymnorhiza*. The genetic information of

this taxa have been evaluated in many countries and hence prediction of miRNA loci can be used as a resourceful technique towards understanding its better salt adaptability [16] through molecular basis. As miRNA regulates gene expression through cleaving its targeted mRNA, the present work is pointing to the identification of miRNAs and their targets leading to understanding their possible roles in plant growth and development. The new miRNAs identified in this study would be worthwhile in understanding the complexity of miRNA-mediated genes network in various stress adaptability.

Materials and Method

Retrieval of data

Available 8442 known plant miRNA sequences were acquired from the publicly available miRNA database, miRBase [17]. The redundant sequences were eliminated using PRINSEQ version 0.20.4 [18]. The remaining 4766 miRNA sequences were used to search their homologs in the EST database of *Bruguiera gymnorhiza*. The available EST database of NCBI (National Centre for Biotechnology Information) are used for this study.

Identification of potential miRNAs in *Bruguiera gymnorhiza*

The sequences of all the above mentioned 4766 miRNA sequences were subjected to BLAST (Basic Local Alignment Search Tool), available on NCBI website, for alignment against the publicly available *Bruguiera gymnorhiza* EST database. The nucleotide match size between query and database was set to 15 with expectation value 0.01, match sequence less than this values are not taken into account. After checking redundancy, the selected ESTs are subjected to blastx [19] against NCBI non-redundant protein sequence collection to eliminate sequences which are protein-coding. RNAs such as tRNA, rRNA, snRNA, or snoRNA were eradicated by subjecting the ESTs to BLAST against NCBI nucleotide collection.

Prediction of RNA secondary structures

The selected candidates were then assessed for secondary structure using the mfold Web Server [20]. The default parameters were used for this study. Potential miRNAs were identified based on the following criteria - a) the position of the miRNA is on hairpin, b) minimum number of residues in miRNA should be 15, c) the maximum number of unpaired residues should be 6, d) the maximum number of G-U pairs in miRNA should be 5, e) the maximum size for a bulge in miRNA sequence should be 5nt, f) the negative minimal folding free energy (MFE) should be low, and g) the minimal folding free energy index (MFEI) should be high. Minimal folding free energy index (MFEI) = [(MFE/length of the RNA sequence) *100] / (G+C) %.

Prediction of potential miRNA targets

Potential targets for the predicted miRNAs from *Bruguiera gymnorhiza* were analyzed using psRNATarget web server

2017 version [21]. Since no miRNAs have been reported from *Bruguiera gymnorhiza* till now, potential target was searched against the database of *Arabidopsis thaliana*.

Result and Discussion

Previously reported 8442 mature miRNA sequences were used in this study to find their homologs in *Bruguiera gymnorhiza*, a mangrove species. Since their discovery, miRNAs have emerged as principal regulators in plant growth and development. miRNAs role in plant stress responses have arisen after the discovery that miR398 target genes have known role in stress responses [22]. In that context we have predicted the miRNAs utilizing *Bruguiera gymnorhiza* expressed sequence tag database.

The redundancy check decreased the total number of miRNA sequences to 4766. These sequences were used as query to find out their homologs present in the *Bruguiera gymnorhiza* EST database. BLAST result produced a total of 272 hits. These were the ESTs which aligned with the query miRNA sequences (Table 1). These ESTs were further subjected to BLAST to eliminate the protein coding sequences and non-coding RNAs. We finally got 14 sequences potential to be miRNA. RNA secondary structure predicting software mfold was used for this study. In the predicted structure the nucleotides that were homologous to the miRNAs were selected and inspected for the above mentioned 7 criterions. The criterion got matched for two ESTs. They were named as bg-miR1029 and bg-miR5021. Both of the sequences were 17 nucleotides long (Table 2). The software generated secondary structure (Figure 1) show -99.67 Kcal/mol minimal folding free energy (MFE) and -13.96 minimal folding free energy index (MFEI). Second one (Figure 1) show -41.1Kcal/mol minimal folding free energy (MFE) and - 8.34 minimal folding free energy index (MFEI).

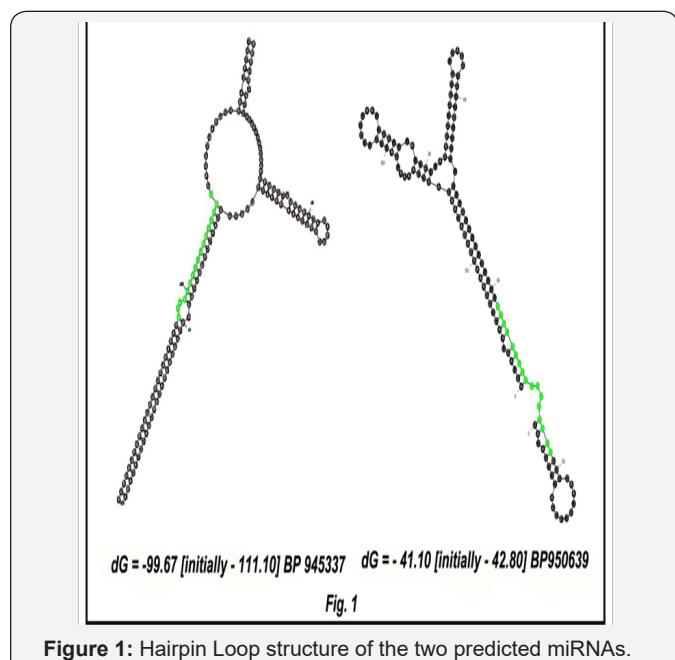


Figure 1: Hairpin Loop structure of the two predicted miRNAs.

Table 1: Predicted Target sequences for the two miRNAs.

miRNA_Acc.	Target_Acc.	Expec tation	UPES	miRNA_ start	miRNA_ end	Target_ start	Target_ end	miRNA_aligned_fragment	Target_aligned_fragment	Inhibition	Target_Desc.	Multiplicity
bg-miR5021	AT2G28100.1	0	-1	1	20	160	179	UGAGAAGAGAAGAAAA	UUUCUCUUCUCUCUCUCA	Cleavage	Symbols: ATFLUC1, FLUC1 alpha-L-fucosidase 1 chr2:11974663-11976600 FORWARD LENGTH=1772	1
bg-miR5021	AT1G30650.1	0	-1	1	20	91	110	UGAGAACAGAGAGAAA	CUUUCUCUUCUCUCUCA	Cleavage	Symbols: WRKY14, ATWRKY14, AR411 WRKY DNA-binding protein 14 chr1:10868303-10871297 FORWARD LENGTH=1926	1
bg-miR5021	AT4G36950.1	1	-1	1	20	29	48	UGAGAACAGAGAGAAA	UUUCUCUUCUCUCUCCU	Cleavage	Symbols: MAPKKK21 mitogen-activated protein kinase kinase kinase 21 chr4:17422572-17423901 REVERSE LENGTH=1330	1
bg-miR5021	AT1G70900.1	1	-1	1	20	6	25	UGAGAACAGAGAGAAA	UUUCUCUUCUCUCUCC	Cleavage	Symbols: unknown protein, BEST Arabidopsis thaliana protein match is: unknown protein (TAIR1AT1G23110.4); Has 57 Blast hits to 57 proteins in 14 species: Archae - 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 57; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLink) chr1:26731B13-26733875 FORWARD LENGTH=1191	1
bg-miR5021	AT3G22950.1	1	-1	1	20	94	113	UGAGAACAGAGAGAAA	UUUCUCUUCUCUCUCCU	Cleavage	Symbols: ATARFC1, ARFC1 ADP-ribosylation factor C1 chr3:8136162-8138084 REVERSE LENGTH=989	1
bg-miR5021	AT3G22950.2	1	-1	1	20	94	113	UGAGAACAGAGAGAAA	UUUCUCUUCUCUCUCCU	Cleavage	Symbols: ARFC1 ADP-ribosylation factor C1 chr3:8136181-8138084 REVERSE LENGTH=973	1
bg-miR5021	AT1G28420.1	1	-1	1	20	111	130	UGAGAACAGAGAGAAA	GUUCUCUUCUCUCUCCU	Cleavage	Symbols: HB-1 homeobox-1 chr1:997802-9987538 FORWARD LENGTH=5430	1
bg-miR5021	AT5G56240.2	1	-1	1	20	322	341	UGAGAACAGAGAGAAA	CUUUCUCUUCUCUCUCC	Cleavage	Symbols: INVOLVED IN: biological process un known; EXPRESSED IN: 22 plant structures; EXPRESSED DURING: 13 growth stages; BEST Arabidopsis thaliana protein match is: hapless 8 (TAIR:AT5G56250.1); chr5:22759805-22764587 REVERSE LENGTH=3532	1

