

Identification and functional analysis of new conserved microRNAs and their targets in potato (*Solanum tuberosum* L.)

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Abstract: MicroRNAs (miRNAs) are tiny, endogenous, nonprotein-coding, and functionally negative regulators of posttranscriptional gene regulation. Potato (*Solanum tuberosum* L.) is a starchy and tuberous crop that is also important for its potassium and vitamin contents. Though potato is the world's fourth largest food crop, its miRNAs and their targets have not been well studied. Here we report a comparative genomics approach that was applied to explore miRNAs and their targeted proteins in potato. A total of 120 new miRNAs from 110 families were identified and characterized from the expressed sequence tags. All 120 miRNAs were observed, along with stable stem-loop precursor structures, whose lengths ranged from 42 to 488 nt with an average of 132 nt. Mature miRNAs lengths ranged from 18 to 26 nt with an average of 21 nt. Later, 10 miRNAs were randomly selected for validation by reverse-transcription polymerase chain reaction. A total of 433 potential targets were predicted for these new 120 potato miRNAs. These targets were involved in regulation, metabolism, transcription factors, growth and development, and other physiological processes. These miRNAs and their targets will be useful to fine-tune potatoes for better food content and higher nutritional value as well biotic and abiotic stress resistance.

Key words: BLAST, comparative genomic, microRNAs, potato (*Solanum tuberosum* L.)

1. Introduction

Potato (*Solanum tuberosum*) belongs to the family Solanaceae and is an important agricultural crop. It is the world's fourth largest food crop, following rice, wheat, and maize. Distributed worldwide with about 5000 varieties, the potato has its center of origin in the high Andes of South America. It is best known for its carbohydrate content in the form of starch, though it also contains important vitamins and minerals (Abbas et al., 2012). It plays a viable role in the worldwide sustainable food system, producing more food energy on less land with low cost of cultivation. It is the most important tuber crop in terms of production, accounting for about 45% of total world tuber crop production (Shewry, 2003) and parental marker studies in anther-derived progeny (Aziz, 2014a, 2014b).

MicroRNAs (miRNAs) are single-stranded RNAs that are noncoding, endogenous, and 18 to 26 nt in length (Bartel, 2004). The intergenic genes of miRNAs are generated from endogenous stem-loop primary transcripts known as precursor miRNAs (pre-miRNAs) through actions of Dicer-like 1 (DCL1), an RNase III enzyme. DCL1, along with other proteins, generates a mature miRNA

from the cleavage of stem-looped pre-miRNA (Ambros, 2003). Mature miRNA further associates with argonaute proteins to develop an RNA-induced silencing complex (RISC). The RISC then functions as a guide molecule in the negative posttranscriptional gene, silencing through 2-way base pairing with target mRNAs. The base pairings are perfect or near perfect and lead to mRNA cleavage or translational repression, respectively. miRNAs are one of the largest gene families, accounting for approximately 1% of the genome (Bartel, 2004). Many recent studies have shown that miRNAs have vital roles in diverse regulatory pathways in plants and animals (Baloch and Din, 2014; Din and Barozai, 2014a). In plants, miRNAs regulate tissue differentiation and development, including leaf, root, stem, and flower tissues. They also regulate developmental timing from the vegetative to reproductive stages, as well as signal transduction and response to biotic and abiotic stresses, such as salinity, drought, cold, heat, and pathogens (Zhang et al., 2006; Baloch et al., 2013; Song et al., 2013). The miRNAs and their targets interact through complex regulatory networks. A single miRNA can bind to regulate several distinct mRNA targets and, conversely,

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many different miRNAs can bind to and cooperatively control a single mRNA target (Lewis et al., 2003).

Since 2002, when the discovery of the first miRNA in plants was reported (Reinhart et al., 2002), enormous numbers of miRNAs have been identified in plants through computational and experimental approaches (Zhang et al., 2006). Due to evolutionary convergence among the families of miRNAs in plants and animals (Bartel, 2004), these are valid sources to identify profiles as well as structurally and functionally analyze them through bioinformatics and experimental approaches in diverse species (Zhang et al., 2006; Din and Barozai, 2014a). Many groups of researchers, using comparative genomic approaches due to the conserved nature of miRNAs, have identified an abundant number of potential miRNAs in various species of plants, including *Panicum virgatum* (Xie et al., 2010); *Gossypium* species (Barozai et al., 2008); *Arabidopsis thaliana*, *Oryza sativa*, and *Populus trichocarpa* (Griffiths-Jones, 2004); *Brassica* (Wang et al., 2012); Euphorbiaceae (Zeng et al., 2009); *Phaseolus* (Barozai et al., 2013a); 71 different plant species (Zhang et al., 2006); *Nicotiana tabacum* (Frazier et al., 2010); and *Solanum melongena* (Din and Barozai, 2014b). This catalog of reported miRNAs suggests that comparative genomic strategies are highly efficient, convenient, valid, and powerful tools to predict further potential miRNAs in other species (Baloch and Din, 2014).

Presently, limited data are available regarding miRNAs in potato, despite its agricultural and economic importance (Griffiths-Jones, 2004; Guo et al., 2007). Recently, Zhang et al. (2013) and Lakhota et al. (2014) reported 28 and 33 miRNA families in potato, respectively. Consequently, this limited information on conserved miRNAs in potato is insufficient considering its overwhelming mass production and nutritional requirements. Therefore, a comprehensive comparative genomics search methodology using all known mature and pre-miRNAs of plants was employed to find 120 new conserved miRNAs from 249,761 potato expressed sequence tags (ESTs) that belonged to 110 families of miRNAs. These new miRNAs were further profiled for structural and functional characterization. In this study, we have also predicted 433 putative protein targets for the 110 miRNA families in potato.

2. Materials and methods

2.1. Reference miRNAs

A methodology described by Zhang et al. (2006) with a few modifications by Barozai et al. (2008) was applied to explore the potential miRNAs from potato ESTs. A total of 3533 known mature plant pre-miRNA sequences were downloaded as reference miRNAs from the microRNA registry database Rfam Release 20 (Griffiths-Jones, 2004) and subjected to the basic local alignment search tool

(BLAST) for alignment with 249,761 publicly available potato (*Solanum tuberosum*) ESTs from a database of ESTs (dbEST, release 130101; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>), using the BLASTn program (Altschul et al., 1990). Repeated ESTs from the same gene were eliminated and a single tone EST per miRNA was produced using the BLASTn program against the potato (*Solanum tuberosum*) EST database with default parameters. Potato (*S. tuberosum* L.) ESTs with 0–4 mismatches with the mature reference miRNA sequences were saved as initial candidate miRNAs.

2.2. Confirmation of potato miRNAs as nonprotein sequences

The miRNA validation as nonprotein-encoding sequences is a crucial criterion for homology-based searches of new miRNAs. The pre-miRNAs sequences, identified by mature miRNAs, were subjected to a protein homology search. The predicted pre-miRNA sequences, in FASTA format, were subjected to BLAST against the NCBI protein database using BLASTx with default parameters (Stephen et al., 1997).

2.3. Generation of stem-loop structures

The initial potential candidate potato miRNA sequences, confirmed as nonprotein-coding with 0–4 mismatches with the reference miRNAs and representing a single tone gene, were stimulated to generate hairpin secondary structures. For this purpose, the publicly available Zuker folding algorithm (<http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi>) known as Mfold (version 3.6) (Zuker, 2003) was applied to predict the secondary structures. The Mfold parameters were adjusted as per published approaches by various researchers for the identification of miRNAs in many plant and animal species (Bai et al., 2012). For physical scrutinizing, self-folded stem-loop structures with minimum free energy (MFE) of no greater than –18 kcal/mol or lower than or equal to the MFE of the reference miRNAs were preferred. The threshold values from Ambros et al. (2003) were applied as a reference to finalize the potential miRNAs. The stem regions of the stem-loop structures were checked and confirmed for mature sequences either at least 16 in number or equal to the reference miRNAs' base pairing involved in Watson–Crick or G/U base pairing between the mature miRNA and the opposite strand (miRNA*).

2.4. Experimental validation by reverse transcription polymerase chain reaction

Ten of the newly discovered miRNAs were randomly selected to be validated by reverse-transcription polymerase chain reaction (RT-PCR). The primers were designed using the Primer3 algorithm for the stem-loop sequences of these selected miRNAs from their ESTs (Table 1). Total RNA from the leaves was extracted using an RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. cDNA was constructed using

Table 1. The potato pre-miRNA primer sequences for RT-PCR experimental validation. Ten randomly selected potato miRNAs subjected to expression analysis through RT-PCR are given here with primers and product size (bp).

Potato miRNAs	Primers	Product size (bp)
stu-miR2869	TGGACTCTTTGTTCCAGGCA TGCTAGACCCCTTCACCAGA	234
stu-miR5538	ACTCAATCACTTGCTGCCGT CCCTACCTTTGAGTGCGGTT	161
stu-miR475	TCAATCAAGTTTCTCTTTTCAGCAGT AGCTGGATGACATGAATCCTTCA	169
stu-miR1870	TGCTACATCAGGCTTGACTIONTCA GCCCACTAGGTCTGGTTCAG	170
stu-miR1873	CATAGGGGCTGTGAAGGACC TGCAGGCAGTGACATAACCC	182
stu-miR2608	GAACCGCCCTAATTCACCCA ACGAAAAAGACGTCGGTGGA	214
stu-miR4993	CCGAAGTGGTGACGGTAGAA GGAGTTGTTCCGAAAAGAGGTC	154
stu-miR1918b	TCAGTGGATTTGACTTCCTCT CAGGGTGGGTATAGGGGAGG	186
stu-miR5040	TGTCTTGCGAAAACGATGGA GTCCTCCTTTGAAGACCTTCCA	259
stu-miR4995	GAATGAGCCGGCGACTCATA CATTTGCGGGGAGAACCAGCT	252

the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas), as per the supplier's protocol. A template of 120 ng of cDNA was used in the following PCR program: initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 59 °C for 30 s, and extension at 72 °C for 35 s, and a final elongation step at 72 °C for 8 min. The PCR products were separated through 2% (w/v) agarose gel.

2.5. Identification of miRNA targets

In the current study, dual approaches were used to predict the potential targets for potato miRNAs. In the first approach, the newly identified potato miRNAs were subjected to a plant miRNA target prediction tool called psRNATarget (<http://bioinfo3.noble.org/psRNATarget>), using default parameters (Dai and Zhao, 2011). The potato miRNAs that did not produce potential targets through psRNATarget were subjected to a secondary approach as described by Barozai (2013). In this approach, the mature miRNA sequences were submitted as queries to the NCBI BLASTn program. The parameters were: database: reference mRNA sequences (refseq_rna); organism: *Solanum tuberosum* (taxid: 4113); and program

selection: highly similar sequences (megablast). The mRNA sequences showing $\geq 75\%$ query coverage were selected and further subjected to RNAhybrid, which is a miRNA target prediction tool (Kruger and Rehmsmeier, 2006). Only those targets with confirmed stringent seed sites located at either positions 2–7 or 8–13 from the 5' end of the miRNAs along with the supplementary site and having MFE of no greater than -20 kcal/mol were selected. For more stringency, these targets were subjected to the NTNU microRNA target prediction tool (http://tare.medisin.ntnu.no/mirna_target/search#results) to confirm the RNAhybrid results. Gene Ontology analysis for the predicted putative targets of potato miRNAs was conducted on the AmiGO website.

2.6. Abbreviations

ath: *Arabidopsis thaliana*, bdi: *Brachypodium distachyon*, BLAST: Basic Local Alignment Search Tool, bra: *Brassica rapa*, csi: *Citrus sinensis*, dbEST: database of ESTs, DCL1: Dicer-like enzyme 1, ESTs: expressed sequence tags, gma: *Glycine max*, mdm: *Malus domestica*, MFE: minimum free energy, miR: mature miRNA, MIR: microRNA gene, miRNA: microRNA, miRNA*: opposite strand of miRNA,

mRNA: messenger RNA, mtr: *Medicago truncatula*, NCBI: National Center for Biotechnology Information, nt: nucleotides, nta: *Nicotiana tabacum*, osa: *Oryza sativa*, ppe: *Prunus persica*, pre-miRNA: precursor-miRNA, pri-miRNA: primary transcripts of mature miRNA, ptc: *Populus trichocarpa*, refseq_rna : reference mRNA sequence, RISC: RNA induced silencing complex, sly: *Solanum lycopersicum*, vvi: *Vitis vinifera*.