bg-miR5021	AT5G56240.1	1	-1	1	20	311	330	UGAGAAAAGAAGAAAGAAAA	CUUUCUUCUUCUUCUUCUCC	Cleavage	Symbols: BEST Arabidopsis thaliana protein match is: hapless 8 (TAR-AT5G56250.1); Has 35333 Blast hits to 34/131 proteins in 2444 species; Archae- 798; Bacteria - 22429; Metazoa- 974; Fungi- 991; Plants- 531; Viruses- 0; Other Eukaryotes- 9610 (source: NCBI Blink); chr5:22759803-22764576 REVERSE LENGTH=3520	1
bg-miR5021	AT5G57870.1	1	-1	1	20	43	62	UGAGAAAAGAAGAAAGAAAA	ACUUCUUCUUCUUCUUCUCA	Cleavage	Symbols: eIF4G1 MIF4G domain-containing protein / MA3 domain-containing protein chr5:23439500-23443781 FORWARD LENGTH=2946	1
bg-miR5021	AT5G57870.2	1	-1	1	20	45	64	UGAGAAAAGAAGAAAGAAAA	ACUUCUUCUUCUUCUUCUCA	Cleavage	Symbols: eIF4G1 MIF4G domain-containing protein / MA3 domain-containing protein chr5:23439500-23443781 FORWARD LENGTH=2946	1
bg-miR5021	AT2G41900.1	1	-1	1	20	45	64	UGAGAAAAGAAGAAAGAAAA	AUUUCUUCUUCUUCUUCU	Cleavage	Symbols: CCCH-type zinc finger protein with ARM repeat domain chr2:17490405-17493922 FORWARD LENGTH=2887	1
bg-miR5021	AT5G67030.1	1	-1	1	20	26	45	UGAGAAAAGAAGAGAAAA	AUUUCUUCUUCUUCUUCU	Cleavage	Symbols: ABA1, LOS6_NPQ2, ATABA1, ZEP, IBS3, ATZEP zeaxanthin epoxidase (ZEP)(ABA1) chr5:26753522-26757236 REVERSE LENGTH=2385	1
bg-miR5021	AT5G67030.2	1	-1	1	20	26	45	UGAGAAAAGAAGAGAAAA	AUUUCUUCUUCUUCUUCU	Cleavage	Symbols: ABA1, LOS6_NPQ2, ATABA1, ZEP, IBS3, ATZEP zeaxanthin epoxidase (ZEP)(ABA1) chr5:26753522-26757236 REVERSE LENGTH=2385	1
bg-miR5021	AT3G12080.1	1	-1	1	20	20	39	UGAGAAAAGAAGAAAA	AUUUCUUCUUCUUCUUCU	Cleavage	Symbols: emb2738_GTP-binding family protein chr3:3847792-3852138 FORWARD LENGTH=2209	1
bg-miR5021	AT3G12080.2	1	-1	1	20	20	39	UGAGAAAAGAAGAAAA	AUUUCUUCUUCUUCUUCU	Cleavage	Symbols: emb2738_GTP-binding family protein chr3:3847792-3852138 FORWARD LENGTH=2205	1
bg-miR5021	AT2G19600.1	1	-1	1	20	154	173	UGAGAAAAGAAGAAAA	CUUUCUUCUUCUUCUCC	Cleavage	Symbols: ATKE44, KE44_K+ efflux transporter 4 chr2:8479198-848330 FORWARD LENGTH=2204	1
bg-miR5021	AT2G19450.1	1	-1	1	20	14	33	UGAGAAAAGAAGAAAA	CUUUCUUCUUCUUCUCC	Cleavage	Symbols: TAG1, AS11, ABX45, DGAT1, RDS1, ATDGAT membrane bound O-acetyltransferase (MBOAT) family protein chr2:8479260-8429736 FORWARD LENGTH=2074	1
bg-miR5021	AT3G21070.1	1	-1	1	20	43	62	UGAGAAAAGAAGAAAA	CUUUCUUCUUCUUCUCC	Cleavage	Symbols: NADK1, ATNADK-1 NAD kinase 1 chr3:7380191-738380 REVERSE LENGTH=1942	1
bg-miR5021	AT3G21070.2	1	-1	1	20	43	62	UGAGAAAAGAAGAAAA	CUUUCUUCUUCUUCUCC	Cleavage	Symbols: NADK1, NAD kinase 1 chr3:7380191-738380 REVERSE LENGTH=1924	1