3. Results and discussion

3.1. New conserved potential potato miRNAs

A comparative genomic approach along with computational and bioinformatic tools produced a total of 120 new conserved miRNAs from the analysis of 249,761 potato ESTs (Table 2). These 120 newly identified pre-miRNAs were also confirmed for stem-loop secondary structures by Mfold (version 3.6) (Zuker 2003). These 120 potential potato miRNAs belong to 110 families, namely stu-miR 473, 475, 535 (a, b), 1320, 1446, 1450, 1510, 1870, 1873, 1879, 1888, 1918 (a, b), 2090, 2586, 2597, 2608, 2616, 2668, 2673, 2678, 2868, 2869, 2939, 3623, 3640, 3948, 3951, 3954, 4351, 4993, 4995, 5012, 5019, 5032, 5035, 5038, 5039, 5040, 5069, 5150, 5171, 5176, 5179, 5183, 5205 (a, b), 5208, 5210, 5219, 5222, 5223, 5226, 5227, 5234, 5237, 5240, 5254, 5261, 5265 (a, b), 5269, 5288, 5301, 5337, 5338, 5368, 5492, 5536, 5538, 5630, 5643, 5649, 5657 (a, b), 5721, 5722, 5747, 5748, 5749, 5754, 5818, 5819, 6019, 6021 (a, b), 6144, 6145, 6148, 6154, 6156, 6164 (a, b, c), 6252, 6270, 6278, 6284, 6287, 6426, 6438, 6439, 6440, 6461, 6468, 6476, 7749, 7777, 7785, 7816, 7822, 7825, 7826, 7828, 7830, 7835, and 8123 (a, b). A maximum of 3 members were found in the stu-miR6164 family; the stu-miR535, 1918, 5205, 5265, 5657, 6021, and 8123 families had 2 members; and the remaining families had only 1 member. On the basis of current available miRNAs reports, all these predicted miRNAs were profiled for the first time in potato. All 120 of these miRNAs were considered as valid candidates after applying the stringent miRNA criteria of Ambros et al. (2003). The potential miRNAs were justified by criteria B, C, and D. According to Ambros et al. (2003), only criterion D is sufficient for homologous sequences to be confirmed as potential miRNAs in other species. The present study is in agreement with the prior studies (Frazier et al., 2010; Xie et al., 2010; Barozai et al., 2012b, 2014). The combination of homology-based investigation and comparative genomics has produced novel and interesting results in plant genomics. Similar approaches were used by many groups of researchers to report novel miRNAs in plants (Frazier et al., 2010; Xie et al., 2010; Barozai et al., 2013a, 2013b).

3.2. Characterization of newly identified potato miRNAs

Characterizing the newly identified candidate miRNAs is a conventional step for their validation, as described

previously (Frazier et al., 2010; Xie et al., 2010; Wang et al., 2012), and MFE is the first significant term of characterization. The pre-miRNAs had MFEs ranging from -8 to -224 kcal/mol with an average of -31 kcal/mol. Profound numbers of newly identified pre-miRNAs (53, or 44%) had MFEs within a range of -8 to -21, followed by -22 to -41 (37, or 31%) and -42 to -224 (31, or 26%). The pre-miRNA lengths of these potato miRNAs ranged from 42 to 488 nt with an average of 132 nt; mature miRNA sequences ranged from 18 to 26 nt with an average of 21 nt. The majority (61 out of 120) of the miRNAs were 21 nt in length, followed by 22 nt (15%), 20 nt (10%), 24 nt (7%), 23 (6%), 19 nt (2%), 25 nt (2%), 26 nt (1%), and 18 nt (1%), respectively. These results of potato pre-miRNAs and mature sequences are in agreement with earlier available data in other plant species (Frazier et al., 2010; Xie et al., 2010; Ji et al., 2012; Barozai et al., 2013a, 2013b). The mature miRNA sequences were found in the form of duplexes in the stem region of pre-miRNA secondary structures, as shown in Figure 1. Similar results were reported by many researchers for various plant and animal species (Frazier et al., 2010; Xie et al., 2010; Chen et al., 2012; Wang et al., 2012). In potato, newly identified conserved mature miRNA sequences showed differences of 4 nt (36%), 3 nt (37%), 2 nt (21%), 1 nt (4%), and 0 (3%); these match perfectly with the corresponding homologous source miRNAs. These values are in agreement with the formerly published values in different plants, where the mature sequences have a difference of 0–4 nucleotides (Wang et al., 2012; Barozai et al., 2013a; Ghani et al., 2013). For the newly reported potato miRNAs, the average GC content was 41%, within a range of 10% to 80%. Fifty-eight out of 120 potato miRNAs sequences were found at the 5' arm, while the remaining 62 were at the 3' arm, as shown in Figure 1. The secondary stem-loop/hairpin structures of the pre-miRNAs had at least 16 nucleotides engaged in Watson–Crick or G/U base pairing between the mature miRNA and the opposite arms (miRNAs*) in the stem region. A few source miRNAs had less base pairing; these precursors did not contain large internal loops or bulges. The relationship between newly discovered miRNAs and known proteins was an important criterion to validate the miRNAs as strong candidates. The pre-miRNAs were compared against the NCBI protein database using the BLASTx algorithm. No homology with known proteins was found. These results confirmed that our identified pre-miRNAs were strong potential miRNA candidates in potato. Similar results were observed in various publications by many researchers (Frazier et al., 2010; Ji et al., 2012). The predicted novel potato miRNAs were also characterized by their organ expression. The majority of the newly discovered miRNAs were expressed in tubers (41 out of 120 (34%)), followed by roots and mixed tissues

Table 2. Characterization of the newly explored potato miRNAs. PL = Precursor miRNA length, MFE = minimum free energy, MS = mature sequence, NM = number of mismatches (represented in red), ML = mature sequence length, MSA = mature sequence arm, SE = source EST, GC% = GC percentage, SL = strand location, OE = organ of expression.

Potato miRNAs	Source miRNAs	PL	MFE	MS	NM	ML	SE	MSA	GC%	SL	OE
stu-miR473	ptc-miR473b	53	-20.30	GATCTCCCTCAGGGCTCTCCA	2	21	CO502083	3'	57.14	-	Roots
stu-miR475	ptc-miR475b	67	-14.00	TTACAGAGCCCAATTGATTTGA	4	21	BG589707	5'	38.09	-	Leaves
stu-miR535a	mdm-miR535b	68	-20.50	TGCCAAGGAGAGAGAGGACGT	3	21	CK852226	5'	57.14	-	Stolon
stu-miR535b	mdm-miR535d	221	-56.88	TGATGACGAGAGAGAAAACGC	3	21	DN743302	3'	47.61	-	Tubers
stu-miR1320	osa-miR1320	168	-40.64	TGGAATGGAGGAATTTGTAA	3	21	CX161644	5'	33.33	-	Floral buds
stu-miR1446	nta-miR1446	129	-38.93	TGAACCTCTCCCTCAATGAGA	3	22	CO502181	5'	45.45	-	Roots
stu-miR1450	ptc-miR1450	112	-22.40	TTTCATGGCTCGGACAGGTTAC	3	22	BM405805	3'	50.00	+	Roots
stu-miR1510	ntr-miR1510a	94	-21.64	TTGTCTTACCTATACCTCCCA	2	21	BQ119248	5'	42.85	-	Mixed tissues
stu-miR1870	osa-miR1870	177	-45.65	ACCTGAACCAGACCTAGTGGGCAT	4	24	CX162202	3'	54.16	+	Leaves
stu-miR1873	osa-miR1873	129	-43.10	GCAACATGGCACCAGACTGGAAG	3	24	CK279106	5'	58.33	+	Leaves and roots
stu-miR1879	osa-miR1879	150	-37.20	GTGTTGGTTTTAAGGAATGAGGAGG	3	25	CK249799	3'	44.00	+	Callus
stu-miR1888	ath-miR1888a	231	-43.70	TAAGTGAAGCTTTGCTTGAAGAA	4	23	CV505970	5'	34.78	-	Floral buds
stu-miR1918a	sly-miR1918	76	-26.60	TGTTGGTGATTTGTCGATTCCTC	2	22	CX161534	3'	40.90	+	Floral buds
stu-miR1918b	sly-miR1918	73	-12.80	TGTTGGTGAGGGTTCCGATTCCTC	1	22	BQ517545	3'	50.00	-	Mixed tissues
stu-miR2090	osa-miR2090	71	-9.90	AACTCTAAATTTAGAATGTTA	4	22	BQ112058	3'	18.18	-	Mixed tissues
stu-miR2586	ntr-miR2586	84	-11.05	CGAGGAATGTTGTGCTTTAT	4	21	BG595781	5'	38.09	-	Tubers
stu-miR2597	ntr-miR2597	105	-26.90	TTTGGTACTTGGTCCATTTT	4	21	DV626256	3'	33.33	+	Tubers
stu-miR2608	ntr-miR2608	56	-12.10	GTTGTTCAATATGTCACACTACTCT	2	22	CK278225	5'	36.36	+	Leaves and roots
stu-miR2616	ntr-miR2616	86	-23.00	ATTGGGTTTGGTTCGGGGGTC	3	22	CK717873	3'	59.09	+	Stolon
stu-miR2668	ntr-miR2668	136	-27.09	TTCAATCCTTGCATTTCCGGGGTT	3	22	JG564427	5'	45.45	+	Roots
stu-miR2673	ntr-miR2673a	82	-9.60	CCCTTCCCTCTTCCCTCTTCCCTT	2	22	CK249851	5'	50.00	-	Callus
stu-miR2678	ntr-miR2678	53	-10.40	TGAAAGTTTTTGGCGAGTATCTT	3	21	DR038075	5'	33.33	+	Tubers
stu-miR2868	osa-miR2868	108	-18.02	TTGGTTTTTGTGTAGGAGAAA	1	20	BI405756	5'	35.00	+	Tubers
stu-miR2869	osa-miR2869	140	-41.80	CCACGACATTAATAATCTGGGA	4	21	BQ518337	3'	42.85	+	Mixed tissues
stu-miR2939	ath-miR2939	242	-38.33	GTAACG-ACAACACTAAGCCAG	2	21	BQ506046	5'	47.61	-	Mixed tissues
stu-miR3623	vvi-miR3623	254	-38.31	TCACTAGTTCATCCAAGCACCA	1	22	BE340934	5'	45.45	-	Stolon
stu-miR3640	vvi-miR3640	52	-13.50	TCCTGATTTGGTATGAAATTTGGG	4	24	CV287270	3'	41.66	-	Tubers
stu-miR3948	csi-miR3948	261	-96.86	GGGAGTGGGAGTGGGAGTGGGGATG	3	25	CV430968	3'	68.00	+	Tubers

Table 2. (Continued).

stu-miR3951	csi-miR3951	168	-42.33	TAGCTAAAAATG-GAGAGAAA	4	20	FG550679	5'	30.00	+	Tubers
stu-miR3954	csi-miR3954	418	-92.51	TGG-CAGAGAAATCACGGTGA	2	20	DN906883	3'	50.00	+	Tubers
stu-miR4351	gma-miR4351	52	-13.70	TTGGGGTTTTAGTTGGAGTTGG	4	22	GH638009	5'	45.45	+	Tubers
stu-miR4993	gma-miR4993	81	-50.40	--GCGGCGGGTGGAGGACTG	3	20	AM908363	5'	80.00	-	Leaves
stu-miR4995	gma-miR4995	83	-31.58	AGGCAGTGGCTTGGTTAAGGG	0	21	FG548901	5'	57.14	-	Mixed tissues
stu-miR5012	ath-miR5012	59	-13.30	TTTTTCCTACTTGTGTTC	2	21	CV494197	5'	38.09	-	Tubers
stu-miR5019	ath-miR5019	99	-46.40	TGTTGGGAAAGAAAAGCTTGT	4	21	CV430833	3'	38.09	+	Tubers
stu-miR5032	gma-miR5032	71	-14.30	AGAGCCACTTTTGGTTCCCAAC	4	23	CN212422	5'	47.82	-	Callus
stu-miR5035	gma-miR5035	212	-72.35	CTTATAAACATTTTTTAACTTA	3	22	CK251928	3'	13.63	-	Callus
stu-miR5038	gma-miR5038a	81	-17.50	TGAGAGTTTGGAGTCTTCCCA	4	21	CK255918	3'	42.85	-	Callus
stu-miR5039	gma-miR5039	137	-18.96	TCCTTTTTTAATCATTGAATG	3	21	GT888549	3'	23.80	+	Tubers
stu-miR5040	gma-miR5040	213	-46.33	ATGATATATGAAAAGCATGAG	2	21	BQ113419	3'	28.57	-	Mixed tissues
stu-miR5069	bdi-miR5069	107	-25.28	GAGGTTATGTATTTGATCAAC	2	21	DR035251	5'	33.33	-	Stolon
stu-miR5150	osa-miR5150	210	-57.25	AGCTTTAACTGCTGCAGTTTCTT	4	24	JG562255	3'	37.50	-	Roots
stu-miR5171	bdi-miR5171	68	-12.30	ACTTAATATGGACCAGAAAGGA	4	21	BQ509356	5'	38.09	+	Mixed tissues
stu-miR5176	bdi-miR5176	78	-19.50	AGAGATGATGTGGCATAAGAAG	4	21	DV624732	3'	42.85	+	Tubers
stu-miR5179	bdi-miR5179	93	-25.60	TTTTTGCTCAAG-CAGCAGCAAC	3	21	DN938331	5'	47.61	+	Callus
stu-miR5183	bdi-miR5183	140	-34.43	TATTTGGATCAAAAGTTTGAGTAT	4	23	BQ505624	5'	26.08	+	Mixed tissues
stu-miR5205a	mtr-miR5205a	73	-20.70	CATATAAATGGGACGGAGGGAG	3	23	DN939649	3'	47.82	-	Tubers
stu-miR5205b	mtr-miR5205b	78	-16.80	CAAAATAAATTTGGACGGAGGGAGT	4	24	DR037177	3'	45.83	-	Tubers
stu-miR5208	mtr-miR5208b	450	-96.27	GACATTGCTGTTAGTGAGTTTGT	4	24	CK854355	5'	37.50	+	Stolon
stu-miR5210	mtr-miR5210	57	-11.10	TAAATGTAATGGAATTAAGGAT	4	22	CK278036	3'	27.27	-	Leaves and roots
stu-miR5219	mtr-miR5219	74	-13.50	CCATGGAATATCAGCAGCAGCA	4	22	EG012851	3'	50.00	+	Tubers
stu-miR5222	mtr-miR5222	68	-11.40	CTTACAGCTGAAGAAATGTAATGGC	3	23	DN908697	3'	43.47	-	Tubers
stu-miR5223	mtr-miR5223	100	-9.48	CAAGGAATTTACTTGAAGATGA	3	22	BG096210	5'	31.81	-	Leaves and petioles
stu-miR5226	mtr-miR5226	109	-33.30	TTTGTACAACTTGGAGGAAAAG	4	22	DV622824	3'	36.36	-	Tubers
stu-miR5227	mtr-miR5227	55	-8.90	TGAAAAGAAGAAGATTTGATGAT	2	22	CV431838	5'	27.27	-	Tubers
stu-miR5234	mtr-miR5234	110	-23.95	TGCAGTTGTGGATGGCTGAAG	4	21	BQ514099	3'	52.38	+	Mixed tissues
stu-miR5237	mtr-miR5237	62	-10.80	TTCAAAAAGATTTAGGTTCCGAT	3	21	JG561426	3'	28.57	+	Petioles
stu-miR5240	mtr-miR5240	145	-32.50	TTGAATAAATTTGTTGGAATTTGT	2	21	CV474616	3'	23.80	-	Tubers
stu-miR5254	mtr-miR5254	66	-15.50	AGGAGGTGGAGGCATTTTTGA	2	21	JG729093	5'	47.61	+	Tubers

Table 2. (Continued).

stu-miR5261	mtr-miR5261	199	-60.33	TCATTGTAGACGGTTTTGGCT	2	21	BQ114536	5'	42.85	+	Mixed tissues
stu-miR5265a	mtr-miR5265a	71	-17.20	AAGTGAATGTTTCATGGTTA	3	20	BQ518704	5'	30.00	-	Mixed tissues
stu-miR5265b	mtr-miR5265a	77	-23.40	AAGTGGATGTTGGAATGGTTG	2	21	JG561423	5'	42.85	+	Petioles
stu-miR5269	mtr-miR5269a	80	-19.60	AAAGTGGTGAGCA-ACTGATTGATT	4	26	BQ508922	3'	38.46	+	Mixed tissues
stu-miR5288	mtr-miR5288	135	-31.60	CAGCTCTTAAAAACATAGGGATTA	4	24	DV624272	3'	33.33	+	Tubers
stu-miR5301	sly-miR5301	85	-19.20	TGTGGATTGGGTGGAAAGCTT	3	21	CV503869	5'	47.61	+	Floral buds
stu-miR5337	osa-miR5337a	152	-23.44	AAATTACTTGTGGTTGTAGGT	3	21	CV286595	3'	33.33	+	Tubers
stu-miR5338	osa-miR5338	133	-33.30	GGAAAGCTTCAGTTCCTTTGTTT	4	21	CV473056	3'	38.09	+	Tubers
stu-miR5368	gma-miR5368	170	-73.19	GGACAGTCTCAGGTAGACA	0	19	CK267196	3'	52.63	-	Leaves and roots
stu-miR5492	osa-miR5492	160	-18.30	TTAAGGAGAATAGAAAATGGT-	4	20	DN906214	3'	30.00	-	Roots
stu-miR5536	osa-miR5536	182	-49.50	TGAGGTAGTGACATATGGTAC	4	22	BI178676	3'	40.90	+	Stem buds
stu-miR5538	osa-miR5538	116	-28.60	ACTGAACCTCAATCACTTGCTGC	0	22	BQ113869	5'	45.45	-	Mixed tissue
stu-miR5630	ath-miR5630a	71	-20.20	GCTAAGAGCGGTACGGATAGA	2	21	JZ168047	3'	52.38	-	Whole plant
stu-miR5643	ath-miR5643a	106	-23.50	AGGCTCTTTAGATCTGGTTGG	3	21	JG729499	5'	47.61	+	Leaves
stu-miR5649	ath-miR5649a	80	-14.80	ATTGAAAATGTT-ATTAATAT	3	20	JG562311	3'	10.00	+	Roots
stu-miR5657a	ath-miR5657a	119	-31.73	TGGACAGGGTGAGATTTGGTG	2	21	JG564198	5'	52.38	+	Roots
stu-miR5657b	ath-miR5657a	59	-8.30	TGGATAAGATTAGATTTGGTG	2	21	DV627149	5'	33.33	+	Tubers
stu-miR5721	bra-miR5721	127	-28.48	AAAAATGGAGTGAGAAAAGAT	4	21	AM908134	3'	28.57	+	Leaves
stu-miR5722	bra-miR5722	145	-42.70	TGGAA---AGTCATGTGGAACG	4	19	BM404303	5'	47.36	+	Roots
stu-miR5747	mtr-miR5747	287	-68.81	AAAAGAATACTCA-ACAACACTTT	4	24	DN938935	5'	25.00	+	Tubers
stu-miR5748	mtr-miR5748	62	-11.80	GAAAAGACATTTGGAAGTCTTT	4	21	AM907421	3'	33.33	+	Leaves
stu-miR5749	mtr-miR5749	130	-19.35	CTCGG-ITGAAAATAATTTTCTT	3	22	JG562288	3'	27.27	-	Roots
stu-miR5754	mtr-miR5754	145	-43.75	TCTTGCACTCATCATCCATGGA	3	22	JG565359	5'	45.45	+	Roots
stu-miR5818	osa-miR5818	94	-19.80	TCAAACTAGAAAGGGACAAGTT	3	21	CN213303	5'	38.09	+	Callus
stu-miR5819	osa-miR5819	131	-41.50	AAGAGGAGAGGAACGGCGGGCG	3	21	CO502612	3'	66.66	+	Roots
stu-miR6019	nta-miR6019	148	-19.69	ACAAGTGACCCGTAATAATGTTT	3	21	BG594326	5'	38.09	-	Tubers
stu-miR6021a	nta-miR6021	381	-224.30	CTCGAAGAGGGTGCTATTAGA	4	21	DN922111	5'	47.61	-	Tubers
stu-miR6021b	nta-miR6021	130	-30.64	CTGGAAGAGGGTGCTATTGGG	2	21	BQ113991	5'	57.14	-	Mixed tissues
stu-miR6144	nta-miR6144	68	-11.90	CTTCAACTTCTTCATCAT-CC	3	20	DN906271	3'	40.00	-	Roots
stu-miR6145	nta-miR6145d	87	-12.55	ATTGTTACATGTAACACCAGA	4	21	BQ120863	3'	33.33	-	Mixed tissues
stu-miR6148	nta-miR6148b	67	-9.20	TTTGTAAATCGTTGTTTCATG	4	21	CK263430	5'	28.57	-	Leaves and roots

Table 2. (Continued).

stu-miR6154	nta-miR6154a	65	-16.80	AGGGTCTCCTGGAGAAAAGTTC	2	21	CN464759	3'	52.38	+	Tubers
stu-miR6156	nta-miR6156	77	-20.10	TTGAAGATTTGTCTATTATGT	4	23	BQ504597	3'	26.08	-	Mixed tissues
stu-miR6164a	nta-miR6164a	68	-8.60	TCACATAAAATGGGACGGAGA	3	21	DN907997	3'	42.85	-	Tubers
stu-miR6164b	nta-miR6164a	488	-55.48	TCACATAAAATGAAAACGGAGG	1	21	CK272906	3'	38.09	-	Leaves and roots
stu-miR6164c	nta-miR6164b	65	-14.80	TCACATAAAATGGGACGGAGG	3	22	DN939649	3'	27.27	-	Leaves and roots
stu-miR6252	osa-miR6252	74	-14.80	ATTGGATGTATTAAGAGAGGGTA	4	21	DN939649	3'	42.85	-	Tubers
stu-miR6270	ppe-miR6270	168	-32.01	TTCTGATATTTGGATTTCAAT	3	23	DN907450	5'	34.78	-	Tubers
stu-miR6278	ppe-miR6278	86	-20.20	CAAACCTTGTGACATATGGCC	3	21	CK851024	3'	23.80	-	Stolon
stu-miR6284	ppe-miR6284	126	-31.40	TTTGGACAATGGATGAAGATG	4	22	BI920700	3'	45.45	+	Stem buds
stu-miR6287	ppe-miR6287	99	-20.38	GAAGAAATGGAAGTTTTGGGT	2	21	EG015285	5'	38.09	-	Tubers
stu-miR6426	ptc-miR6426a	312	-62.26	GTGAAGAAAATG-AAAGTGAAGA	2	21	DN921108	3'	42.85	-	Tubers
stu-miR6438	ptc-miR6438b	149	-27.00	ATGCACACAGAATAAGGTGAAAA	3	20	BG095629	3'	35.00	-	Leaves
stu-miR6439	ptc-miR6439b	84	-11.00	CCCAGAAAGCCATCCTAGCTA	3	22	BF186839	3'	36.36	-	Stolon
stu-miR6440	ptc-miR6440c	55	-10.30	AAGTTTGCTTGATTTCCGAGTT	4	21	BG599111	3'	52.38	-	Tubers
stu-miR6461	ptc-miR6461	238	-41.55	TAGCTAGCAA-TACATAGAAC	3	21	CN462150	5'	33.33	+	Tubers
stu-miR6468	ptc-miR6468	103	-20.30	GTTTTCTCTGAAATGGCTCCCC	4	20	CK863677	3'	35.00	-	Roots
stu-miR6476	ptc-miR6476a	42	-14.70	CAAGTGGAGATGAGACATGA	4	21	CV502876	3'	52.38	-	Floral buds
stu-miR7749	bdi-miR7749	57	-24.20	ACCGCGATGGCGGCGATTGCGGC	3	20	BG594207	3'	45.00	-	Tubers
stu-miR7777	bdi-miR7777	130	-24.70	GCTGACAAAACACCATCCTCAA	4	24	CK257023	5'	75.00	+	Callus
stu-miR7785	bdi-miR7785	86	-22.90	GTAGTATTGGTGATGGGAGAAGGC	3	21	JG563516	5'	47.61	-	Roots
stu-miR7816	ptc-miR7816	271	-61.08	AAATGTTGTTATATCACTGTT	2	24	DN743351	5'	50.00	-	Stolon and tubers
stu-miR7822	ptc-miR7822	273	-52.46	TTTGAAAATTGAAAAAAAATGGA	2	21	DN587501	5'	23.80	-	Tubers
stu-miR7825	ptc-miR7825	146	-24.43	TTGAAGAAAAGG-AGAAAAAATAG	4	21	JG558460	3'	19.04	-	Leaves
stu-miR7826	ptc-miR7826	167	-34.05	TTACCAGTTTTCAAATTTCTGG	3	21	CK262341	5'	28.57	-	Leaves and roots
stu-miR7828	ptc-miR7828	287	-58.95	GATGACATGGGAGAACAAAAATG	3	21	JG566250	5'	38.09	+	Roots
stu-miR7830	ptc-miR7830	187	-42.30	TGATCTAGAGAAAATTTG-T	3	21	CK265039	5'	38.09	+	Leaves and roots
stu-miR7835	ptc-miR7835	138	-31.15	GATGGGATTTGGGGGAAGTG	3	19	JG563947	5'	31.57	+	Roots
stu-miR8123a	ppe-miR8123	53	-13.10	TGAGCAATGGCACAGAGACCC	2	21	CV471760	5'	52.38	+	Tubers
stu-miR8123b	ppe-miR8123	112	-39.03	CTGTGCCAATTGCTCAAGC	3	21	BE343819	5'	57.14	-	Stolon
					1	18	BE343819	5'	55.55	+	Stolon



Figure 1. The newly identified potato miRNA secondary structures. The potato pre-miRNA secondary structures were developed through the Mfold algorithm. These structures clearly show that the mature miRNAs are in the stem portion of the stem-loop structures.