bg-miR5021	AT1G67410.1	1	-1	1	20	57	76	UGAGAAGAAGAAGAAGAAAA	CUUUCUUCUUCUUCUUCU	Cleavage	Symbols: Exostosin family protein chr1:25251193-25253667 REVERSE LENGTH=1805	1
bg-miR5021	AT5G67420.1	1	-1	1	20	494	513	UGAGAAGAAGAAGAAGAAA	CUUUCUUCUUCUUCUCC	Cleavage	Symbols: LBD37, ASL39 LOB domain-containing protein 37/ ch5:2690489-26906026 REVERSE LENGTH=1651	1
bg-miR5021	AT3G25110.1	1	-1	1	20	174	193	UGAGAAGAAGAAGAAGAAAA	GUUUCUUCUUCUUCUCC	Cleavage	Symbols: AtFtA, FtA ftA, acyl-ACP thioesterase chr3:9146271-9148486 REVERSE LENGTH=1620	1
bg-miR5021	AT3G48070.1	1	-1	1	20	43	62	UGAGAAGAAGAAGAAGAAA	GUUUCUUCUUCUUCUCC	Cleavage	Symbols: RING/U-box superfamily protein chr3:17750711-17752416 FORWARD LENGTH=1565	1
bg-miR5021	AT2G27701	1	-1	1	20	141	160	UGAGAAGAAGAAGAAGAAA	CAUUCUUCUUCUUCUCA	Cleavage	Symbols: Plant protein of unknown function (DUF868) chr2:1832809-1183465 REVERSE LENGTH=1561	1
bg-miR5021	AT4G32590.1	1	-1	1	20	202	221	UGAGAAGAAGAAGAAGAAA	UCUUCUUCUUCUUCUCA	Cleavage	Symbols: 2Fe-2S ferredoxin-like superfamily protein chr4:15721324-15722908 FORWARD LENGTH=747	1
bg-miR5021	AT4G32590.3	1	-1	1	20	202	221	UGAGAAGAAGAAGAAGAAA	UCUUCUUCUUCUUCUCA	Cleavage	Symbols: 2Fe-2S ferredoxin-like superfamily protein chr4:15721324-15722869 FORWARD LENGTH=724	1
bg-miR5021	AT4G32590.4	1	-1	1	20	202	221	UGAGAAGAAGAAGAAGAAA	UCUUCUUCUUCUUCUCA	Cleavage	Symbols: 2Fe-2S ferredoxin-like superfamily protein chr4:15721324-15722869 FORWARD LENGTH=712	1
bg-miR5021	AT3G48070.2	1	-1	1	20	42	61	UGAGAAGAAGAAGAAAA	GUUUCUUCUUCUUCUCA	Cleavage	Symbols: RING/U-box superfamily protein chr3:17750712-17752761 FORWARD LENGTH=1307	1
bg-miR5021	AT4G32590.2	1	-1	1	20	183	202	UGAGAAGAAGAAGAAAA	UCUUCUUCUUCUUCUCA	Cleavage	Symbols: 2Fe-2S ferredoxin-like superfamily protein chr4:15721343-15722832 FORWARD LENGTH=646	1
bg-miR5021	AT1G28070.1	1	-1	1	20	33	52	UGAGAAGAAGAAGAAAA	GUUUCUUCUUCUUCUCC	Cleavage	Symbols: unknown protein; BEST Arabidopsis thaliana protein match is; unknown protein (TAIR:AT2G33510.1); Has 85 Blast hits to 77 proteins in 15 species: Archae- 0; Bacteria - 0; Metazoa - 0; Fungi - 0; Plants - 85; Viruses - 0; Other Eukaryotes - 0 (source: NCBI Blink); chr1:9782996-9783998 FORWARD LENGTH=751	1
bg-miR5021	AT1G14200.1	1.5	-1	1	20	20	39	UGAGAAGAAGAAGAAAA	UUUCUUCUUCUUCUUA	Cleavage	Symbols: RING/U-box superfamily protein chr1:4854384-4885200 REVERSE LENGTH=817	1

bg-miR5021	AT3G21320.1	1.5	-1	1	20	291	310	UGAGAACAGAGAGAAAA	CUUUCUUCUUCUUCUUA	Cleavage	Symbols: BEST Arabidopsis thaliana protein match is: hydroxyproline-rich glycoprotein family protein (TAIR:AT2G25930.1); Has 631 Blast hits to 58 proteins in 21 species: Archaea - 0; Bacteria - 0; Metazoa - 3; Fungi - 0; Plants - 58; Viruses - 0; Other Eukaryotes - 2 (source: NCBI BLINK); LENGTH=2419
bg-miR5021	AT1G55570.1	1.5	-1	1	20	1982	2001	UGAGAAGAGAGAGAAAA	uuuucuucuucuucuua	Cleavage	Symbols: SKU5 similar to 12 chr1:20757767-20760076 FORWARD LENGTH=2088
bg-miR5021	AT2G18910.1	1.5	-1	1	20	128	147	UGAGAAGAGAGAGAAAA	cuuucuucuucuucuua	Cleavage	Symbols: hydroxyproline-rich glycoprotein family protein chr2:812708-8194338 REVERSE LENGTH=911
bg-miR5021	AT3G51550.1	2	-1	1	20	177	197	UGAGAAGAGAGAGAA-	uucuucuucuucuucuua	Cleavage	Symbols: Malectin/receptor-like protein kinase family protein chr3:1917594-19120881 REVERSE LENGTH=3123
bg-miR5021	AT4G05020.2	2	-1	1	20	85	105	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: NDB2 NAD(PH) dehydrogenase B2 chr4:2572621-2576595 FORWARD LENGTH=2364
bg-miR5021	AT4G05020.1	2	-1	1	20	85	105	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: NDB2 NAD(PH) dehydrogenase B2 chr4:2572621-2576595 FORWARD LENGTH=2253
bg-miR5021	AT5G24670.2	2	-1	1	20	140	160	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: Cytidine/ deoxyctidylate deaminase family protein chr5:8447954-8451078 FORWARD LENGTH=1800
bg-miR5021	AT3G06660.1	2	-1	1	20	110	130	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: PAP-1 like family protein / zinc finger (HTF type) protein chr3:2102402-2104906 FORWARD LENGTH=1755
bg-miR5021	AT1G73740.1	2	-1	1	20	29	49	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: UDP-Glycosyltransferase superfamily protein chr1:27734279-27736189 FORWARD LENGTH=1649
bg-miR5021	AT5G26670.1	2	-1	1	20	25	45	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: Pectinacetyl esterase family protein chr5:9318282-9320987 FORWARD LENGTH=1596
bg-miR5021	AT5G18760.1	2	-1	1	20	173	193	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: RING-U-box superfamily protein chr5:6258295-6260929 FORWARD LENGTH=1550
bg-miR5021	AT5G26670.2	2	-1	1	20	27	47	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: Pectinacetyl esterase family protein chr5:9318280-9320991 FORWARD LENGTH=1519
bg-miR5021	AT1G53650.1	2	-1	1	20	55	75	UGAGAAGAGAGAGAA-	uuucuucuucuucuuucuca	Cleavage	Symbols: CIDB CTC-interacting domain 8 chr1:20029029-20031566 REVERSE LENGTH=1501

bg-miR5021	AT1G50780.1	2	-1	1	20	507	UGAGAAGAGAAGAGAA- AA	uuucuucuucuucuuucuca	Cleavage	Symbols: 2Fe-2S ferredoxin-like superfamily protein chr1:18821267-18823077 FORWARD LENGTH=972	1
bg-miR5021	AT1G76460.1	2	-1	1	20	238	UGAGAAGAGAAGAAAA	acuuucuucuucuucuucu	Cleavage	Symbols: RNA-binding (RRM/RBD/RNP motifs) family protein chr1:28666181-286889121 REVERSE LENGTH=2123	1
bg-miR5021	AT1G74410.1	2	-1	1	20	153	UGAGAAGAGAAGAAAA	uuuuuuucuucuucuucu	Cleavage	Symbols: RING/U-box superfamily protein chr1:2796551-27967926 FORWARD LENGTH=1089	1
bg-miR5021	AT4G38600.1	2	-1	1	20	49	UGAGAAGAGAAGAAAA	ucuucuucuucuucuucucc	Cleavage	Symbols: KAK, UPL3 HEAT repeat (HETC-domain (ubiquitin-transferase) chr4:18041031-18049252 REVERSE LENGTH=6139	2
bg-miR5021	AT4G38600.2	2	-1	1	20	9	UGAGAAGAGAAGAAAA	ucuuucuucuucuucucc	Cleavage	Symbols: KAK, UPL3 HEAT repeat (HETC-domain (ubiquitin-transferase) chr4:18041031-18049252 REVERSE LENGTH=5954	2
bg-miR5021	AT3G24550.1	2	-1	1	20	88	UGAGAAGAGAAGAAAA	uucucuucuucuucuucug	Cleavage	Symbols: ATPERK1, PERK1 proline extensin-like receptor kinase 1 chr3:8960258-8963568 FORWARD LENGTH=2377	1
bg-miR5021	AT1G80370.1	2.5	-1	1	20	94	UGAGAAGAGAAGAAAA	uuuuuuucuucuucuucung	Cleavage	Symbols: CYCA2:4 Cyclin A2:4 chr1:30213705-30216861 FORWARD LENGTH=1588	1
bg-miR5021	AT1G19980.1	2.5	-1	1	20	70	UGAGAAGAGAAGAAAA	uuuuuuucuucuucuucuua	Cleavage	Symbols: cytomatrix protein-related (chr1:6933016-6935002 FORWARD LENGTH=1534	1
bg-miR5021	AT4G00440.1	2.5	-1	1	20	536	UGAGAAGAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Protein of unknown function (DUF3741) chr4:193879-198579 FORWARD LENGTH=3802	1
bg-miR5021	AT4G00440.2	2.5	-1	1	20	536	UGAGAAGAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Protein of unknown function (DUF3741) chr4:193879-198579 FORWARD LENGTH=3708	1
bg-miR5021	AT4G00440.3	2.5	-1	1	20	536	UGAGAAGAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Protein of unknown function (DUF3741) chr4:193879-198579 FORWARD LENGTH=3703	1
bg-miR5021	AT4G24480.1	2.5	-1	1	20	17	UGAGAAGAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Protein kinase superfamily protein chr4:12649987-12654984 FORWARD LENGTH=3523	1
bg-miR5021	AT3G23900.3	2.5	-1	1	20	2	UGAGAAGAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: RNA recognition motif (RRM)-containing protein chr3:863569-8636059 REVERSE LENGTH=3446	1