(17 (14% each)); stolon (9 (7%)); leaves, leaf callus, and root (8 (6.6% each)); floral buds (5 (4%)); petioles and stem buds (2 (2% each)); and leaves, petioles, whole plant, and stolon tuber (1 (1% each)). These findings are similar to those of other reports (Zhang et al., 2013; Lakhotia et al., 2014) and suggest an organ-dependent expression pattern. The organ-specific miRNA expression could be utilized to manage organogenesis in potato. Due to availability of genome and mRNA sequences, genome-wide searches for sense-antisense transcripts have been reported (Orlov et al., 2012). In the same study, 65 (54%) of the 120 novel miRNAs were found on antisense and 55 (46%) on sense strands of the transcripts. RT-PCR experimental validation was also performed for the newly discovered miRNAs. Ten randomly selected miRNAs were subjected to RT-PCR validation studies: stu-miR2869, stu-miR5538, stu-miR475, stu-miR1870, stu-miR1873, stu-miR2608, stu-miR4993, stu-miR1918b, stu-miR5040, and stu-miR4995. All 10 miRNAs were validated (Figure 2). This experimental confirmation strengthens the expressed nature for bioinformatically explored potato miRNAs. The novel miRNAs were characterized in terms of source miRNAs, pre-miRNA length, MFE, mature miRNA sequence, number of nucleotide mismatches, mature sequence length, source EST, mature sequence arm, GC%, strand location, and organ of expression (Table 2).

3.3. Sense/antisense miRNA genes in potato

In both vertebrates and invertebrates, including humans and fruit flies, another class of miRNA, known as sense/antisense miRNA, was identified for the first time in 2008 by Stark et al. In this class, miRNAs are transcribed and processed from both sense and antisense transcripts derived from the same genomic loci. In this study, we found miRNA miR8123 with both an antisense (miR8123a) and sense (miR8123b) strand orientation in the same potato (Figure 3). This result is in agreement with Zhang et al. (2008). Since the mature and precursor sequences of sense/antisense miRNAs are different, it was proposed that they might have different targets or implement their functions through different mechanisms in plants (Zhang et al., 2008).

3.4. Cluster miRNAs gene in potato

In animals, a large number of miRNAs have been found in clusters and have been predicted to have similar expression

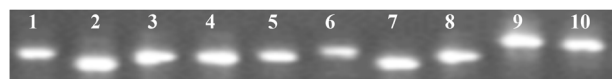
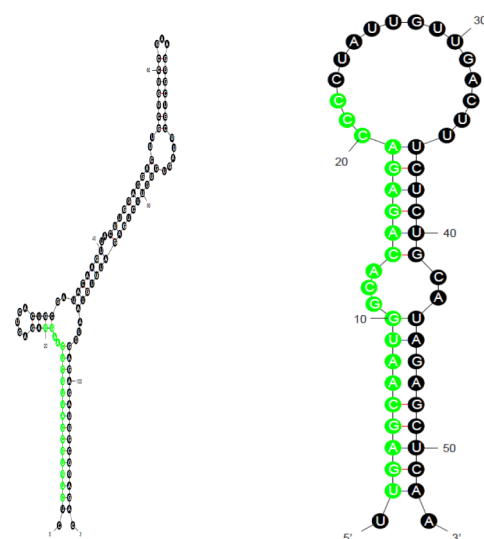


Figure 2. Experimental validation of potato miRNAs by RT-PCR. The potato miRNAs (1: stu-miR2869, 2: stu-miR5538, 3: stu-miR475, 4: stu-miR1870, 5: stu-miR1873, 6: stu-miR2608, 7: stu-miR4993, 8: stu-miR1918b, 9: stu-miR5040, 10: stu-miR4995) were selected and subjected to experimental validation by RT-PCR. The product of each sample was separated on a 2% (w/v) agarose gel.



stu-mir8123b (sense)

stu-mir8123a (antisense)

Figure 3. Potato sense/antisense miRNA gene. The newly explored potato miRNA miR8123 was observed with an antisense (miR8123a) and sense (miR8123b) stand orientation in the same EST (*BE343819*). Mfold (version 3.6) was applied to predict their secondary structures, showing mature sequences in green.

profiles and functions (Yu et al., 2006). miRNA clusters have rarely been detected in plants; they were first reported by Jones-Rhoades and Bartel (2004). In this study, we also identified 1 pre-miRNA cluster in potato including 2 mature miRNAs (miR6164b and miR6164c) within the same pre-miRNA sequence with a distance of 137 nt separating them (Figure 4). On the basis of current

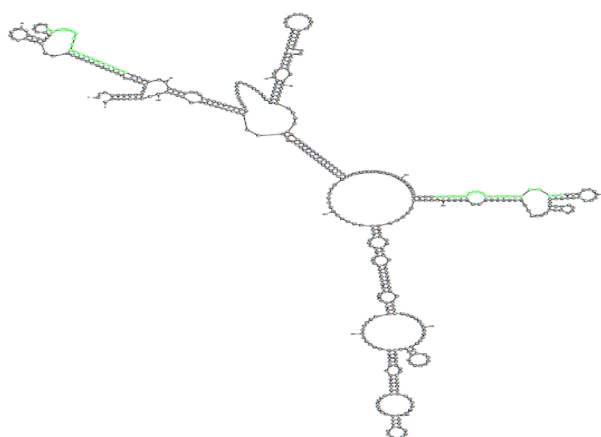


Figure 4. Potato pre-miRNA cluster. The potato miRNA (stu-miR6164) was found as a pre-miRNA cluster with 2 mature miRNAs (miR6164b and miR6164c). The pre-miRNA cluster secondary structure was created by Mfold (version 3.6), showing mature sequences in green within the same pre-miRNA sequence with a distance of 137 nt apart from each other.

available literature, this miRNA family (miR6164) was found for the first time in potato as a cluster.

3.5. Convergence and phylogenetic studies

The newly identified potato miRNA stu-miR535 was further considered for convergence and phylogeny analyses due to its conserved nature. The alignment and cladogram tree of stu-miR535 was created using the neighbor-joining clustering method with *Malus domestica* (mdm), *Theobroma cacao* (tcc), *Citrus sinensis* (csi), *Ricinus communis* (rco), and *Manihot esculenta* (mes) by the publicly available ClustalW, a multiple sequence alignment tool (Larkin et al., 2007), and Web-Logo, a sequence logo generator (Crooks et al., 2004). stu-miR535 was observed in convergence with *Malus domestica*, *Theobroma cacao*, *Citrus sinensis*, *Ricinus communis*, and *Manihot esculenta*, as shown in Figure 5. The phylogenetic cladogram (Figure 6) clearly shows that on the basis of sharing a more recent common ancestor the potato miRNA is more closely related to *Theobroma cacao* than *Malus domestica*, *Citrus sinensis*, *Ricinus communis*, or *Manihot esculenta*. The same result for the conserved nature of miRNA among its homologs was also reported by Zhang et al. (2009) in plants.

3.6. Targeted genes of new potato miRNAs

Target prediction is the crucial criterion for the screening of newly discovered miRNAs. In this study, we retrieved a total of 433 novel potential proteins for the 120 newly discovered potential candidate miRNAs in potato. This also strengthens the philosophy that a single miRNA can bind to and regulate many different mRNA targets and that, on the contrary, several different miRNAs can bind to and cooperatively control a single mRNA target (Lewis et al., 2003). The predicted genes were categorized as: metabolism (34%), transcription factors (23%), hypothetical proteins (9%), transporter (8%), signal

transduction (7%), disease-related (6%), structural protein (6%), stress-related (6%), and growth and development (2%) (see Supplementary Table, online only). Such protein targets were also found in previous studies (Frazier et al., 2010; Xie et al., 2010; Bai et al., 2012).

Out of 433 of the novel miRNAs, 147 were observed to regulate metabolic proteins, such as carotenoid cleavage dioxygenase (CCD), dihydrodipicolinate reductase, fructose-bisphosphate aldolase, glycosyl transferase family 48, peptidyl-prolyl cis-trans isomerase, and UDP-glucuronic acid decarboxylase (Supplementary Table). Metabolism-related genes targeted by miRNAs were also found in prior publications in plants and animals (Barozai, 2012; Ji et al., 2012).

Plant carotenoids act as precursors to C13-norisoprenoids, a set of apocarotenoid complexes with different biological activities. Enzymatic processing of carotenoids catalyzed by members of the CCD family has been observed to generate a number of industrially significant volatile flavor and aroma apocarotenoids (Auldridge et al., 2006). This means that by regulating the members of the CCD family, one can manage the carotenoid-derived flavor and aroma profile in both fruits and flowers. In our exploration, the potato miRNA family miR5219 putatively targeted the CCD gene. Fructose 1,6-bisphosphate aldolase (FBA) is another significant enzyme of glycolysis and gluconeogenesis in plants. Recently, Lu et al. (2012) found a responsive expression behavior for FBA in *Arabidopsis thaliana* under salt, drought, abnormal temperature, and abscisic acid (ABA), suggesting a protective role for it. In this study, FBA was predicted as a putative target for stu-miR6164. By managing the expression of FBA using stu-miR6164, one can not only fine-tune the glycolysis and gluconeogenesis for better yields, but can also manage the plant under different stresses.

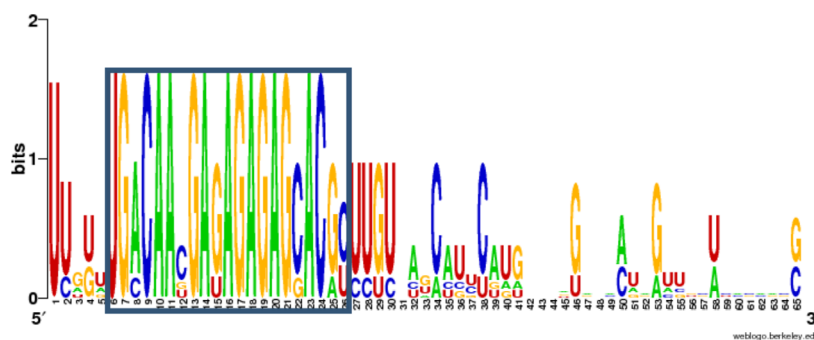


Figure 5. Potato miRNA convergence study. Alignment of potato miRNA stu-miR535 with *Malus domestica* (mdm), *Theobroma cacao* (tcc), *Citrus sinensis* (csi), *Ricinus communis* (rco), and *Manihot esculenta* (mes) was done by Web-Logo, a sequence logo generator, showing a conserved nature for the miRNA. The mature sequences are highlighted within a box.

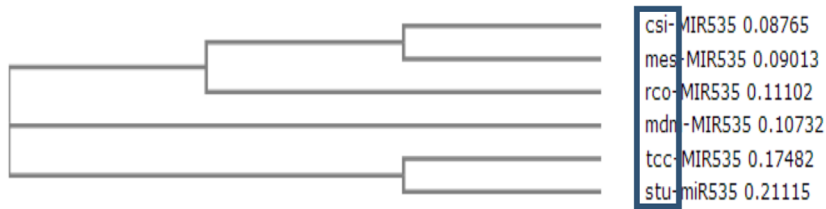


Figure 6. Potato miRNA phylogenetic study. The phylogenetic study of the potato miRNA stu-miR535 with *Malus domestica* (mdm), *Theobroma cacao* (tcc), *Citrus sinensis* (csi), *Ricinus communis* (rco), and *Manihot esculenta* (mes) was done with the help of ClustalW and a cladogram tree was generated using the neighbor-joining method. The phylogenetic tree showed that, on the basis of sharing a more recent common ancestor, the potato miRNA is more closely related to *T. cacao* than to *M. domestica*, *C. sinensis*, *R. communis*, or *M. esculenta*.

In addition, 98 of the novel miRNAs were involved in targeting transcription factors, such as MYB transcription factor, BZIP transcription factor bZIP21, homeobox-leucine zipper protein, translation initiation factor 6-2, transcription factor BIM2, heat stress transcription factor HSFA9, and homeobox protein Nkx-6.1, which are targeted by stu-miR5254, stu-miR1450, stu-miR1879, stu-miR2673, and stu-miR5819, respectively. This class of protein has been considered as a miRNA target in various studies in different plant species (Zeng et al., 2009; Frazier et al., 2010; Xie et al., 2010; Barozai et al., 2012).

The transcription factor MYB has an important regulatory role in many developmental and physiological mechanisms in plants by regulating the transcription pathway. This suggests that MYB will be an important family of transcription factors to use for inducing desirable traits in potato. Another family of transcription factors, zinc finger, is thought to be involved in many biotic and abiotic stresses, as it is the responding gene to help the plant cope under assorted stresses (Kodaira et al., 2011). The C2H2-type of zinc finger was found to be involved in potato responses to salt and dehydration stresses through an ABA-dependent pathway (Tian et al., 2010). In the same study, 6 newly identified potato miRNA families, miR3951, 5240, 5649, 6284, 6468, and 7830, were found to target the zinc finger transcription factor family. These miRNA families will be an important resource for regulating the zinc finger family proteins for the fine-tuning of potato under various biotic and abiotic stresses.

Thirty-nine of 433 novel miRNAs of potato were found to target hypothetical proteins. Such findings were also published earlier (Wang et al., 2012). Similarly, 34 of the 433 genes targeted by potato miRNAs are engaged in transport pathway (Supplementary Table). Some examples of the transporter proteins targeted by newly profiled potato miRNAs are sucrose transporter-like protein, ABC transporter A, potassium channel NKT2, ammonium transporter 1 member 3, glucose/galactose transporter, amino acid transporter, and the drug/metabolite exporter

family. Such findings are in agreement with the other works in the miRNA field (Ji et al., 2012; Wang et al., 2012). The ABC transporters are significant regulators of plants under biotic and abiotic stresses. The ABC transporters are also thought to shuttle xenobiotic molecules across the cell membrane and cytosol to vacuole, thus playing a vital role in detoxification processes (Kretschmar et al., 2011). Here the stu-miR1446, stu-miR2090, stu-miR3951, stu-miR5150, stu-miR5205, stu-miR5449, and stu-miR6438 miRNAs were also newly explored to target the ABC transporter. By using these miRNAs, potato tolerance for biotic, abiotic, and xenobiotic stresses can be enhanced.

Twenty-eight of the miRNAs targeted were involved in disease-related proteins, such as late blight resistance protein R3a, TIR-NBS-LRR type disease resistance protein, bacterial spot disease resistance protein 4, enhanced disease susceptibility 1 protein, and disease resistance protein RPP13 (Supplementary Table).

The main destructive disease of potato is late blight, which can cause losses of 16% in total yield. Late blight is caused by *Phytophthora infestans*, which can infect the whole plant, as well as stems, leaves, and tubers (Fry, 2008). The plants have 2 main families of disease-resistance proteins, sharing the Toll/interleukin-1 receptor domain in common. A nucleotide-binding site and a variable number of C-terminal LRRs are encoded by a huge number of disease-resistance genes (R-genes). The plant's first layer of immune system has the ability to identify plant pathogens by interaction of the R-genes with virulence effectors released by pathogens (Fry, 2008). The recognition of a virulence factor from pathogens by the related R proteins initiates a series of reactions that stimulate the levels of salicylic acid, jasmonic acid, phenyl ammonium lyase, and systemin. The production of these biochemical signals is known to induce many common R-gene signaling cascades, causing cell apoptosis and modification in gene expression patterns (Vlot et al., 2008). The tu-miR1450, 1510, 2597, 2678, 3623, 5183, 5261, 5722, and 6019 miRNAs were found to target these disease-

related proteins. With the help of these potato miRNAs, one can better manage the plant against late blight and other disease-causing pathogens.

Structural proteins like flotillin-like protein 2, ribosomal protein L11-like protein, hydroxyproline-rich cell wall protein, and 60S acidic ribosomal protein P2 were also explored as putative targets regulated by the novel identified miRNAs (Supplementary Table). The plant flotillin-like proteins have a key role in establishing the symbiotic relationship between plants and bacteria. Haney and Long (2010) used artificial miRNAs to elucidate the role of the flotillin-like gene family in symbiotic interactions in *Medicago truncatula*. stu-miR1446 was found to target this important symbiotic relationship creator protein. By utilizing this information, one can further elucidate the role of miRNAs in plant symbiotic relationships.

Some potato miRNAs were discovered to target the protein functioning in the process of cell signaling pathways. Some of these are serine/threonine kinase-like protein, DELLA protein RGL1, receptor-like protein kinase, cyclic nucleotide-gated channel beta, and receptor protein kinase. Similar findings were observed by many researchers in various organisms (Chen et al., 2012; Ji et al., 2012). This will help us to regulate cell signaling via miRNAs in plants.

The newly discovered miRNAs were also engaged in the targeting of growth- and development-related proteins, such as auxin-induced protein X10A, gibberellin-

regulated protein, aux/IAA protein-like, and fruit-ripening protein-like (Supplementary Table). Such proteins, which play a function in growth and development, are already recognized in a large number of plant species (Zeng et al., 2009; Barozai et al., 2013a).

In the same study, conserved novel potato miRNAs were also observed to target the stress-related proteins. One such important protein group is heat shock proteins (HSPs), which are targeted by stu-miR1320 and 5035. The fine-tuning of HSPs via plant miRNAs can enhance plant tolerance for cold, heat, salt, and drought stresses (Mahmood-ur-Rahman et al., 2013). This finding is in agreement with many other studies (Gao et al., 2011; Bai et al., 2012; Chen et al., 2012).

3.7. Conclusion

In the current study, a total of 120 miRNAs belonging to 110 miRNA families and their 433 targeted genes were identified and functionally analyzed in potato, an economically important solanaceous plant species. All of these miRNAs have been reported here for the first time. These results will serve as reference data to elucidate the regulation, management, and modification of this economically important crop plant at the micromolecular level. This will help us to improve potato production and biotic and abiotic stress tolerance in the near future. Furthermore, these miRNAs and their targets are also powerful functional genomic resources in the plant kingdom.

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Supplementary Table. The potato miRNA families and their putative protein targets, predicted with the help of miRU, BLASTn, and RNAhybrid tools, are presented. The miRNA families, GenBank accession numbers, targeted protein names, functions, and alignment results are provided.

miRNA	Target acc.	Target description	Function	Alignment
stu-miR473	TC216696	Serine/threonine kinase-like protein	Metabolism	miRNA 20 CCUCUCGGGACUCCUCUAG 1 Target 97 UGAGAGUCCUGGGGGAGAUG 116
stu-miR475	TC217886	MGC69562 protein	Transcription factor	miRNA 21 AGUUUAGUUACCCGAGACAUU 1 Target 198 UUGAAUCA AUGGGUUUUGUGA 218
stu-miR475	TC213251	Thioredoxin	Metabolism	miRNA 20 GUUUAGUUACCCGAGACAUU 1 Target 78 CAAAUCAAUGGGUUGUGUAC 97
stu-miR475	CN465472	ATP binding	Transcription factor	miRNA 21 AGUUUAGUUACCCGAGACAUU 1 Target 238 UUGAAUCA AUGGGUUCUGAAA 258
stu-miR475	BE339603	Os01g0279200 protein	Hypothetical protein	miRNA 20 GUUUAGUUACCCGAGACAUU 1 Target 52 CAUAUCA AAGGGCUCUGUAC 71
stu-miR535	CK852226	Peroxidase	Metabolism	miRNA 21 UGCAGGAGAGAGAGGAACCGU 1 Target 555 ACGUCCUCUCUCUCCUUGGCA 575
stu-miR535	TC215818	Sucrose transporter-like protein	Transporter	miRNA 20 GCAGGAGAGAGAGGAACCGU 1 Target 1519 UGUUUUCUCUCUUCUUGGCA 1538
stu-miR535	TC222072	Proteinase inhibitor 1	Metabolism	miRNA 20 GCAGGAGAGAGAGGAACCGU 1 Target 47 UGUUUUCUUCUUCUUGGCA 66
stu-miR535	DN921124	ATP-dependent Clp protease proteolytic	Transcription factor	miRNA 20 GCAGGAGAGAGAGGAACCGU 1 Target 285 UGUUCUUUCUUCUUGGUG 304
stu-miR1320	TC219292	Heat shock cognate 70 kDa protein	Stress-related	miRNA 20 AUGUUUUAAGGAGGUAAGGU 1 Target 500 UAUUAGAUUCCUCCAUUUCA 519
stu-miR1320	TC206427	Subtilisin-like protease	Metabolism	miRNA 20 AUGUUUUAAGGAGGUAAGGU 1 Target 257 UACAAAGUUUCUUAUCCU 276
stu-miR1320	TC226468	Protein phosphatase 2C	Metabolism	miRNA 20 AUGUUUUAAGGAGGUAAGGU 1 Target 777 UACAGAGUUUCUCCAUUUUG 796
stu-miR1446	XM_006351501	DELLA protein RGL1	Transcription factor	target 5' A U A U 3' UCUU GGAGA AGUUCA AGAG CCUCU UCAAGU miRNA 3' UAACUC C 5'
stu-miR1446	XM_006347925	Flotillin-like protein 2	Structural protein	target 5' G G U U A G 3' UUCAU G GGA AG AGUUUA GAGUA C CCCU UC UCAAGU miRNA 3' A A U C 5'
stu-miR1446	XM_006359544	Polyprenol reductase 2	Metabolism	target 5' A GUA AU A 3' UC GUUGA GAGAGGGUUCA AG UAACU CUCUCUCAAGU miRNA 3' AG CC 5'
stu-miR1446	XM_006345618	Protein GPR107	Structural protein	target 5' A U A AA C 3' AUUGGG GGA GAGA GUUCA UAACUC CCU CUCU CAAGU miRNA 3' AGAG 5'

Supplementary Table. (Continued).

stu-miR2673	CK854379	Cyclic nucleotide-gated channel beta	Signal transduction	miRNA 22 UUCCUUCUCCUUCUCCUUCUCC 1	Target 530 AAGGAGGAGGAAGAGGAAGAGG 551
stu-miR2673	TC218494	Heat stress transcription factor HSFA9	Transcription factor	miRNA 22 UUCCUUCUCCUUCUCCUUCUCC 1	Target 484 AAGGAGGAGGAGGAGGAGGAGG 505
stu-miR2678	TC203592	TIR/NBS/LRR protein	Disease-related	miRNA 21 UUCUAUGAGCGUUUUUGAAGU 1	Target 218 AAGAUACUCGCAAAACCUUCA 238
stu-miR2678	TC222251	Resistance gene	Stress-related	miRNA 21 UUCUAUGAGCGUUUUUGAAGU 1	Target 95 AAGAUACUCGCAAAAUUUUA 115
stu-miR2678	TC197676	Biotin carboxylase	Metabolism	miRNA 21 UUCUAUGAGCGUUUUUGAAGU 1	Target 820 AAGAUGCUUUCAAAAACUUCA 840
stu-miR2678	BG095680	Resistance gene	Stress-related	miRNA 20 UCUAUGAGCGUUUUUGAAGU 1	Target 170 AGAUACUCGCAAAAUUUUA 189
stu-miR2678	CX161632	TAGL1 transcription factor	Transcription factor	miRNA 21 UUCUAUGAGCGUUUUUGAAGU 1	Target 80 AAGAUGCUCGCAAAACUUC 100
stu-miR2678	CK263552	Predicted protein	Hypothetical protein	miRNA 21 UUCUAUGAGCGUUUUUGAAGU 1	Target 321 AAGAAACUUGUCAAAACUUCA 341
stu-miR2868	CN463660	Transcriptional regulator, LysR family	Transcription factor	miRNA 20 AAAGAGGAUGUGUUUUGGUU 1	Target 662 UUUUUUAUACACAAAACUAA 681
stu-miR2868	TC214205	NIM1-like protein 2	Hypothetical protein	miRNA 20 AAAGAGGAUGUGUUUUGGUU 1	Target 18 UUUUUCCUACUCAAAAUCAU 37
stu-miR2868	CV429377	Predicted protein	Hypothetical protein	miRNA 20 AAAGAGGAUGUGUUUUGGUU 1	Target 1314 UUUUUUUUACACAACACCAA 1333
stu-miR2868	TC219925	HEAT repeat-containing protein	Transcription factor	miRNA 20 AAAGAGGAUGUGUUUUGGUU 1	Target 346 AUUCUUUUACACAAAACCAU 365
stu-miR2868	TC218468	Probable pectate lyase P18 precursor	Metabolism	miRNA 20 AAAGAGGAUGUGUUUUGGUU 1	Target 799 AUUCUUUUACACAGGACCAA 818
stu-miR2869	TC221203	Arsenical-resistance protein	Stress-related	miRNA 21 AGGGUCUUAAAUAACAGCACC 1	Target 510 UUUCAGAUUUUGAUGUUGUGG 530
stu-miR2939	DN589431	Nitrilase/cyanide hydratase and apolipoprotein N	Metabolism	miRNA 20 ACCGAAUCACAACAGCAAUG 1	Target 633 UGGCUUAG-GUUGUUGUAC 651
stu-miR2939	AW907320	Per1-like family protein	Metabolism	miRNA 21 GACCGAAUCAC-AACAGCAAUG 1	Target 451 CUGCCUUAGUGAUUGUCGUUAC 472
stu-miR2939	CK267456	Ammonium transporter 1 member 3	Transporter	miRNA 20 ACCGAAUCACAACAGCAAUG 1	Target 150 UGGCUUAUUAUGUUGUUGC 169
stu-miR3623	BE340934	Resistance gene	Stress-related	miRNA 22 ACCACGAACCUACUUGAUCACU 1	Target 569 UGGUGCUUGGAUGAACUAGUGA 590

Supplementary Table. (Continued).