bg-miR5021	AT4G35790.3	2.5	-1	1	20	248	267	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: ATP1DDETA, P1DDETA phosphotipase D delta chr4:169555518-16960176 REVERSE LENGTH=3264	1
bg-miR5021	AT4G35790.1	2.5	-1	1	20	248	267	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: ATP1DDETA, P1DDETA phosphotipase D delta chr4:169555518-16960176 REVERSE LENGTH=3158	1
bg-miR5021	AT4G35790.2	2.5	-1	1	20	248	267	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: ATP1DDETA, P1DDETA phosphotipase D delta chr4:169555518-16960176 REVERSE LENGTH=3125	1
bg-miR5021	AT3G22150.1	2.5	-1	1	20	53	72	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: Tetra-tricopeptide repeat (TPR)-like superfamily protein chr3:781294-7815928 FORWARD LENGTH=2985	1
bg-miR5021	AT3G13460.1	2.5	-1	1	20	218	237	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUU	Cleavage	Symbols: Evolutionarily conserved C-terminal region 2 chr3:4384714-4388501 REVERSE LENGTH=2869	1
bg-miR5021	AT3G13460.3	2.5	-1	1	20	194	213	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUU	Cleavage	Symbols: ECT2 evolutionarily conserved C-terminal region 2 chr3:4384714-4388501 REVERSE LENGTH=2847	1
bg-miR5021	AT3G13460.4	2.5	-1	1	20	170	189	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUU	Cleavage	Symbols: ECT2 evolutionarily conserved C-terminal region 2 chr3:4384714-4388477 REVERSE LENGTH=2848	1
bg-miR5021	AT3G13460.2	2.5	-1	1	20	170	189	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUU	Cleavage	Symbols: ECT2 evolutionarily conserved C-terminal region 2 chr3:4384714-4388477 REVERSE LENGTH=2842	1
bg-miR5021	AT1G03190.1	2.5	-1	1	20	25	44	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: ATXPD, UHVH6 RA33-like DNA-binding helicase protein chr1:775527-78027 FORWARD LENGTH=2578	1
bg-miR5021	AT3G07810.1	2.5	-1	1	20	204	223	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: RNA-binding (RRM/ RBD/RNP motifs) family protein chr3:2492273-2495750 FORWARD LENGTH=2518	1
bg-miR5021	AT3G07810.2	2.5	-1	1	20	184	203	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: RNA-binding (RRM/ RBD/RNP motifs) family protein chr3:2492293-2495750 FORWARD LENGTH=2451	1
bg-miR5021	AT2G16750.1	2.5	-1	1	20	2140	2159	UGAGAAGAAGAGAAGAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: Protein kinase protein with adenine nucleotide alpha-hydrolases-like domain chr2:7271012-7274693 FORWARD LENGTH=2428	1

bg-miR5021	AT3G20770.1	2.5	-1	1	20	70	89	UGAGAAGAAGAAGAAGAAAA	uuuuucuucucuucuucuuc	Cleavage	Symbols: EIN3, ATEIN3 Ethylene insensitive 3 family protein chr3:7260432-7263352 REVERSE LENGTH=2413	1
bg-miR5021	AT4G32250.3	2.5	-1	1	20	1	22	UGAGAAGAAGAAGAA- -AA	uuuccuucuucuucuucuca	Cleavage	Symbols: Protein kinase superfamily protein chr4:15570044-15573325 REVERSE LENGTH=2223	1
bg-miR5021	AT3G60680.1	2.5	-1	1	20	239	258	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Plant protein of unknown function (DUF641) chr3:72429960-72432042 FORWARD LENGTH=2083	1
bg-miR5021	AT3G26370.1	2.5	-1	1	20	1883	1902	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: O-fucosyltransferase family protein chr3:3956744-16925935 FORWARD LENGTH=2010	1
bg-miR5021	AT2G40520.4	2.5	-1	1	20	1831	1850	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Nucleotidyltransferase family protein chr2:16923318-16925914 FORWARD LENGTH=1971	1
bg-miR5021	AT2G40520.1	2.5	-1	1	20	1777	1796	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Nucleotidyltransferase family protein chr2:16923068-16925914 FORWARD LENGTH=1917	1
bg-miR5021	AT3G60520.2	2.5	-1	1	20	1767	1786	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT5G66480.1); Has 76 Blast hits to 75 proteins in 28 species: Archae - 0; Bacteria - 10; Metazoa - 7; Fungi - 2; Plants - 49; Viruses - 0; Other Eukaryotes - 8 (source: NCBI BlinR) chr3:18919513-18922238 FORWARD LENGTH=1861	1
bg-miR5021	AT3G50910.1	2.5	-1	1	20	104	123	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT5G66480.1); Has 76 Blast hits to 75 proteins in 28 species: Archae - 0; Bacteria - 10; Metazoa - 7; Fungi - 2; Plants - 49; Viruses - 0; Other Eukaryotes - 8 (source: NCBI BlinR) chr3:18919513-18922238 FORWARD LENGTH=1861	1
bg-miR5021	AT2G40520.3	2.5	-1	1	20	1691	1710	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Nucleotidyltransferase family protein chr2:16923350-16925914 FORWARD LENGTH=1831	1
bg-miR5021	AT3G48160.2	2.5	-1	1	20	1530	1549	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: DEL1, E213, E22E DPE-E2F-like 1 chr3:1778331-17786099 FORWARD LENGTH=1775	2
bg-miR5021	AT1G17940.1	2.5	-1	1	20	63	82	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: Endosomal targeting protein chr1:16169341-617322 REVERSE LENGTH=1734	1
bg-miR5021	AT2G01290.1	2.5	-1	1	20	18	37	UGAGAAGAAGAAGAAAA	uuuuucuucuucuucuuc	Cleavage	Symbols: RP12 ribose-5-phosphate isomerase 2 chr2:148919-150382 REVERSE LENGTH=1464	1

bg-miR5021	AT1G05800.1	2.5	-1	1	20	176	197	UGAGAAAGAGAGAGAA- .AA	uccuuucucucuucuuucuca	Cleavage	Symbols: DGL alpha/beta-hydrolases superfamily protein chr1:1741204-1742619 FORWARD LENGTH=1416	1
bg-miR5021	AT3G10260.3	2.5	-1	1	20	151	170	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: Reticulon family protein chr3:3171222-3172959 REVERSE LENGTH=1386	1
bg-miR5021	AT5G38220.3	2.5	-1	1	20	9	28	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: alpha/beta-hydrolases superfamily protein chr5:15268827-15271386 FORWARD LENGTH=1361	1
bg-miR5021	AT3G15140.1	2.5	-1	1	20	224	243	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: Polyubiquitin transferase; ribonuclease H-like superfamily protein chr3:509532-5101781 REVERSE LENGTH=1258	1
bg-miR5021	AT1G16060.1	2.5	-1	1	20	27	46	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: ADAP ARIA-interacting double AP2 domain protein chr5:5508507-5511735 FORWARD LENGTH=1220	1
bg-miR5021	AT3G10260.1	2.5	-1	1	20	158	177	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: Reticulon family protein chr3:3171222-3172966 REVERSE LENGTH=1130	1
bg-miR5021	AT5G25810.1	2.5	-1	1	20	528	547	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: tiny Integrase-type DNA-binding superfamily protein chr5:8986771-8987787 REVERSE LENGTH=1017	1
bg-miR5021	AT4G38600.1	2.5	-1	1	20	121	140	UGAGAAAGAGAGAGAAA	cuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: KAK, UPL3, HEAT repeat; HECT-domain (ubiquitin-transferase) chr4:18041031-18049292 REVERSE LENGTH=6139	2
bg-miR5021	AT4G38600.2	2.5	-1	1	20	81	100	UGAGAAAGAGAGAGAAA	cuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: KAK, UPL3, HEAT repeat; HECT-domain (ubiquitin-transferase) chr4:18041031-18049252 REVERSE LENGTH=5954	2
bg-miR5021	AT5G66440.1	2.5	-1	1	20	4	23	UGAGAAAGAGAGAGAAA	uuuuuuuuuuuuuuuuuuuuuu	Cleavage	Symbols: unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT4G34560.1); Has 1807 Blast hits to 1807 proteins in 277 species; Archae - 0; Bacteria - 0; Metazoa - 736; Fungi - 347; Plants - 385; Viruses - 0; Other Eukaryotes - 339 (source: NCBI Blink). chr5:26530235-26531097 REVERSE LENGTH=863	1

bg-miR5021	AT5G10946.1	2.5	-1	1	20	20	39	UGAGAAGAAAGAAGAAAA	UUUUCUUCUUCUUCUUCUUC	Cleavage	Symbols: unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: endomembrane system; Has 30201 Blast hits to 1732 proteins in 780 species; Archaea - 12; Bacteria - 1396; Metazoa - 17338; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996 (source: NCBI BLink) chr5:3455607-3457306 FORWARD LENGTH=833
bg-miR5021	AT1G51355.1	2.5	-1	1	20	552	571	UGAGAAGAAAGAAGAAAA	UUUUUCUUCUUCUUCUUC	Cleavage	Symbols: unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: chloroplast; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT3G20899) Has 52 Blast hits to 52 proteins in 9 species: Archaea - 0; Bacteria - 0; Metazoa - 2; Fungi - 0; Plants - 50; Viruses - 0; Other Eukaryotes - 0 (source: NCBI BLink) chr1:19041397-19042146 FORWARD LENGTH=750
bg-miR5021	AT5G49680.2	2.5	-1	1	20	640	659	UGAGAAGAAAGAAGAAAA	UCUUCUUCUUCUUCUUCUCA	Cleavage	Symbols: Golgi-body localisation protein domain; RNA pol II promoter Fnp27 protein domain chr5:20176385-2018307 FORWARD LENGTH=7764
bg-miR5021	AT4G32600.1	2.5	-1	1	20	155	174	UGAGAAGAAAGAAGAAAA	UUUUUCUUCUUCUUCUUCUGC	Cleavage	Symbols: RING/U-box superfamily protein chr4:157234-15726109 FORWARD LENGTH=2027
bg-miR5021	AT2G47820.1	2.5	-1	1	20	58	77	UGAGAAGAAAGAAGAAAA	GUUUCUUCUUCUUCUUCUUG	Cleavage	Symbols: unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT1G09040.1) Has 628 Blast hits to 543 proteins in 149 species: Archaea - 0; Bacteria - 106; Metazoa - 145; Fungi - 69; Plants - 97; Viruses - 10; Other Eukaryotes - 201 (source: NCBI BLink) chr2:19587408-19590822 FORWARD LENGTH=2845
bg-miR5021	AT5G40300.1	2.5	-1	1	20	397	416	UGAGAAGAAAGAAGAAAA	UUUUUCUUCUUCUUCUAC	Cleavage	Symbols: Uncharacterised protein family (UPF0497) chr5:1610088-16112968 FORWARD LENGTH=1170
bg-miR5021	AT1G77680.1	2.5	-1	1	20	80	99	UGAGAAGAAAGAAGAAAA	UUUUCAUCUUCUUCUUA	Cleavage	Symbols: Ribonuclease III/R family protein chr1:29191834-29196287 FORWARD LENGTH=3846
bg-miR5021	AT3G25660.1	2.5	-1	1	20	1908	1927	UGAGAAGAAAGAAGAAAA	CUUUCUUCUUCUUCUUCUG	Cleavage	Symbols: Amidase family protein chr3:9339423-9342204 FORWARD LENGTH=1991
bg-miR5021	AT2G21320.1	2.5	-1	1	20	31	50	UGAGAAGAAAGAAGAAAA	CUUUCUUCUUCUUCUUC	Cleavage	Symbols: B-box zinc finger family protein chr2:9126263-912843 FORWARD LENGTH=949

bg-miRS021	AT1G04310.1	2.5	-1	1	20	212	231	UGAGAAGAAGAAGAGAAAA	UCUUCUUCUCUCUCUUCUA	Cleavage	Symbols: ERS2 ethylene response sensor 2 chr1:1154764-1157711 REVERSE LENGTH=2470	1
bg-miRS021	AT1G69960.1	2.5	-1	1	20	42	61	UGAGAAGAAGAAGAGAAAA	UCUUCUUCUCUCUCUUCA	Cleavage	Symbols: Chaperone Dual-domain superfamily protein chr1:25964468-25967450 FORWARD LENGTH=2309	1
bg-miRS021	AT1G20970.1	3	-1	1	20	59	79	UG- AGAGAGAGAGAGAAAA	UUUCUCUUCUCUCUCUUCUA	Cleavage	Symbols: FUNCTION IN: molecular function unknown; INVOLVED IN: biological process unknown; LOCATED IN: plasma membrane; vacuole; EXPRESSED IN: guard cell; cultured cell; BEST Arabidopsis thaliana protein match is: proton pump interactor 1 (TAIR:AT4G27500.1); Has 3021 Blast hits to 17322 proteins in 780 species; Arthropoda - 12; Bacteria - 1396; Metazoa - 1733B; Fungi - 3422; Plants - 5037; Viruses - 0; Other Eukaryotes - 2996; Source: NCBI Blink chr1:7313946-7319574 FORWARD LENGTH=4673	1
bg-miR5021	AT2G1910.1	3	-1	1	20	115	135	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: SLP3 subtilisin-like serine protease 3 chr2:8314005-8318596 REVERSE LENGTH=2906	1
bg-miR5021	AT1G33980.2	3	-1	1	20	54	74	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: ATUPF3; UPF3 Smg-4; UPF3 family protein chr1:12351593-12355023 FORWARD LENGTH=2126	1
bg-miR5021	AT1G33980.1	3	-1	1	20	54	74	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: ATUPF3; UPF3 Smg-4; UPF3 family protein chr1:12351593-12355023 FORWARD LENGTH=2120	1
bg-miR5021	AT5G25040.2	3	-1	1	20	14	34	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: Major facilitator superfamily protein chr5:8621954-8624337 FORWARD LENGTH=2017	1
bg-miR5021	AT5G25040.3	3	-1	1	20	14	34	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: Major facilitator superfamily protein chr5:8621954-8624337 FORWARD LENGTH=1845	1
bg-miR5021	AT5G25040.1	3	-1	1	20	14	34	UG- AGAGAGAGAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: Major facilitator superfamily protein chr5:8621954-8624337 FORWARD LENGTH=1837	1
bg-miR5021	AT3G16850.1	3	-1	1	20	81	101	UGA- GAAGAAGAAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: Pectin lyase-like superfamily protein chr3:5748570-5751297 FORWARD LENGTH=1806	1
bg-miR5021	AT2G61735.1	3	-1	1	20	106	126	UGA- GAAGAAGAAGAAAA	UUUCUCUUCUCUCUCA	Cleavage	Symbols: RUE1 RING-finger protein for embryogenesis chr2:334472-336094 FORWARD LENGTH=1306	1