stu-miR5205	TC204968	Cytosolic nucleoside diphosphate kinase	Metabolism	miRNA 23 GAGGGAGGCAGGGUAAAAUUAUAC 1 : : : : : : : : : : : : Target 993 CUCCCUCUGUCUCAUUUUACAUG 1015
stu-miR5205	TC217058	50S ribosomal protein L21	Structural protein	miRNA 23 GAGGGAGGCAGGGUAAAAUUAUAC 1 : : : : : : : : : : : : Target 633 CUCCCUCUACUCCAUUUUGAUGUG 655
stu-miR5205	TC209413	Ribosomal protein S8	Structural protein	miRNA 23 GAGGGAGGCAGGGUAAAAUUAUAC 1 : : : : : : : : : : : : Target 386 CUCUCUCUGUUCUCAAUUUUUGUG 408
stu-miR5205	TC213482	IAA-alanine resistance protein 1	Stress-related	miRNA 23 GAGGGAGGCAGGGUAAAAUUAUAC 1 : : : : : : : : : : : : Target 874 CUCCUUCGUAUCAAUUUAUGUG 896 target 5' A G C AUUG U 3' GGA UCACUG CAGCAGU GUC UUU AGUGAU GUCGUUA CAG
stu-miR5208	XM_006346488	CTP synthase-like	Metabolism	miRNA 3' UUG G U 5' target 5' U AGG G G 3' AACAAAUUCA GAUG AAUG UUGUUUGAGU UUGUC UUAC
stu-miR5208	XM_006360774	Disease resistance protein RPP13	Disease-related	miRNA 3' GA G AG 5' target 5' U UG UGGUGA UCUGAUA A 3' AGCUU UGAC AGCA GUGUC UUGAG AUUG UCGU UACAG
stu-miR5208	XM_006355736	Nuclear transcription factor Y subunit B-5	Transcription factor	miRNA 3' UUGU UG 5' target 5' A AGAA AA A 3' AACAAA UCA UUAACAGUAA UUGUUU AGU GAUUGUCGUU
stu-miR5208	XM_006344365	Histone-lysine N-methyltransferase ATX4	Metabolism	miRNA 3' G ACAG 5' miRNA 20 GGAAUUAAGGUAUGGUAUUU 1 : : : : : : : : : : : : Target 756 CCUUGAUUGCAUACCAUUUU 775
stu-miR5210	TC197054	Disease resistance protein B11	Disease-related	miRNA 20 GGAAUUAAGGUAUGGUAUUU 1 : : : : : : : : : : : : Target 203 UCUUAACUCCUACCAUUUA 222
stu-miR5210	TC209645	Resistance-like protein I2GA-SH23-1	Stress-related	miRNA 21 AGGAAUUAAGGUAUGGUAUUU 1 : : : : : : : : : : : : Target 563 UCCUUGAUUGCAUACCAUUUU 583
stu-miR5210	TC224268	NBS/LRR resistance protein-like protein	Disease-related	miRNA 21 AGGAAUUAAGGUAUGGUAUUU 1 : : : : : : : : : : : : Target 75 UUCUUUUUUCCAUAUUAUUU 95
stu-miR5210	DN942201	Ubiquitin-protein ligase-like protein	Metabolism	miRNA 22 ACGACGACGACUAUAAGGUACC 1 : : : : : : : : : : : : Target 80 UGUUGGUGCUGAUUUUCUGUGG 101
stu-miR5219	EG012650	Arginine biosynthesis bifunctional	Metabolism	miRNA 21 CGACGACGACUAUAAGGUACC 1 : : : : : : : : : : : : Target 1214 GCUGCUGUUGAUUUUCCACGG 1234
stu-miR5219	TC216300	Carotenoid cleavage dioxygenase	Metabolism	miRNA 21 GUAUGUAAGAAGUCGACAUUC 1 : : : : : : : : : : : : Target 299 UAUGUGUUUUUCAGUUGUGAG 319
stu-miR5222	TC218401	Predicted protein	Hypothetical protein	miRNA 22 AGUAGAAGUUCAUUUUAGGAAC 1 : : : : : : : : : : : : Target 266 UCAUCUUCUCAAUUUUCCUUG 287
stu-miR5223	BG096210	PHAVOLUTA-like HD-ZIPIII protein	Transcription factor	miRNA 20 AAAGGAGGUUCAACAUGUUU 1 : : : : : : : : : : : : Target 201 AUUUCUCCAACUUGUACAAA 220
stu-miR5226	BQ515842	CYP72A52v1	Metabolism	miRNA 20 AAAGGAGGUUCAACAUGUUU 1 : : : : : : : : : : : : Target 134 UUUUUUCGAAGUUGUGCAAAA 153
stu-miR5226	CK854211	Peptidyl-prolyl cis-trans isomerase	Metabolism	

Supplementary Table. (Continued).

stu-miR5254	TC198813	BZIP transcription factor bZIP21	Transcription factor	miRNA 20 GUUUUUACGGAGGUGGAGGA 1 ::: ::::: ::::: Target 291 CAAAGUUGCCUCCAUCUCCU 310
stu-miR5254	TC195569	Progesterone 5-beta-reductase	Metabolism	miRNA 20 GUUUUUACGGAGGUGGAGGA 1 : ::::: ::::: : Target 1158 CAAAAAUGCCUUCAUCUCAU 1177
stu-miR5254	TC224727	Beta-galactosidase	Metabolism	miRNA 21 AGUUUUUACGGAGGUGGAGGA 1 : ::::: ::::: :: Target 864 UCAAAAAUGGCUCUACCUACU 884
stu-miR5254	CK861831	MYB transcription factor	Transcription factor	miRNA 20 GUUUUUACGGAGGUGGAGGA 1 : ::::: : : : : : Target 273 CAAAAAUUCUUCUACCUUUU 292
stu-miR5261	TC194598	Resistance-like protein I2GA- SH194-2	Stress-related	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 652 GCUAAAGCGGUUACAAUGA 671
stu-miR5261	TC194600	Resistance-like protein I2GA- SH23-1	Stress-related	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 652 GCUAAAGCGGUUACAAUGA 671
stu-miR5261	TC194599	Late blight resistance protein R3a	Disease-related	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 652 GCUAAAGCGGUUACAAUGA 671
stu-miR5261	CK278238	Tobacco fibrillar homolog	Metabolism	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 329 GGUGAAGCUGUCUACAAUGA 348
stu-miR5261	TC194597	Resistance-like protein I2GA- SH23-3	Stress-related	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 652 GCUAAAGCCGUUACAAUAA 671
stu-miR5261	DN940362	70 kDa microtubule associated protein Type 2	Structural protein	miRNA 20 CGGUUUUGGCAGAUGUUACU 1 : : : : : : : : : : Target 410 GCCAAAACCGUUUGCGUUGA 429
stu-miR5265	BQ518704	DNA-directed RNA polymerase II subunit RPB7	Transcription factor	miRNA 20 AUUGGUACUUUUGUAGUGAA 1 : : : : : : : : : : Target 431 UAACCAUGAAAACAUCACUU 450
stu-miR5265	TC225509	Salicylic acid/benzoic acid	Metabolism	miRNA 20 AUUGGUACUUUUGUAGUGAA 1 : : : : : : : : : : Target 25 UAAAAUUGAAAACAUAUACUA 44
stu-miR5265	BQ511009	NB-ARC domain containing protein	Transcription factor	miRNA 20 AUUGGUACUUUUGUAGUGAA 1 : : : : : : : : : : Target 408 UAACUGUGAACGCAUCAUUU 427
stu-miR5265	BQ114348	Polysialic acid capsule expression protein	Disease-related	miRNA 20 AUUGGUACUUUUGUAGUGAA 1 : : : : : : : : : : Target 261 UAAUAUUGAAAACAUCAUUU 280
stu-miR5265	DV627551	Pyridoxine biosynthesis protein	Metabolism	miRNA 20 AUUGGUACUUUUGUAGUGAA 1 : : : : : : : : : : Target 602 UAACCGGAAAACAUCAUUC 621
stu-miR5269	TC205338	Prolyl-tRNA synthetase	Metabolism	miRNA 21 UUAGUCAACGAGGUGGUGAAA 1 : : : : : : : : : : Target 560 GAUUAGUUGUUGCAUCACUUU 580
stu-miR5288	TC199088	Photosystem I subunit XI	Metabolism	miRNA 22 UAGGGAUACAAAAAUUCUCGAC 1 : : : : : : : : : : Target 300 GUCCCUA-GUUUUUAAAGAGUUG 320
stu-miR5288	TC216656	Transcriptional regulator TyrR	Transcription factor	miRNA 23 UUAGGGAUACAAAAAUUCUCGAC 1 : : : : : : : : : : Target 9 AAGCUCUAUGUUUCUGAGAGUUG 31

Supplementary Table. (Continued).

stu-miR5649	NP005727	CAA57772 putative DNA/RNA binding protein	Transcription factor	miRNA 20 UAUAUUUAUUGUAAAAGUUA 1 :::..... Target 1511 AUACUGAUGACAUUUUCAAC 1530
stu-miR5657	DN907843	ARF_CRYNE ADP-RIBOSYLATION FACTOR	Transcription factor	miRNA 20 UGGUUUUAGAGUGGGACAGGU 1 Target 492 GCCGGAUCUUUUCUUGUCCA 511
stu-miR5657	AM908825	Phosphomannomutase	Metabolism	miRNA 21 GUGGUUUAGAGUGGGACAGGU 1 :::..... Target 656 CACCUAUUCUCACCUUCUCCA 676
stu-miR5721	TC208668	Nicotiana tabacum wound inducive mRNA	Disease-related	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 Target 25 AUCUUUUUCUCACACAUUUUUAU 45
stu-miR5721	CN515643	Patatin-B1 precursor	Stress-related	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 Target 60 AUCUUUUUUUAAUUUUUUAUUUUU 80
stu-miR5721	TC211142	Patatin-15 precursor	Stress-related	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 Target 34 AUCUUUUUUUAAUUUUUUAUUUUU 54
stu-miR5721	TC197668	Patatin-B2 precursor	Stress-related	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 Target 39 AUCUUUUUUUAAUUUUUUAUUUUU 59
stu-miR5721	CN513476	Patatin-03 precursor	Stress-related	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 Target 27 AUCUUUUUUUAAUUUUUUAUUUUU 47
stu-miR5721	BG888822	Glutaredoxin	Metabolism	miRNA 21 UAGAAAAGAGUGAGGUAAAAA 1 :::..... Target 141 AUUUCUUCUCACUCCAUGUU 161
stu-miR5721	TC215304	Aspartic protease inhibitor 8	Metabolism	miRNA 20 AGAAAAGAGUGAGGUAAAAA 1 Target 608 CCUUUUUCUUGCUCUCCAUUUUG 627
stu-miR5722	XM_006353702	Kinesin protein NACK2	Metabolism	target 5' C GAG UG C A A 3' G CU CUGA ACUG CC C GA GACU UGAC GG miRNA 3' A A UG C A 5' target 5' G C A A 3' GUC ACU GGGA GUCC CAG UGG CUCU CAGG
stu-miR5722	XM_006339122	Calcium-dependent protein kinase 20	Metabolism	miRNA 3' A A A GA 5' target 5' A UUA A 3' UCU CUGGGA UGUCC AGA GACUCU ACAGG
stu-miR5722	XM_006365516	Putative disease resistance protein Atlg50180	Disease-related	miRNA 3' AC UG G 5' target 5' A UCU UG G 3' UGUCU CCUGAGAUU CC ACAGA GGACUCUGA GG
stu-miR5722	XM_006365278	RAS-related protein RABD2a	Disease-related	miRNA 3' U CA 5' target 5' A UAU AGUGG A 3' GCCUGAGA CUG CC UGGACUCU GAC GG
stu-miR5722	XM_006356655	2-oxoisovalerate dehydrogenase alpha 1	Metabolism	miRNA 3' ACAGA A 5' target 5' A AC A A 3' UGCC UGAG C GUCC AUGG ACUC G CAGG
stu-miR5722	XM_006353640	TMV resistance protein N	Disease-related	miRNA 3' ACAG U A 5' target 5' U GUGCUCCAU U U 3' UCUACU GGC UGUCC AGAUGG CUG ACAGG
stu-miR5722	XM_006353543	TMV resistance protein	Disease-related	miRNA 3' AC ACU 5'

Supplementary Table. (Continued).