bg-miR5021	AT2G13770.1	3	-1	1	20	76	96	UG- AGAAGAAGAAAGAAAA	uuuucuuucuuucuuuuuca	Cleavage	Symbols: CONTAINS InterPro DOMAIN/s: Putative harbinger transposase-derived nuclelease (InterPro:IPR006912); Has 451 Blast hits to 352 proteins in 33 species; Archive: 0; Bacteria - 0; Metazoa - 28; Fungi - 8; Plants - 388; Viruses: 0; Other Eukaryotes - 27 (source: NCBI BLink) chr2:5736570-5737847 FORWARD LENGTH=1065	1
bg-miR5021	AT1G71970.1	3	-1	1	20	52	72	UGA- GAAGAAGAAAGAAAA	uuuucuuucuuucuuuccuca	Cleavage	Symbols: unknown protein; FUNCTIONS IN: molecular function unknown; INVOLVED IN: biological process unknown; LOCATED IN: chloroplast; EXPRESSED IN: plant structures; EXPRESSED DURING: 13 growth stages; BEST match is: unknown protein (TAIR:AT1G22680.1); Has 58 Blast hits to 58 proteins in 13 species; Archae - 0; Bacteria - 0; Metazoa - 4; Fungi - 0; Plants - 49; Viruses - 0; Other Eukaryotes - 5 (source: NCBI BLink); (chr1:27093028-27094027 FORWARD LENGTH=1000	1
bg-miR5021	AT3G48160.2	3	-1	1	20	1556	1576	UGAGAAGAAGAGAA- AA	uucuuucuuucuucucuucu	Cleavage	Symbols: DEIL1, E2L13, E2FE DR-E2F-like 1 chr3:1778331-1778609 FORWARD LENGTH=1775	1
bg-miR5021	AT4G02235.1	3	-1	1	20	16	36	UGA- GAAGAAGAAAGAAAA	uuuucuuucuuucuucuuguca	Cleavage	Symbols: AGL51 ACAMOUS-like 51 chr4:980955-98171 FORWARD LENGTH=591	1
bg-miR5021	AT5G11390.1	3	-1	1	20	15	35	UGAGAAGAAGAGAGAA- AA	uucuuucuuucuucuucuug	Cleavage	Symbols: WIT1 WPP domain-interacting protein 1 domain-interacting protein 1 chr5:3633667-3634570 FORWARD LENGTH=2387	2
bg-miR5021	AT2G46830.1	3	-1	1	20	79	99	UGAGAAGAAGAGAA- AA	uucuuucuuucuucuucuug	Cleavage	Symbols: CCA1 circadian clock associated 1 chr2:19245672-19248909 FORWARD LENGTH=2268	1
bg-miR5021	AT2G34500.1	3	-1	1	20	126	146	UGAGAAGAAGAGAA- AA	uucuuucuuucuucuucuug	Cleavage	Symbols: CYP710A1 cytochrome P450, family 710, subfamily A, polypeptide 1 chr2:14539479-14541406 FORWARD LENGTH=1928	1
bg-miR5021	AT5G66320.1	3	-1	1	20	146	166	UGAGAAGAAGAGAA- AA	uucuuucuuucuucuucuug	Cleavage	Symbols: GATA5 GATA transcription factor 5 chr5:26495955-26497835 FORWARD LENGTH=1494	1
bg-miR5021	AT3G23890.1	3	-1	1	20	45	65	UGAGAAGAAGAGAA- AA	wucuuucuuucuucuucuuc	Cleavage	Symbols: TOPIL, ATTOPIL topoisomerase II chr3:8624750-8631327 FORWARD LENGTH=4824	1
bg-miR5021	AT3G23890.2	3	-1	1	20	45	65	UGAGAAGAAGAGAA- AA	uucuuucuuucuucuucu	Cleavage	Symbols: TOPIL, ATTOPIL topoisomerase II chr3:8624750-8631327 FORWARD LENGTH=4719	1

bg-miR5021	AT1G19835.1	3	-1	1	20	96	116	UGAGAAGAGAGAGAA- AA	UUCUUCUCUCUCUCUCU	Cleavage	Symbols: Plant protein of unknown function (DUF869) chr1:6855952-6860532 REVERSE LENGTH=3444	1
bg-miR5021	AT3G61780.1	3	-1	1	20	613	633	UG- AGAGAGAGAGAAAAA	GUUUUCUUCUUCUUCUUCUCA	Cleavage	Symbols: emb1703 embryo defective chr3:32867814- 22871462 REVERSE LENGTH=3366	1
bg-miR5021	AT1G65920.1	3	-1	1	20	61	81	UGAGAAGAGAGAGAA- AA	UUCUUCUCUCUCUCU	Cleavage	Symbols: Regulator of chromosome condensation (RCC1) family with FVFE zinc finger domain chr1:24525102-24529294 REVERSE LENGTH=2276	1
bg-miR5021	AT4G13918.1	3	-1	1	20	438	458	UGAGAAGAGAGAGAA- AA	UUUAUCUCUCUCUCUCUCC	Cleavage	Symbols: Potential natural antisense: locus overlaps with AT-G13920 chr4:48043587- 8047176 REVERSE LENGTH=3225	1
bg-miR5021	AT1G69440.1	3	-1	1	20	51	71	UGAGAAGAGAGAGAA- AA	UUCUUCUCUCUCUCU	Cleavage	Symbols: AG07_ZIP Argonaute family protein chr1:26101527- 26105166 REVERSE LENGTH=3161	1
bg-miR5021	AT4G19610.1	3	-1	1	20	49	69	UGAGAAGAGAGAGAA- AA	UUCUUCUCUCUCUCUCC	Cleavage	Symbols: Nudt6/otide binding/nucleic acid binding;RNA binding chr4:1067729-10851771 FORWARD LENGTH=2852	1
bg-miR5021	AT3G60750.1	3	-1	1	20	241	261	UGAGAAGAGAGAA- AA	UUCUUCUCUCUCUCU	Cleavage	Symbols: Transketolase chr3:22453719-22457152 FORWARD LENGTH=2839	1
bg-miR5021	AT1G10850.1	3	-1	1	20	242	262	UG- AGAGAGAGAGAAAAA	CUUUCUUCUUCUUCUCA	Cleavage	Symbols: Leucine-rich repeat protein kinase family protein chr1:361747-36184613 FORWARD LENGTH=2743	1
bg-miR5021	AT4G21880.1	3	-1	1	20	76	96	UGAGAAGAGAGAGAA- AA	UUCUUCUUCUUCUUCUCC	Cleavage	Symbols: Pentatricopeptide repeat (PPR) superfamily protein chr4:11604986-11610651 FORWARD LENGTH=2702	1
bg-miR5021	AT3G07060.1	3	-1	1	20	370	390	UGAGAAGAGAGAGAA- AA	UUCUUCUUCUUCUUCUCC	Cleavage	Symbols: containing protein chr3:223206-2237118 FORWARD LENGTH=2684	1
bg-miR5021	AT3G60750.2	3	-1	1	20	264	284	UGAGAAGAGAGAA- AA	UUCUUCUUCUUCUUCUCC	Cleavage	Symbols: Transketolase chr3:22453696-2245690 FORWARD LENGTH=2597	1
bg-miR5021	AT1G08520.1	3	-1	1	20	95	115	UGAGAAGAGAGAGAA- AA	UUCUUCUUCUUCUUCUCC	Cleavage	Symbols: AIB1, AIB-1V_V157, PD1166, CHLD, ALBNA1 chr1:2696412-2700959 FORWARD LENGTH=2549	1
bg-miR5021	AT3G16000.1	3	-1	1	20	279	299	UGAGAAGAGAGAGAA- AA	UUCUUCUUCUUCUUCUCC	Cleavage	Symbols: MFP1, MAR binding filament-like protein 1 chr3:5430889-5433817 REVERSE LENGTH=2537	1
bg-miR5021	AT3G27580.1	3	-1	1	20	134	154	UGAGAAGAGAGAGAA- AA	UUCUUCUUCUUCUUCU	Cleavage	Symbols: ATPK7, D6PKL3 Protein kinase superfamily protein chr3:10217417-10219976 REVERSE LENGTH=2483	1

bg-miR5021	AT3G49160.1	3	-1	1	20	84	104	UGAGAACAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: pyruvate kinase family protein chr3:18221994-18224885 REVERSE LENGTH=2445	1
bg-miR5021	AT4G37890.1	3	-1	1	20	369	389	UG- AGAGAGAGAGAGAAA	GUUUUCUCUCUCUCUC	Cleavage	Symbols: EDA40 Zinc finger (C3HC4-type RING finger) family protein chr4:17812736-17815084 REVERSE LENGTH=2349	1
bg-miR5021	AT2G23300.1	3	-1	1	20	46	66	UGAGAAGAAGAAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: leucine-rich repeat protein kinase family protein chr2:9914608-9917130 FORWARD LENGTH=2322	1
bg-miR5021	AT3G15095.1	3	-1	1	20	168	188	UGAGAAGAAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: unknown protein; FUNCTIONS IN: molecular function unknown; INVOLVED IN: biological process unknown; LOCATED IN: chloroplast; EXPRESSED IN: 22 plant structures; EXPRESSED DURING: 13 growth stages; Has 9762 Blast hits to 6439 proteins in 764 species; Archaea - 77; Bacteria - 1339; Metazoa - 3211; Fungi - 718; Plants - 437; Viruses - 131; Other Eukaryotes - 3849 (source: NCBI Blink); chr3:5081780-5084081 FORWARD LENGTH=2302	1
bg-miR5021	AT4G37890.2	3	-1	1	20	380	400	UG- AGAGAGAGAGAGAAA	GUUUUCUCUCUCUCUC	Cleavage	Symbols: EDA40 Zinc finger (C3HC4-type RING finger) family protein chr4:17812736-17815095 REVERSE LENGTH=2276	1
bg-miR5021	AT2G27030.2	3	-1	1	20	21	41	UGAGAAGAAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: CAM5 (calmodulin 5) chr2:11532007-11534272 FORWARD LENGTH=2266	1
bg-miR5021	AT5G64816.2	3	-1	1	20	481	501	UGAGAAGAAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: unknown protein; Has 35333 Blast hits to 34131 proteins in 2444 species; Archaea - 798; Bacteria - 22429; Metazoa - 974; Fungi - 991; Plants - 531; Viruses - 0; Other Eukaryotes - 9610 (source: NCBI Blink); chr5:25913000-25914027 FORWARD LENGTH=659 FORWARD LENGTH=2302	1
bg-miR5021	AT3G27580.2	3	-1	1	20	58	78	UGAGAACAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: ATPK7 Protein kinase superfamily protein chr3:10217430-10219900 REVERSE LENGTH=2163	1
bg-miR5021	AT5G12120.1	3	-1	1	20	32	52	UGAGAACAGAACAGAA- AA	UUCUCUCUCUCUCUC	Cleavage	Symbols: Ubiquitin-associated/ translation elongation factor EF1B protein chr5:3916048-3918859 REVERSE LENGTH=2154	1
bg-miR1029	AT1G28610.2	2.5	-1	1	20	1323	1342	UCUCUCUCACCAACCAUC	GUAUUGUUGUUGAGAGAGA	Translation	Symbols: GDSL-like Lipase/Acylhydrolase superfamily protein chr1:10033617-10055731 REVERSE LENGTH=1478	1
bg-miR1029	AT1G28610.1	2.5	-1	1	20	1377	1396	UCUCUCUCACCAACCAUC	GUAUUGUUGUUGAGAGAGA	Translation	Symbols: GDSL-like Lipase/Acylhydrolase superfamily protein chr1:10033617-10055681 REVERSE LENGTH=1532	1

bg-miR1029	ATIG28610.2	2.5	-1	1	20	1323	1342	UCUCUCUACCAACCAUAC	GUAUUGUUUGUAGAGAGA	Translation	Symbols: GDSL-like Lipase/ Acylhydrolase superfamily protein chr1:10053617-10055731 REVERSE LENGTH=1478	1
bg-miR1029	ATIG28610.1	2.5	-1	1	20	1377	1396	UCUCUCUACCAACCAUAC	GUAUUGUUUGUAGAGAGA	Translation	Symbols: GDSL-like Lipase/ Acylhydrolase superfamily protein chr1:10053617-10055681 REVERSE LENGTH=1532	1
bg-miR1029	Potri.013G114400.3	2.5	-1	1	20	1048	1067	UCUCUCUACCAACCAUAC	GAUUGGUUGGUAAAGAGA	Cleavage	pacid=26995417 locus=Potri.013G114400.3.v3.0 annot-version=v3.0	1
bg-miR1029	Potri.013G114400.2	2.5	-1	1	20	1048	1067	UCUCUCUACCAACCAUAC	GAUUGGUUGGUAAAGAGA	Cleavage	pacid=26995416 locus=Potri.013G114400.2.v3.0 annot-version=v3.0	1
bg-miR1029	Potri.013G114400.1	2.5	-1	1	20	1051	1070	UCUCUCUACCAACCAUAC	GAUUGGUUGGUAAAGAGA	Cleavage	pacid=26995415 locus=Potri.013G114400.1.v3.0 annot-version=v3.0	1
bg-miR1029	Potri.013G114400.5	2.5	-1	1	20	1054	1073	UCUCUCUACCAACCAUAC	GAUUGGUUGGUAAAGAGA	Cleavage	pacid=26995419 locus=Potri.013G114400.5.v3.0 annot-version=v3.0	1
bg-miR1029	Potri.013G114400.4	2.5	-1	1	20	1286	1305	UCUCUCUACCAACCAUAC	GAUUGGUUGGUAAAGAGA	Cleavage	pacid=26995418 locus=Potri.013G114400.4.v3.0 annot-version=v3.0	1
bg-miR1029	Potri.001G438100.1	2.5	-1	1	20	87	106	UCUCUCUACCAACCAUAC	CUAUGGAUGGUUAGAGAGA	Cleavage	pacid=27047900 locus=Potri.001G438100.1.v3.0 annot-version=v3.0	1
bg-miR1029	AM822656	2.5	-1	1	20	109	128	UCUCUCUACCAACCAUAC	CUACGGUUGGAUGAGAGAGA	Translation	similar to UniRef100_A6YB88 Cluster: Pax3/7; n=1; -Platynereis dumerilii Rep. Pax3/7 - Platynereis dumerilii (Dumeril's clam worm), partial (9%)	1
bg-miR1029	EH665658	2.5	-1	1	20	389	408	UCUCUCUACCAACCAUAC	CUUUGGUUGGUAGAGAGA	Cleavage	similar to UniRef100_Q9M7J4 Cluster: MAR-binding filament-like protein 1-1; n=1; Nicotiana tabacum [Rep: MAR-binding filament-like protein 1-1 - Nicotiana tabacum (Common tobacco) partial (11%)	1