stu-miR5819	EG011776	Predicted protein	Hypothetical protein	miRNA 21 GCGGCGGCAAGGAGAGGAGAA 1 :::.. :::::..	Target 29 CGUCGUUCUUCUUCUCCUCUU 49
stu-miR5819	TC197710	Vitellogenin II	Transporter	miRNA 21 GCGGCGGCAAGGAGAGGAGAA 1 ::: :..:..:..	Target 138 CGCCUCUGUUUCUCCUCUC 158
stu-miR5819	CK251904	Lissencephaly type-1-like homology motif	Transcription factor	miRNA 20 CGGCGGCAAGGAGAGGAGAA 1 :..:..:..:..	Target 425 GUCGCGUUCUUUUUCUUUU 444
stu-miR5819	TC195751	Homeobox protein Nkx-6.1	Transcription factor	miRNA 21 GCGGCGGCAAGGAGAGGAGAA 1 :..:.. :..:..	Target 60 UGCUGCCUUCUUCUCUCUC 80
stu-miR6019	TC212181	Bacterial spot disease resistance protein 4	Disease-related	miRNA 21 UUGUAAAUGCCCAGUAACA 1 :..:..:..:..	Target 155 AAACAUUACGGGUCACUUGU 175
stu-miR6021	TC225640	Hcr9-OR3A	Hypothetical protein	miRNA 21 AGAUUAUCGUGGGAGAAGCUC 1 :..:..:..:..	Target 585 UCUAUAGCACCCUCUUCGAG 605
stu-miR6144	TC212209	Chitinase 3 precursor	Metabolism	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 402 UGAUGAUGAAAAGUUGAAG 421
stu-miR6144	TC211419	TonB family protein	Signal transduction	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 378 UGAUGAUGAAAAGUUGAAG 397
stu-miR6144	CV470320	Predicted protein	Hypothetical protein	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 407 UGAUGAUGAAAAGUUGAAG 426
stu-miR6144	TC212847	HD-ZIP protein	Transcription factor	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 519 UGAUGAUGAAGAAGAUGAAG 538
stu-miR6144	TC196282	Cellulose synthase	Metabolism	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 147 GGAUGAUGAAGAAGAUGAAU 166
stu-miR6144	CK279523	Sulfate transporter	Transporter	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 721 AGGUGAUGAAGAAGUUGAAC 740
stu-miR6144	TC201086	Eukaryotic translation initiation factor 2 beta	Transcription factor	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 115 AGAUGAUGAAGAAGCUGGAG 134
stu-miR6144	CK863165	Cyclic nucleotide-gated ion channel 1	Transporter	miRNA 20 CCUACUACUUCUUCUACUUC 1 :..:..:..:..	Target 119 AGAUGAUGAGGAAGAUGAAG 138
stu-miR6145	TC214868	ATPase	Transcription factor	miRNA 20 GACCACAAUGUACAUUGUUA 1 :..:..:..:..	Target 402 UUGGUGUCACAUUAACAAA 421
stu-miR6145	TC201839	Lipoic acid synthase	Metabolism	miRNA 20 GACCACAAUGUACAUUGUUA 1 :..:..:..:..	Target 1400 CUGUUGUUUAUCUAAUAAU 1419
stu-miR6145	TC196493	26S proteasome subunit 7	Metabolism	miRNA 20 GACCACAAUGUACAUUGUUA 1 :..:..:..:..	Target 547 CUGAUGUUACAUAAUUGAU 566
stu-miR6145	TC210556	Predicted protein	Hypothetical protein	miRNA 21 AGACCACAAUGUACAUUGUUA 1 :..:..:..:..	Target 35 UCUCGUGUUGCGUGUAGCAGU 55

Supplementary Table. (Continued).

stu-miR6148	TC206628	Calcium-binding protein	Transcription factor	miRNA 21 GUACUUGUUUGCUAAUUGUUU 1 .. : : : : : : : : : : : : Target 518 UGUAAACAAAUGAUUAAACAAA 538
stu-miR6148	EG013613	Short chain alcohol dehydrogenase-like	Metabolism	miRNA 20 UACUUGUUUGCUAAUUGUUU 1 : : : : : : : : : : : : Target 156 AUGAAGAAGCGCUUAAACAAA 175
stu-miR6148	TC211792	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase	Metabolism	miRNA 20 UACUUGUUUGCUAAUUGUUU 1 : : : : : : : : : : : : Target 1272 AUGAACAAGCGAUUGAAAGA 1291
stu-miR6148	DN590149	Acyl-CoA dehydrogenase	Metabolism	miRNA 21 GUACUUGUUUGCUAAUUGUUU 1 : : : : : : : : : : : : Target 360 CAUGAACAGAAGAUUAAUGAG 380
stu-miR6148	DN940390	Class III HD-Zip protein 5	Transcription factor	miRNA 20 UACUUGUUUGCUAAUUGUUU 1 : : : : : : : : : : : : Target 48 AUGAACAAGCUAUUAAUGGA 67
stu-miR6154	CV477984	BYJ15	Metabolism	miRNA 20 UUGAAAGAGGUCCUCUGGGA 1 : : : : : : : : : : : : Target 182 AACUUUCUUCAGAAAGACCCA 201
stu-miR6154	DN907678	Neuropeptide Y receptor type 5	Signal transduction	miRNA 20 UUGAAAGAGGUCCUCUGGGA 1 : : : : : : : : : : : : Target 302 AACUUGCACUAGGAGAUCCU 321
stu-miR6154	BG595832	Peroxidase	Metabolism	miRNA 20 UUGAAAGAGGUCCUCUGGGA 1 : : : : : : : : : : : : Target 631 GACUCUCUCCAGCGGACCCU 650
stu-miR6156	DN921675	Uncharacterized protein At5g55000	Hypothetical protein	miRNA 23 UGUUUUUUUAUCCUGUUUAGAAGUU 1 : : : : : : : : : : : : Target 662 AUGUAAAGAGGGUGAAUCUUCGA 684
stu-miR6156	DN849907	Cytochrome b5	Transporter	miRNA 22 GUUUUUUAUCCUGUUUAGAAGUU 1 : : : : : : : : : : : : Target 18 UAUAAAUGGGUAAAGUCUUCAA 39
stu-miR6164	TC209721	Dehydration-responsive protein RD22	Signal transduction	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 894 CCUCCGUUUUCAUUUUUAUGUGA 914
stu-miR6164	TC223233	Chlorophyll a-b binding protein 6A	Transcription factor	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 617 CCUUCGUUUUCAUUUUUAUGUGA 637
stu-miR6164	TC213482	IAA-alanine resistance protein	Stress-related	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 877 CUUCCGUUUUCAUUUUUAUGUGA 897
stu-miR6164	BQ047226	Biotin carboxyl carrier protein	Transporter	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 420 CCUCCGUCCCAUUUUUAUGUGA 440
stu-miR6164	CK860784	F1N18.14 protein	Transporter	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 458 CCUCUGUUUCAUUUUUAUGUGG 478
stu-miR6164	TC200659	Peroxidase	Metabolism	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 758 CCUCUAUUUCAGUUUUUAUGUGA 778
stu-miR6164	BG096235	Fructose-bisphosphate aldolase	Metabolism	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 129 UCUCCAUUUCAUUUUUAUGUGA 149
stu-miR6164	TC203064	Small GTP binding protein Rab2A	Transcription factor	miRNA 21 GGAGGCAAAGUAAAAUACACU 1 : : : : : : : : : : : : Target 84 CCUCCGUCCCAUUUUUAUGUGG 104

Supplementary Table. (Continued).

stu-miR6426	TC201561	Eukaryotic translation initiation factor	Transcription factor	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 9 UCUUUGCUUCGUUUUCUUCAU 28
stu-miR6426	TC197359	Cytochrome P450 71A2	Metabolism	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 46 UCUUCAUUUUUUUCUUCAU 65
stu-miR6426	CV505427	Nodulin-like protein	Metabolism	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 38 UUUUCACUAAUUUCUUCAA 57
stu-miR6426	BE340580	MATE efflux family protein	Disease-related	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 180 UCUUCACUUCAUUUUCUUCAU 199
stu-miR6426	TC215764	50S ribosomal protein L18	Structural protein	miRNA 20 AGAAGUGAA-GUAAAGAAGUG 1 : : : : : : : : : : Target 46 UUUUCACUUUCAUUUUCUUCAC 66
stu-miR6426	CN213114	Methyl-accepting chemotaxis protein	Signal transduction	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 431 UCUUUAGUUUUUUUCUUUAC 450
stu-miR6426	TC204373	Heme oxygenase 1	Metabolism	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 103 UUUUCACUUCUUUUUUUCAA 122
stu-miR6426	BG591877	Protein yippee-like	Transcription factor	miRNA 20 AGAAGUGAAGUAAAGAAGUG 1 : : : : : : : : : : Target 400 UCUUCAUUUCAUUUUUAUCAU 419
stu-miR6438	XM_006361408	Cytochrome c oxidase assembly protein COX15	Metabolism	target 5' A UAA ACAAGG A 3' UCAUCU UC GUGUGCAU AGUGGA AG CACACGUA miRNA 3' AAA UA A 5' target 5' A A C A 3' GCCUG UCUG UGUG UGGAU AGAC ACAC miRNA 3' AAAAG A GUA 5' target 5' G GU GUGUUGU G 3' U CCUG UUCU UGUGCA A GGAU AAGA ACACGU miRNA 3' AAA GU C A 5' target 5' A G CA C 3' UAU UGU UGUGUGUA GUG AUA ACACACGU miRNA 3' AAAA G AG A 5' target 5' C CCAACA U A 3' UCAUC UGUGUG CAU AGUGG ACACAC GUA miRNA 3' AAA AUAAG 5'
stu-miR6438	XM_006357667	Light-inducible protein CPRF2	Transcription factor	miRNA 3' AAAAG A GUA 5' target 5' G GU GUGUUGU G 3' U CCUG UUCU UGUGCA A GGAU AAGA ACACGU miRNA 3' AAA GU C A 5' target 5' A G CA C 3' UAU UGU UGUGUGUA GUG AUA ACACACGU miRNA 3' AAAA G AG A 5' target 5' C CCAACA U A 3' UCAUC UGUGUG CAU AGUGG ACACAC GUA miRNA 3' AAA AUAAG 5'
stu-miR6438	XM_006367139	Peroxidase	Metabolism	miRNA 3' AAAAG A GUA 5' target 5' G GU GUGUUGU G 3' U CCUG UUCU UGUGCA A GGAU AAGA ACACGU miRNA 3' AAA GU C A 5' target 5' A G CA C 3' UAU UGU UGUGUGUA GUG AUA ACACACGU miRNA 3' AAAA G AG A 5' target 5' C CCAACA U A 3' UCAUC UGUGUG CAU AGUGG ACACAC GUA miRNA 3' AAA AUAAG 5'
stu-miR6438	XM_006365779	SNF1-related protein kinase	Metabolism	miRNA 3' AAAAG A GUA 5' target 5' G GU GUGUUGU G 3' U CCUG UUCU UGUGCA A GGAU AAGA ACACGU miRNA 3' AAA GU C A 5' target 5' A G CA C 3' UAU UGU UGUGUGUA GUG AUA ACACACGU miRNA 3' AAAA G AG A 5' target 5' C CCAACA U A 3' UCAUC UGUGUG CAU AGUGG ACACAC GUA miRNA 3' AAA AUAAG 5'
stu-miR6438	XM_006361371	ABC transporter B	Transporter	miRNA 3' AAAAG A GUA 5' target 5' G GU GUGUUGU G 3' U CCUG UUCU UGUGCA A GGAU AAGA ACACGU miRNA 3' AAA GU C A 5' target 5' A G CA C 3' UAU UGU UGUGUGUA GUG AUA ACACACGU miRNA 3' AAAA G AG A 5' target 5' C CCAACA U A 3' UCAUC UGUGUG CAU AGUGG ACACAC GUA miRNA 3' AAA AUAAG 5'
stu-miR6439	BQ505153	Homeobox protein knotted-1-like LET6	Transcription factor	miRNA 20 UCGAUCACUACCGAAGACCC 1 : : : : : : : : : : Target 386 AGCUAGUGCAAGCUUCUGGG 405
stu-miR6440	TC224448	Membrane protein	Signal transduction	miRNA 21 UUGAGCUUUAGUUCGUUUGAA 1 : : : : : : : : : : Target 599 AACUCGGAAUCAAGCAAACUC 619
stu-miR6440	TC226042	Boron transporter	Transporter	miRNA 21 UUGAGCUUUAGUUCGUUUGAA 1 : : : : : : : : : : Target 799 GACUUGAAAUAAGCAAACUC 819
stu-miR6440	TC203104	Hcr2-0A	Transcription factor	miRNA 21 UUGAGCUUUAGUUCGUUUGAA 1 : : : : : : : : : : Target 332 AACUUGAAAUCAAAUAACUG 352

Supplementary Table. (Continued).

stu-miR6440	CK863901	Adenosine deaminase-like protein	Metabolism	miRNA 20 UGAGCUUUAGUUCGUUUGAA 1 .: :.....:.	Target 427 GUUGGAAGUCAAGCAAUUU 446
stu-miR6440	TC214939	Transport protein-related	Transporter	miRNA 21 UUGAGCUUUAGUUCGUUUGAA 1 : :.....:.	Target 48 AACCCGGAUUCGAGCAAACUC 68
stu-miR6440	TC204085	Xanthine/uracil permease	Metabolism	miRNA 21 UUGAGCUUUAGUUCGUUUGAA 1 : :.....:.	Target 159 AACUCAGAAUCGAGCAAACUC 179
stu-miR6461	TC220557	RD20 protein	Stress-related	miRNA 20 CAAGAUACAUAACGAUCGAU 1 : :.....:.	Target 187 GUUCUAUGUAUUGCUAGCUA 206
stu-miR6461	BG096187	NADH-ubiquinone oxidoreductase chain 1	Metabolism	miRNA 19 AAGAUACAUAACGA-UCGAU 1 : :.....:.	Target 122 UUUUAUGUAUUGCUUAGCUA 141
stu-miR6461	TC207439	Dem protein	Transcription factor	miRNA 20 CAAGAUACAUAACGAUCGAU 1 : :.....:.	Target 636 GUUCAUGUAUUGCUACUUA 655
stu-miR6461	TC203938	Predicted protein	Hypothetical protein	miRNA 20 CAAGAUACAUAACGAUCGAU 1 : :.....:.	Target 1055 CUUUUAUGUAUGGCUAGUUA 1074
stu-miR6468	CV502876	Os06g0561800 protein	Transcription factor	miRNA 21 CCCUCGGUAAGUCUCUUUUG 1 : :.....:.	Target 71 GGGGAGCCAUUCAGAGAAAAC 91
stu-miR6468	BQ112786	Zinc finger A20	Transcription factor	miRNA 20 CCCUCGGUAAGUCUCUUUUG 1 : :.....:.	Target 346 GGUAGCCAUCAGAGGAAGC 365
stu-miR6476	TC208272	T6J4.11 protein	Hypothetical protein	miRNA 20 AGUACAGAGUAGAGGUGAAC 1 : :.....:.	Target 35 UCAUCUCUCAUUUUCGUUUG 54
stu-miR6476	TC214932	Amino acid transporter	Transporter	miRNA 20 AGUACAGAGUAGAGGUGAAC 1 : :.....:.	Target 949 UCCUGUCUUGUCUUCUUUUG 968
stu-miR6476	CV497077	TPR Domain containing protein	Transcription factor	miRNA 20 AGUACAGAGUAGAGGUGAAC 1 : :.....:.	Target 196 UCAAGUCUCAAUUCAUUUG 215
stu-miR7749	TC208224	Homeobox protein SIX5	Transcription factor	miRNA 20 GUUAGCGCGGUAGCGGCCA 1 : :.....:.	Target 415 UUAUCGCCGCUAUCGCCGGU 434
stu-miR7749	CK251696	ACRE 132-like protein	Transcription factor	miRNA 20 GUUAGCGCGGUAGCGGCCA 1 : :.....:.	Target 368 GCGUCGCCGCGUCGCCGGU 387
stu-miR7777	TC201708	Retrotransposon gag protein	Transcription factor	miRNA 20 ACUCCUACCACAAACAGUCG 1 : :.....:.	Target 767 UGAGGAUGUUGUUUUGUUG 786
stu-miR7785	DN743351	ORF91	Hypothetical protein	miRNA 24 CGGAAGAGGGUAGUGGUUAUGAUG 1 : :.....:.	Target 26 GCCUUCUCCCAUCACCAAUACUAC 49
stu-miR7785	CN516169	Metalloproteinase inhibitor	Metabolism	miRNA 23 GGAAGAGGGUAGUGGUUAUGAUG 1 : :.....:.	Target 422 CCUUUCCCAUCAACAGUACUUA 444
stu-miR7816	CN516640	MFS transporter	Transporter	miRNA 21 UUGUCACUAUUUUGUUGUAA 1 : :.....:.	Target 109 AACCAUGAUAGUAACAACAUU 129

Supplementary Table. (Continued).

stu-miR7816	TC203240	BEL1-related homeotic protein 5	Transcription factor	miRNA 21 UUGUCACUAUUUUGUUGUAA 1 ::: ::::: :::::	Target 208 AAUAGUAAUAAUAAUAAU 228
stu-miR7816	DV626534	Uncharacterized protein At4g36980	Hypothetical protein	miRNA 21 UUGUCACUAUUUUGUUGUAA 1 ::: ::::: ::::: :	Target 13 AACAGUGAUGAUAGCAACGAU 33
stu-miR7816	CV495690	Cysteine protease	Metabolism	miRNA 21 UUGUCACUAUUUUGUUGUAA 1 ::: ::::: ::::: :	Target 644 AAUAGUGAUCAUACAACA 664
stu-miR7816	TC205085	Proton/peptide symporter	Transporter	miRNA 20 UGUCACUAUUUUGUUGUAA 1 ::: ::::: :::::	Target 525 AUAGUGAUAAUAAUAAUGUC 544
stu-miR7816	TC217836	Glycosyl transferase, family 48	Metabolism	miRNA 20 UGUCACUAUUUUGUUGUAA 1 ::: ::::: :::::	Target 1830 CUAGUGGUAAUAGCAGCAU 1849
stu-miR7822	TC210260	FtsH-like protein Pftf	Transcription factor	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 968 UCCAUUUUUCUCAUUUCAGG 988
stu-miR7822	TC203839	Chloroplast protease precursor	Metabolism	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 400 UCCAUUUUUCUCAUUUCAGG 420
stu-miR7822	TC218861	Glucose-6-phosphate isomerase	Metabolism	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 310 UCCAUUUUUUUCUUUCAA 330
stu-miR7822	CK268102	LRRGT00097	Metabolism	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 : : ::::: :::::	Target 403 UCUCUUUUUUCGAUUUCGAA 423
stu-miR7822	EG015522	Transcriptional regulator, GntR family	Transcription factor	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 : : ::::: :::::	Target 491 UUCACUUUUUUCAGUUUCAAG 511
stu-miR7822	TC201663	Predicted protein	Hypothetical protein	miRNA 21 AGGUAAAAAAGU-UAAAGUUU 1 ::: ::::: :::::	Target 173 UCCAUUUUUUUCACAUUCAAG 194
stu-miR7822	BM405181	Chromosome segregation ATPases-like protein	Transcription factor	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 : : ::::: :::::	Target 660 UCAUUUUUUUUAAUUUCAAA 680
stu-miR7822	TC207201	Osmotin-like protein	Stress-related	miRNA 20 GGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 751 UCAUUUUUUUCAAAUUCAAG 770
stu-miR7822	TC204399	Cytoplasmic ribosomal protein S13-like	Structural protein	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 635 UCCAUUUUUUUGAAUUUGAU 655
stu-miR7822	BI432396	Portal protein B (GpB)	Transporter	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 ::: ::::: :::::	Target 59 UCCAUUUUUUUGAAUUUGAU 79
stu-miR7822	TC217838	ATP binding / DNA binding / helicase / protein	Transcription factor	miRNA 21 AGGUAAAAAAGUUAAGUUU 1 : : ::::: :::::	Target 374 UUAUUUUUUUUAAUUUGAA 394
stu-miR7825	CK262341	Castasterone 26-hydroxylase	Metabolism	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 ::: ::::: :::::	Target 724 UAUUUUUCUCCUUUCUCAA 743
stu-miR7825	TC206590	Lectin-like protein kinase	Metabolism	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 ::: ::::: :::::	Target 82 UAUUUUUCUUCUUUUUCA 101

Supplementary Table. (Continued).

stu-miR7825	TC201609	Remorin	Signal transduction	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 789 UAUUUUUUCUUUCUUUCUCAA 808
stu-miR7825	TC196963	60S ribosomal protein L6-like protein	Structural protein	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 780 UAUUUUUUCUGUUUUUCUUCGG 799
stu-miR7825	TC216642	Pyruvate kinase	Metabolism	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 6 CAUUUUUUUUUCUUUCUUCAG 25
stu-miR7825	DR035452	Ribosomal protein S27	Structural protein	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 668 UGUCUUUUUCCUUUUUCAA 687
stu-miR7825	TC219861	Calcineurin B-like protein 4-1	Transcription factor	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 471 UAUUUUUUCUUUCUUUCUU 490
stu-miR7825	DN940673	ATP-dependent DNA helicase RecG	Transcription factor	miRNA 20 AUAAAAAGAGGAAAGAAGUU 1 : : : : : : : : : : Target 962 GGUUUUUCUCUUUCUUUGG 981
stu-miR7826	CK263936	Glycolate oxidase	Metabolism	miRNA 21 GGUCUUAAACUUUGGACCAUU 1 : : : : : : : : : : Target 529 UCAGAAUUGGCAACUUGGUA 549
stu-miR7826	TC208775	Acyltransferase homolog	Metabolism	miRNA 21 GGUCUUAAACUUUGGACCAUU 1 : : : : : : : : : : Target 41 CCAGGAUUUGAUACCUUGUA 61
stu-miR7826	TC196351	Tobacco fibrillarin homolog	Metabolism	miRNA 20 GUCUUAAACUUUGGACCAUU 1 : : : : : : : : : : Target 352 AAGAAUUUGGUACCUGGUGA 371
stu-miR7826	TC220412	Arginase 2	Metabolism	miRNA 20 GUCUUAAACUUUGGACCAUU 1 : : : : : : : : : : Target 54 GAGAAUCUGAAACUUGGUGA 73
stu-miR7828	TC217866	Dihydrodipicolinate reductase	Metabolism	miRNA 21 GUAAAAACAAGAGGUACAGUAG 1 : : : : : : : : : : Target 553 UAUUUUUUUUCCAUGUCAGC 573
stu-miR7828	TC220910	NADH-ubiquinone oxidoreductase	Metabolism	miRNA 20 UAAAACAAGAGGUACAGUAG 1 : : : : : : : : : : Target 271 UUUUUGUUUCCAUGUCAAC 290
stu-miR7830	XM_006362168	Enoyl-[acyl-carrier-protein] reductase [NADH] 1	Transporter	target 5' A CAUUG UGCAGUUUUU A A 3' GUCUACC GA GAC GUCC CAGAUGG CU CUG CAGG miRNA 3' A A A 5'
stu-miR7830	XM_006350328	Disease resistance protein At4g27220	Disease-related	target 5' A A GUGAAAUG GUU A 3' UG CUGCC GAGAC UGUCC AC GAUGG CUCUG ACAGG miRNA 3' A A A 5'
stu-miR7830	XM_006345228	Disease resistance protein At4g27190	Disease-related	target 5' A AAAA UGUG G 3' UGUC UGCCUGA GUCC ACAG AUGGACU CAGG miRNA 3' CUGA 5'
stu-miR7830	XM_006344194	Disease resistance protein At1g62630	Disease-related	target 5' G C C 3' GUC ACC GAGACUG CC CAG UGG CUCUGAC GG miRNA 3' A A A A 5'
stu-miR7830	XM_006356387	F-box/FBD/LRR-repeat protein At1g13570	Disease-related	target 5' G CUCCAAA AC A 3' GUUU ACUUGAGA UUGUCU CAGA UGGACUCU GACAGG miRNA 3' A A 5'

Supplementary Table. (Continued).

stu-miR7830	XM_006355507	Transcription factor GTE12	Transcription factor	target 5' G UCAU AUG C G C 3' UGUCUG CC GAG C GUCC ACAGAU GG CUC G CAGG miRNA 3' A U A 5'
stu-miR7830	XM_006343958	Zinc finger protein CONSTANS 2	Transcription factor	target 5' A ACGAACCCUUG C G 3' UCUGCC GA GAUUGUCC AGAUGG CU CUGACAGG miRNA 3' AC A 5'
stu-miR7830	XM_006365151	Ubiquitin-specific protease 1D	Metabolism	target 5' C G G U G 3' UC ACC GAGGC UGUC AG UGG CUCUG ACAG miRNA 3' AC A A G 5'
stu-miR7835	CN515313	Aspartic protease inhibitor	Metabolism	miRNA 20 UGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 80 ACUUUCCCCAAAAUCCCAUC 99
stu-miR7835	CV473305	Kunitz-type protease inhibitor	Metabolism	miRNA 21 GUGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 77 CACUUCCCC-AAAAUCCCAUC 96
stu-miR7835	CN514489	Aspartic protease inhibitor	Metabolism	miRNA 21 GUGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 67 CACUUCCCCAAAAUCCCAUC 87
stu-miR7835	TC202659	CXE carboxylesterase	Metabolism	miRNA 20 UGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 1491 GCAUUCCCCAAAAUCCCAU 1510
stu-miR7835	TC213762	Signal recognition particle 54 kDa protein 1	Signal transduction	miRNA 20 UGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 37 ACUUCCUUCAAAAUCCUGUU 56
stu-miR7835	TC220751	Spindle pole body protein-like	Structural protein	miRNA 21 GUGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 19 UGCUUCCCAAAAAUUUCAUC 39
stu-miR7835	CV496351	Solute carrier family 39 (Zinc transporter)	Transporter	miRNA 20 UGAAGGGGGUUUUAGGGUAG 1 : : : : : : : : : : : : : : : : Target 373 UCUUCCUUCAAAGUCCAUC 392
stu-miR8123	TC195281	40S ribosomal protein-like protein	Structural protein	miRNA 21 CCCAGAGACACGGUAACGAGU 1 : : : : : : : : : : : : : : : : Target 390 GGGUCUCUGUGCCAUUGCUC 410