Web server psRNA Target was used to predict the target genes for these two miRNAs. The cDNA library of *Arabidopsis thaliana* was used for this study, as cDNA library of *Bruguiera gymnorhiza* was not available; and being a genetically well studied model organism *Arabidopsis thaliana* can give the most accurate prediction. Default parameters were set for this study. The specificity of target binding for these two miRNAs might provide some useful information towards understanding the stress response. bg-miR5021 showed a total of 146 hits (Table 1). Target genes for this miRNA include NAD kinase1, fatA acyl- ACP thioesterase, Calmodulin S, Topoisomerase, Circadian clock associated 1, Pectin-lyase like superfamily protein, NADPH dehydrogenase B, Ferrodoxin – like superfamily protein among others. All of these genes have some role in growth and development of plants. The binding of the miRNAs to these genes results in despaired activity. On the other hand some of these genes have plant stress responsive activities. Ca²⁺-dependent protein kinases (CDPKs) and sucrose non-fermentation 1 (SNF1)-related kinases (SnRKs) regulate stress responsive gene expression which include ABA-responsive transcription factors and LEA genes [23]. Several protein kinases involved in stress tolerance are stimulated by ABA, which have a immense role in stress response [24]. Calmodulin (CaM) is a major Ca²⁺-sensing protein, involved in transduction of Ca²⁺ signals. CaM undergoes conformational change after interacting with Ca²⁺ and stimulates the activities of a diverse range of CaM-binding proteins. Most important role played by CaM is in adaptation to adverse environmental conditions [25]. bg-miR1029 showed a total of 12 hits, which includes GDSL-like Lipase/Acylhydrolase superfamily protein among others.

The salinity is increasing all over the globe, this rapid salinization may pose serious threat to the growth and development of the plants [26]. Genes and miRNAs involved in salinity stress have been identified to some extent [27]. But the molecular data of salt responsive genes and miRNAs available currently is very limited. Genome wide molecular level study on the halophytes and mangroves could provide clear insight for coping with the rapid salinization all along the globe. Hence the present work predicted that the target genes for the two miRNAs bg-miR1029 and bg-miR5021, include some genes very important for plant growth and development, as well as plant stress responses. Experimental validation and characterization of these two miRNAs expression and their predicted target genes along the salinity gradient would give us more clear understanding of adaptability within saline atmosphere for the species *Bruguiera gymnorhiza*.

References

1. Mallory AC, Vaucheret H (2006) Functions of microRNAs and related small RNAs in plants. *Nature Genet* 38: S31-S36.
2. Bushati N, Cohen SM (2007) microRNA functions. *Annu Rev Cell Dev Biol* 23: 175-205.
3. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116(2): 281-297.
4. Carrington JC, Ambros V (2003) Role of microRNAs in plant and animal development. *Science* 301(5631): 336-338.
5. Djuranovic S, Nahvi A, Green R (2011) A parsimonious model for gene regulation by miRNAs. *Science* 331(6017): 550-553.
6. Vaucheret H (2006) Post-transcriptional small RNA pathways in plants: mechanisms and regulations. *Genes Dev* 20(7): 759-771.
7. Bottino MC, Rosario S, Grativol C, Thiebaut F, Rojas CA, Farrinelli L, et al. (2013) High-throughput sequencing of small RNA transcriptome reveals salt stress regulated microRNAs in sugarcane. *PloS one* 8(3): e59423.
8. Ren Y, Chen L, Zhang Y, Kang X, Zhang Z, et al. (2013) Identification and characterization of salt-responsive microRNAs in *Populus tomentosa* by high-throughput sequencing. *Biochimie* 95(4): 743-750.
9. Zhu J, Li W, Yang W, Qi L, Han S (2013) Identification of microRNAs in *Caragana intermedia* by high-throughput sequencing and expression analysis of 12 microRNAs and their targets under salt stress. *Plant cell Rep* 32(9): 1339-1349.
10. Mondal TK, Ganje SA (2014) Identification and characterization of salt responsive miRNA-SSR markers in rice (*Oryza sativa*). *Gene* 535(2): 204-209.
11. Tomlinson PB (1986) The botany of mangroves. Cambridge University Press, pp. 413.
12. FAO (2007) The World's Mangroves 1980-2005. FAO Forestry Paper, Rome, Italy, p. 153.
13. Hogarth P (2007) The biology of mangroves and sea grasses. In: Hogarth P (Ed.), (2nd edn), Oxford University Press, New York, USA.
14. Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, et al. (2011) Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography* 20(1): 154-159.
15. Dasgupta N, Nandy P, Sengupta C, Das S (2010) RAPD and ISSR marker mediated genetic polymorphism of two mangroves *Bruguiera gymnorhiza* and *Heritiera fomes* from Indian Sundarbans in relation to their sustainability. *Physiology and Molecular Biology of Plants* 21(3): 375-384.
16. Dasgupta N, Sengupta C, Das S (2014) Role of Secondary Metabolites and Radical Scavenging Aptitude for Better Adaptability of Mangroves in Varying Salinity of Sundarbans, India. *Annals of Tropical Research* 36(2): 1-22.
17. Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ (2008) miRBase: tools for microRNA genomics. *Nucleic acids research* 36(suppl 1): D154-D8.
18. Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27(6): 863-864.
19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of molecular biology* 215(3): 403-410.
20. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31(13): 3406-3415.
21. Dai X, Zhao PX (2011) psRNATarget: a plant small RNA target analysis server. *Nucleic Acids Res* 39(suppl 2): W155-159.
22. Sunkar R, Chinnusamy V, Zhu J, Zhu JK (2007) Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. *Trends in plant science* 12(7): 301-309.
23. Saito Y, Hata S, Kyozuka J, Shimamoto K, Izui K (2000) Over-expression of a single Ca²⁺-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant J* 23(3): 319-327.

24. Wasilewska A, Vlad F, Sirichandra C, Redko Y, Jammes F, et al. (2008) An update on abscisic acid signaling in plants and more.... Mol plant 1(2): 198-217.
25. Virdi AS, Singh S, Singh P (2015) Abiotic stress responses in plants: roles of calmodulin-regulated proteins. Front plant sci 6: 809.
26. Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51(1): 463-499.
27. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, et al. (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31(3): 279-292.



This work is licensed under Creative Commons Attribution 4.0 Licens
DIO: [10.19080/IJCSMB.2017.2.555579](https://doi.org/10.19080/IJCSMB.2017.2.555579)

**Your next submission with Juniper Publishers
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats
(Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission

<https://juniperpublishers.com/online-submission.php>